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THE DISTRIBUTION AND BIOGEOGRAPHY OF THE CRYPTIC MULTIMAMMATE MICE, *MASTOMYS NATALENSIS* (A. SMITH, 1834) AND *MASTOMYS COUCHA* (A. SMITH, 1836) IN SOUTHERN AFRICA

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by

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The distribution and biogeography of the cryptic multimammate mice, *Mastomys natalensis* (A. Smith, 1834) and *Mastomys coucha*(A. Smith, 1836) in southern Africa.

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ABSTRACT

Univariate and multivariate analyses were performed on 17 cranial and dental measurements taken from cytogenetically identified specimens of *Mastomys natalensis* and *M. coucha.* Interpopulation and intrapopulation variation were investigated, and, craniometric discrimination technique for distinguishing between the two species was developed. This discrimination technique was used to identify specimens of *M. natalensis s.l.* from the southern African and distributions of the two species were determined.

Intrapopulation analyses indicated a lack of significant sexual dimorphism, but size related age variation was pronounced. Subsequent analyses of inter - population variation indicated a high degree of geographic variation in both species, with subtle but significant shape related differences between Zimbabwe samples and South African and Namibian samples. Interspecific analyses supported the existence of two shape- related morphometrically distinct taxa within *M. natalensis (sensu lato)* in southern Africa, which are herein taxonomically equated to *M. natalensis* and *M. coucha*. Since geographic variation was substantial it was necessary to develop regionally based discrimination techniques. The distributions of the two species based on this revised data, shows that both species are broadly sympatric in southern Africa. The current data also shows positive correlation between rainfall data and the distribution of the two species, but lack thereof with altitude, vegetation or plague data.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION.

The multimammate mice, previously classified within the *Mastomys natalensis* species complex, are common and widely distributed in subSaharan Africa (Figure 1.1; Skinner & Smithers, 1990; Smithers, 1983; de Graaff, 1981). Throughout Africa, member species of the genus *Mastomy*s have been subject of many studies, mainly because of their role in transmitting diseases to man (Higgins-Opitz, Bhoola & Dettman, 1987; Keogh & Price, 1981; Monath, Newhouse, Kemp, Setzer & Cacciapuoti, 1974; Davis, 1964), and their capacity to cause severe agricultural damage (Leirs, 1992; de Graaff, 1981; Nandwa, 1973). They are also invaluable as models in biomedical research, since they are uniquely susceptible to develop spontaneous tumours, some of which are of human similitude (Murray, 1980).

In southern Africa, *Mastomys* acts as an intermediary host for the plague bacillus, *Yersinia pestis* (Davis, 1964). In Sierra Leone, Lassa virus was isolated from these rodent species during an epidemic (Monath *et al.* 1974). *Mastomys* is also susceptible to experimental infections, such as *Schistosoma mansoni* and *Borelli duttoni* (Higgins-Opitz *et al.* 1987; Keogh & Price, 1981). Other diseases associated with *Mastomys* species include rabies, Witwatersrand and Banzi viruses (Keogh & Price, 1981). Since members of the genus *Mastomys* are commensal with humans (Skinner & Smithers, 1990; Coetzee, 1965), they may transmit pathogens to humans, and thus are of public health concern.

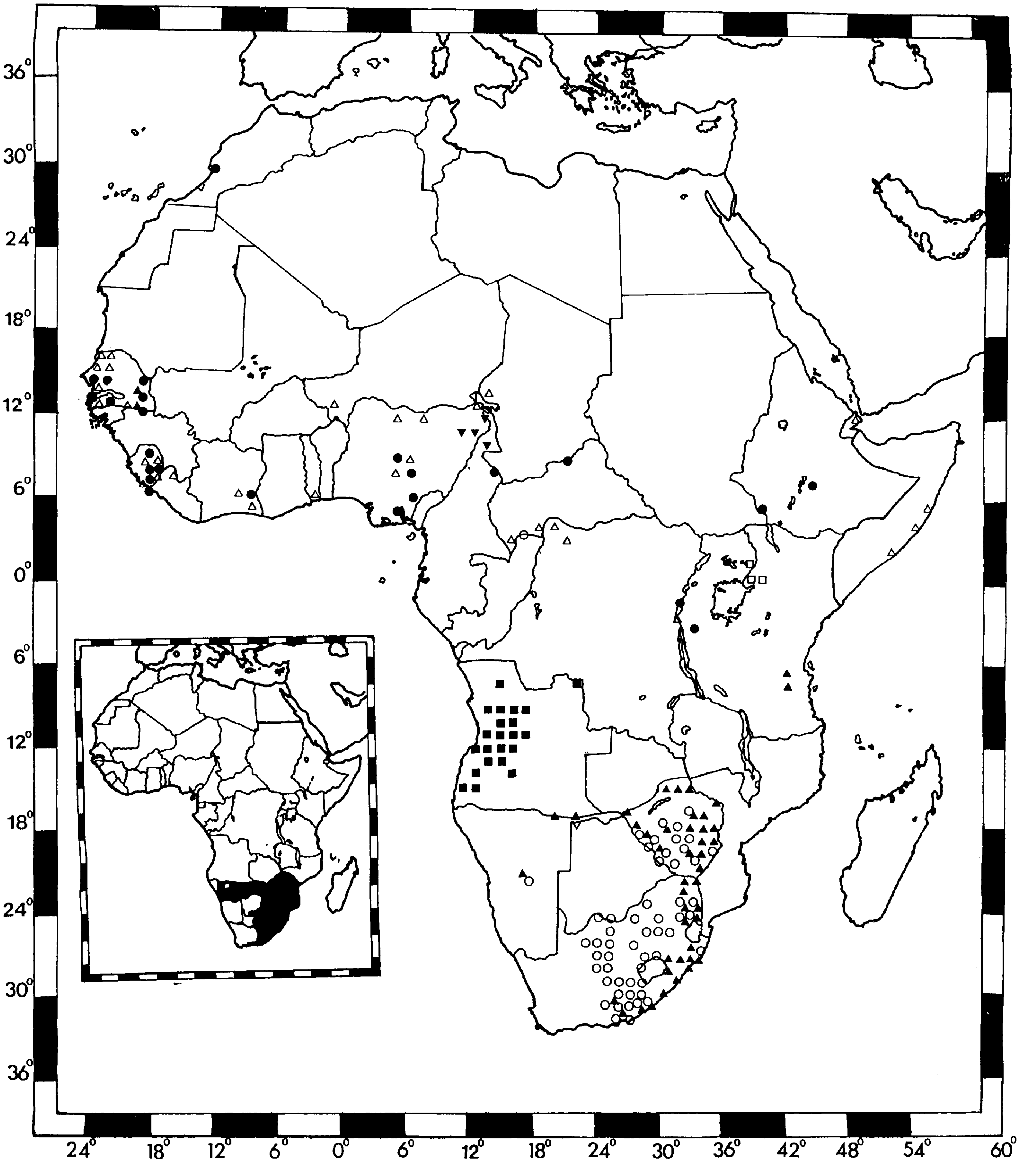

Figure 1.1 : Geographic distribution of the eight species recognised within the genus *Mastomys* in Africa. ■ = *M. angolensis*; ○ = *M. coucha* (2n = 36); ● = *M. erythroleucus*; (2n = 38) △ = *M. hildebrandtii* (2n = 32); ▲ = *M. natalensis* (2n =32); □ = *M. pernanus*; ▽ = *M. shortridgei* (2n = 36); ▼ = *M. verheyeni*. Insert shows the distribution of *M. natalensis s.l.* in southern Africa (Smithers, 1983).

In several countries in Africa, *Mastomys* is known to cause extensive damage to crops like maize, wheat, barley, rice, peanuts and sorghum (de Graaff, 1981; Nandwa, 1973). During rodent population explosions, damage to standing crops can be as high as 80% (Leirs, 1992). They also dig up and consume seeds during planting, resulting in lower yields during harvest time, even if replanting is done (Leirs, 1992).

Internationally, *Mastomys* is used as laboratory model in cancer research. Studies of laboratory colonies of the taxon at the South African Institute of Medical Research (SAIMR) have shown that this taxon is susceptible to adenocarcinoma of the glandular stomach (Oettle, 1955) - a tumour that is rare in species other than humans, thus making multimammate mice useful models for cancer research (Stewart, 1953). Colonies of *Mastomys* have since been distributed to various international laboratories (Coetzee, 1980b). Hepatic tumours, renal lesions, and adrenal tumours have also been studied using *Mastomys* as a model (Fujii & Sato, 1980; Kozima, Soga, Morita, Oite, Yamamoto, Kihara, Murohashi & Tazawa, 1980; Soga & Karaki, 1980; Soga & Tazawa, 1980).

The importance of the role (s) played by member species of the genus *Mastomys* in agriculture, public health and in biomedical research necessitates a proper knowledge of their taxonomy, biology, behaviour and ecology. A broad biological knowledge base of the genus will, therefore, help facilitate the design of effective measures for countering the spread of diseases, as well as the formulation of effective strategies for agricultural control of these problematic rodents.

1.2. TAXONOMIC STATUS OF *M. NATALENSIS (SENSU LATO).*

Cytogenetic and protein electromorph studies conducted during the past two decades revealed that specimens of *M. natalensis (sensu lato)* comprises of a complex of cryptic species, instead of the one species once thought to occur throughout subSaharan Africa (Duplantier, Britton - Davidian, Granjon, 1990; Robbins, Krebs & Johnson, 1983; Green, Gordon & Lyons, 1978; Gordon, 1978; Petter, 1977). In southern Africa, *M. natalensis s.l.* shows two distinct cytotypes (2n = 32 and 2n = 36), and two distinct cytotype specific protein electromorphs described as "slow" and "fast" with respect to human Hb (Robbins *et al.*, 1983; Hallett, 1979; Green *et al.* 1978; Gordon, 1978). Green, Keogh, Gordon, Pinto & Hartwig (1980) referred specimens with 2n = 32 and a "slow" protein electromorph to *M. natalensis* (A. Smith, 1834), and those with 2n = 36 and a "fast" protein electromorph to *M. coucha* (A. Smith, 1836). In addition, comparison of the G - banding chromosome analysis indicated few obvious homologies between the two cytotypes (Lyons, Gordon & Green, 1980). A study of chromosomal phylogeny in the genus *Mastomys* in Africa has also shown *M. natalensis* to be a sister species to the west African *M. huberti* rather than closely allied to *M. coucha (* Britton-Davidian, Catalan, Granjon & Duplantier, 1995), thereby indicating that the two southern African species may not be genealogically closely related.

In addition to molecular and biochemical evidence, current distribution maps based on karyotyped material show that the two species separate geographically, although in some areas they occur in broad sympatry (Keogh & Price, 1981; Green *et al.* 1980). In areas of sympatry no hybrids have been found in nature (Gordon, 1984). Furthermore, laboratory - crossed specimens of the two cytotypes in southern Africa produced hybrids with an intermediate diploid number of chromosomes (2n = 34), but the hybrids were infertile when backcrossed, and showed reduced development of testes, and disruption of meiosis (Hallett, 1977). Green *et al.* (1980) and Gordon (1978) interpreted all the above data as indication of positive assortative mating, evidence that the two cytotypes represent distinct species.

In light of these findings behavioural differences, particularly in relation to reproduction and ultrasonic signalling, were investigated by Gordon (1984). Quantitative analyses of male behaviour in *M. coucha* and *M. natalensis* revealed species - specific behaviour patterns and frequency. During interaction with oestrous females, *M. natalensis* showed a high degree of male initiated allogrooming and little antagonistic behaviour between pairs. Tactile interactions in this species commenced very soon after the introduction of females, and males displayed secondary behavioural patterns including capering about the cage, and springing upwards from a stationary position, after which they would start twitching. *Mastomys coucha* males, on the other hand, remained predominantly motionless away from females, or chased females during the test encounters. Mounting behaviour was characterised by flank grasp and drumming in *M. natalensis,* and single brief thrusts in *M. coucha*. Courtship behaviour thus differs substantially between the two species.

Gordon (1984) also studied ultrasonic vocalizations produced during mating in both species. Call structure (defined in terms of frequency modulated or constant - frequency pulses) were present in both species, but were longer and more frequent in *M. natalensis* than in *M. coucha*.

Differences relating to agonistic behaviour have also been noted between the two species. Leirs (1994) noted that laboratory - kept *M. coucha* is more docile, and easy to handle. Some field workers have also reported that wild-caught *M. natalensis* is more aggressive than *M. coucha* to humans (Leirs, 1994).

The past failure to detect interspecific variation within the southern African *M. natalensis* species complex was a consequence of the lack of obvious observable and measurable morphological differences. Until recently, known morphological differences between the two species included only differences in urethral lappets on the distal surface of the urethra (Gordon,1984) and spermatozoa morphology (Gordon & Watson, 1985). Dippenaar, Swanepoel & Gordon (1993), however, showed that the two species can be distinguished cranially using multivariate statistical procedures. Their study is important as it opens the door to the identification of these two cryptic species in the absence of cytogenetic data.

1.3. GENERAL OVERVIEW OF THE GENUS *MASTOMYS*.

This section gives a general review of the taxonomy, ecology, reproduction and behavioural studies done on the genus *Mastomys*. Taxonomy forms the basis of the current study, and although peripheral to this study, the ecology, reproduction and behaviour of *Mastomys* have also been intensively studied owing to the role (s) of the species as disease carriers and being agricultural pests. Since most of the past research, particularly in southern Africa, was carried out prior to the discovery of the two species within *M. natalensis s.l.*, results of these studies may refer to either species. The present study shall attempt to clarify which of the two species were studied during these investigations.

1.3.1. Taxonomic overview of the genus *Mastomys*.

Generic status of *Mastomys*.

The generic affiliation of the multimammate mice has been a subject of historical uncertainty (Meester, Rautenbach, Dippenaar & Baker, 1986). Previous authors treated *Mastomys* as a subgenus of either *Rattus* (Meester, 1960; Davis & Oettle, 1958, Oettle, 1955) or that of *Praomys* (Swanepoel, 1980; Hallett, 1979; Green *et al.* 1978), owing to its possession of characteristics typical of both rats and mice, some that are intermediate, as well as traits distinct to these murid rodents (Dettman, Higgins - Opitz & Bronner, 1987). Meester *et al.* (1986) summarized the use of *Mastomys* in past literature. They and several recent authors (Dippenaar *et al.* 1993; Leirs, 1992; Bronner, Rautenbach & Meester, 1988; Dettman *et al.* 1987; Isaacson, Artzen & Taylor, 1981; Green *et al.* 1980) recognized *Mastomys* as a distinct genus following evidence based on the chromosomal studies by Matthey (1958), and multivariate analyses of cranial features by Van der Straeten (1979).

Currently recognized species and distributions.

Systematic problems within *Mastomys* are more acute now than ever before, as no practical morphological characters have been found to define and identify many of the cryptic species known to exist (Robbins & Van der Straeten, 1989), and recent systematic studies based on chromosomal data (Duplantier, Britton-Davidian, Granjon, 1990; Green *et al.* 1980; Gordon, 1978) have revealed karyotypic diversity within the genus. Furthermore, the majority of the systematic studies on *Mastomys* have been limited only to some African countries or geographic regions (Duplantier *et al.* 1990 - Senegal; Dippenaar *et al.* 1993; Gordon 1984; Green *et al.* 1980 - southern Africa; Leirs, Verheyen, Michiels, Verhagen & Stuyck, 1989 - Tanzania; Robbins & Van der Straeten, 1989 - west Africa). No comprehensive Pan African taxonomic revision of the genus has been undertaken to date. Robbins & Van der Straeten (1989) reviewed 56 taxa associated with *Mastomys*, and assigned 44 taxa to the genus *Mastomys,* but did not allocate any of them to species. For the purpose of this review, I follow Musser & Carleton (1993), who listed the following eight species within the genus *Mastomys* throughout Africa:

Mastomys angolensis Bocage, 1890

Some authors considered this species to be closely related to or conspecific with

M. shortridgei but the two are currently treated as distinct species (Meester *et al.* 1986). There is currently no published chromosomal data on *M. angolensis,* which is known to occur in Angola and the southern part of Zaire (Figure 1.1; Musser & Carleton, 1993; Crawford - Cabral, 1983).

Mastomys coucha Smith, 1836

This is one of the three species known to occur in the southern African subregion and a detailed account of the taxonomic characters attributed to this species is given in Section 1.2. Based on current published information, *M. coucha* occurs in south and western parts of Zimbabwe and in central Namibia (Skinner & Smithers, 1990; Gordon, 1984; Meester *et al.* 1986). In South Africa, the species is predominantly distributed in the Southern Savanna Grassland, the South West Arid, and the Southern Woodland biotic zones, with the marginal intrusion into the South West Cape Biotic Zone (Gordon, 1984; Keogh & Price, 1981; Green *et al.* 1980). With the exception of a single specimen from the Central African Republic (Figure 1.1; Hubert, Meylan, Petter, Poulet & Trainer, 1983), the extralimital range of the species is currently unknown.

Mastomys erythroleucus Temminck, 1853

This species is distinct from other *Mastomys* species in diploid number of chromosomes ($2n = 38$), allozyme properties (Musser & Carleton, 1993 and references therein), serum proteins (Robbins & Van der Straeten, 1989) and fundamental number ($NF = 50 - 56$; Granjon, Duplantier, Britton - Davidian, Catalan, Ducroz & Volobouev, 1996). It ranges from Morocco, Gambia and Senegal eastwards through west Africa to southern Ethiopia and Somalia, and southwards through east Africa to eastern Zaire, and Burundi (Figure 1.1, Musser & Carleton, 1993; Duplantier *et al.* 1990; Hubert *et al.* 1983; Monath *et al.* 1974).

Mastomys hildebrandtii Peters, 1878

This taxon is characterised by the same diploid number of chromosomes (2n = 32) as *M. natalensis*, but the two taxa differ in haemoglobin patterns (Robbins *et al.* 1983), serum proteins (Robbins & Van der Straeten, 1989) and fundamental number (NF = 44 - 46; Granjon *et al.*, 1996). There are currently nomenclatural problems concerning this taxon. Musser & Carleton (1993) considered *M. huberti* Wroughton, 1908 to be a synonym of *M. hildebrandtii*, and referred this taxon to *M. hildebrandtii* following Qumsiyeh, King, Arroyo - Cabreles, Aggundey, Schlitter, Baker & Morrow (1990). They, therefore, considered this taxon to range from Senegal and Gambia eastwards through west Africa to the Central African Republic and northern Zaire, extending to Djibouti and Somalia, then southwards to Burundi and Kenya (Figure 1.1). Recent publications, however, refer to this taxon as *M. huberti* (Granjon, Duplantier, Catalan, Britton - Davidian & Bronner (in press); Duplantier *et al.* 1990; Britton -Davidian *et al.* 1995). Granjon[1] (pers comm, 1996), consider *M. huberti* as a distinct species from *M. hildebrandtii*, and maintain that the distribution of the latter species distribution is restricted to west Africa. Oguge[2] (pers comm, 1996), however, karyotyped *Mastomys* specimens with 2n =32, and NF = 44 from Kenya, and this suggests that the distribution of this taxon does indeed extend to east Africa.

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[2] Dr N Oguge, Department of Zoology, Kenyatta University, P.O Box 43844, Nairobi, Kenya.

Mastomys natalensis Smith, 1834

Further details pertaining to diagnostic characters of this species are given in Section 1.2. *Mastomys natalensis* seems to be more widely distributed in subSaharan Africa than other species in the genus. This species has been recorded from the east - central part of Tanzania (Figure 1.1; Leirs, 1992), and the south - eastern Senegal (Granjon & Duplantier, 1993). It also occurs widely in Zimbabwe, and in central and north - eastern Namibia (Skinner & Smithers, 1990; Meester *et al.* 1986). The distribution range of this species in South Africa correlates mainly with the Southern Savanna Woodland biotic zone, although it occurs peripherally in the South West Arid and the Southern Savanna Grassland biotic zones (Gordon, 1984). Granjon *et al.* (in press) demonstrated conspecificity of *M. natalensis* populations from Senegal and South Africa, which suggests that the distribution of the species may cover most of Africa south of the Sahelian zone.

Mastomys pernanus Kershaw, 1921

There are no published chromosomal data for *M. pernanus*. Robbins & Van der Straeten (1989) have speculated that this species should be referred to *Myomys,* but Musser & Carleton (1993) currently assigns it to *Mastomys*. This species is known to occur in south - western Kenya, north - western Tanzania and in Rwanda (Figure 1.1; Musser & Carleton, 1993; Missone & Verschuren, 1964)

Mastomys shortridgei St Leger, 1933

Mastomys shortridgei has same diploid number of chromosomes ($2n = 36$) as *M. coucha* (Gordon, 1984), but the two species differ in autosomal configuration, morphology of the sex chromosomes, and the shape of the spermatozoon head (Skinner & Smithers, 1990). This species differs also from its two southern African counterparts in possessing only five pairs of mammae, which are not clearly separated into pectoral and inguinal sets (Skinner & Smithers, 1990). It occurs in the north - western parts of Botswana and north - eastern Namibia at the confluence of the Okavango and Kwito rivers (Figure 1.1; Skinner & Smithers, 1990; Meester *et al.* 1986).

Mastomys verheyeni Robbins and Van der Straeten, 1989

This newly described species differs from other *Mastomys* by the larger size of the molar teeth (Robbins &Van der Straeten, 1989). No chromosal data are available for this species, which is known to occur in Nigeria and Cameroon savanna area surrounding the southern part of Lake Chad (Figure 1.1; Robbins & Van der Straeten, 1989).

1.3.2 Ecology.

Mastomys natalensis s.l. occurs in a wide variety of habitats, climates and geographic ranges (Meester, Lloyd & Rowe-rowe, 1979). The ecological distribution range of this taxon includes open grassland, mixed savanna and clearings in West African forests (Coetzee, 1975). In southern Africa it is common in dense bush and cultivated areas (Veenstra, 1958), as well as around human habitations, where population densities often exceed those in the wild (Coetzee, 1980a). Multimammate mice thrive in transitory and seasonal habitats, and in areas which are confined in size, discontinuous and separated by marginal or sub-marginal areas (Willan & Meester, 1989). It is, however, rare in arid, winter rainfall areas, and mountainous plains (Coetzee, 1975). In Tanzania, multimammate mice have been reported to occur in such diverse habitats as forests, vegetation along a water courses, grassland and rocky environments (Vesey - Fitzgerald, 1966).

Mastomys tends to nest in sheltered places, like rocks and crevices, or in deserted underground burrows of other rodents (Coetzee, 1975 ; Choate, 1972). Choate (1972) observed that their nests are simple, consisting of grass or leaves, and that they are often occupied by more than one adult. In the field, *M. natalensis* may utilize the vertical component of a habitat, as the species is adapted for arboreal feeding (Willan, 1982).

Mastomys natalensis s.l. co-exists with other rodent species, such as *Tatera, Mus, Aethomys, Thamnomys* and *Rhabdomys,* but is excluded from areas where *Rattus* is established (Coetzee, 1975). Swanepoel (1976) and Veenstra (1958) observed that *M. natalensis s.l.* is a pioneer species which seems to prefer areas suffering from some sort of destruction, but that it is displaced in favour of more specialized species (such as *Otomys* and *Rhabdomys pumilio)* as the habitat recovers and ecological succession continues (Meester *et al.* 1979). In a post-mining rehabilitation study, *M. natalensis s.l.* densities were shown to have dropped from about 50 animals\ha at about six months after rehabilitation to about 15 animals\ha after eight months of rehabilitation (Neluvhalani, Ferreira & Van Aarde, 1995). While *M. natalensis s.l.* tends to dominate "young" rehabilitating stands, it is replaced in older stands by *Saccostomus campestris* (Ferreira & Van Aarde, 1996). In burned habitats, *M. natalensis* may predominate for up to 8 months after burning before eventually being displaced by more specialized species (Bronner *et al.* 1988). Given their predilection for disturbed areas, Meester *et al.* (1979) suggested numbers of these rodent species can be expected to rise as human impact on environments increases.

1.3.3. Reproduction.

Population explosions of *M. natalensis s.l.* species can have profound implications for agriculture and public health. Several authors have, therefore, studied the breeding patterns of *Mastomys* to understand factors associated with their reproduction (Bronner *et al.* 1988; Swanepoel, 1980; Coetzee, 1965 - South Africa; Leirs, 1992; Telford, 1989 -

Tanzania).

Mastomys natalensis s.l. are characterized by 10 to 12 pairs of mammae (de Graaff, 1981). Their reproductive rate is two and four times that of *R. pumilio* and *O. irroratus* respectively (Willan & Meester, 1989). Litter size can range from an average of 7.32 (Oliff, 1953) to 8.53 (Meester, 1960) young per litter, and females can give birth to up to 20 young (Willan, 1992). Consequently, population sizes can increase rapidly when favourably environmental conditions prevail. Furthermore, the gestation period is only 23 days, and post-partum oestrus may start within 2.6 days after parturition (Willan, 1992). Young show rapid post-natal development and reach sexual maturity within three months (Willan, 1992), so that they have a short generation time of about three months (Willan & Meester, 1989).

Breeding in *M. natalensis s.l.* normally peaks during warmer and wetter months (Bronner *et al.* 1988; Swanepoel, 1979; Coetzee, 1965). Food availability, or some secondary plant compounds such as 6-MBOA, appear to be the main factors regulating reproduction (Linn, 1991; Leirs, 1992). Willan & Meester (1989) and Bronner *et al.* (1988) maintained that *M. natalensis* are opportunistic breeders that breed throughout the year when conditions allow, and thus that they do not use seasonal or climatic predictors to regulate reproduction. Leirs (1992) however, argued that in equatorial Africa reproduction is distinctly seasonal, and that *M. natalensis* uses rainfall as a short term cue to ensure that young are born when optimal conditions prevail.

1.3.4. Life history strategies.

An important aspect in pest control management of problematic rodents, is the study of their life history strategies (Leirs, 1994). The only such studies done on *M. natalensis* (Leirs, 1994; Willan, 1982) adopted the r - K selection theory (Pianka, 1970). The r -K selection theory characterises r - selected strategists as those species which tend to maximize productivity by reducing body size, longevity and age at sexual maturity. Such species also tend to show poor parental care, and have short generation time. Litter size are usually large and these species have a tendency to semelparity.

Willan & Meester (1989) compared life history traits of three southern African rodent (*Mastomys natalensis, Otomys irroratus* and *Rhabdomys pumilio*) according to the r - K selection theory. Their study concluded that *M. natalensis* has the shortest life span, largest litter size, highest reproductive effort and fecundity. Furthermore *M. natalensis* is an opportunistic breeder, it shows poor parental care, and population fluctuations in this taxon tend to be conspicuous. These traits as well as the good colonizing ability of the taxon (Table 1.1), led Willan & Meester (1989) to conclude that *M. natalensis* is the most r -selected of the three species they compared. Leirs (1994) compared data of *M. natalensis* populations from Tanzania to that from South Africa. His study showed that the life history traits of *M. natalensis* from the two countries are largely comparable. His findings supported Willan & Meester (1989)'s conclusions that *M.natalensis* is an r - selected strategist (Table 1.1). The only differences between data from the two countries related to population fluctuations, which tended to be more marked in Tanzania. Furthermore, fecundity in populations from tropical Africa is higher, maximal body size is larger and generation time is longer than in the South African populations (Leirs, 1994; Table 1.1).

1.3.5. Behaviour .

Mastomys natalensis s.l. has been described as gregarious, non - aggressive, and generally non - territorial (Leirs, 1994; Willan, 1982; Coetzee, 1975; Choate, 1972; Veenstra, 1958). Both in the field and in captivity several adults can live together with little aggression

Table 1.1 :Comparison of life history traits in *M. natalensis* from South Africa and Tanzania (data from Leirs, 1994; Willan & Meester, 1989). Cited values of some of the traits are given in parentheses. SA denotes South Africa.

Trait	Willan & Meester (1989). Conclusions	Leirs (1994). Conclusions and Comparison with SA data
Habitat	unpredictably unstable	predictable at short term
Maximum body weight	intermediate (81g)	larger (99 g)
Maximum body length	intermediate (145mm)	longer (171mm)
Longevity	short (11 months)	short (11 months)
Reproduction		
gestation period	short (23 days)	short (23 days)
generation time	short (54 days)	longer (60 - 180 days)
litter size	large (7.30 -11.27)	large (11.31)
fecundity	high (24 - 37 young\adult)	higher (up to 68 young\adult)
parental care	little	no data
seasonality	opportunistic	opportunistic
Population fluctuations	large (4.7 - 9.6 \hectare)	larger (19.2 \ hectare)
Colonizing ability	good	good
Competitive ability	poor	poor

and strangers are readily accepted in cages (Leirs, 1994; Veenstra, 1958).

Mastomys natalensis s.l. is nervous with respect to handlers, is quick to escape and is conscious of danger, both in the laboratory and the field (Veenstra, 1958; Choate, 1972). In Senegal, however, *M. natalensis* has been reported to show aggressive behaviour but other congenerics are less belligerent (Granjon, Duplantier & Cassaing, 1986 in Leirs, 1994).

Several studies have suggested that territoriality in *M. natalensis s.l.* is poorly developed (Cilliers, 1972; De Wit, 1972; Veenstra, 1958). In captivity, however, Cilliers (1972) observed struggle for dominance and killing of wild - caught males of *M.natalensis s.l.* during the first phase of his study. He attributed such behaviour to demographic factors such as population density and age structure. On the basis of his study, that of Cilliers (1972) and of De Wit (1972), Willan (1982) concluded that *M. natalensis* may be partially territorial, with social organization based on a dominance hierarchy whereby dominant males make the greatest reproductive contribution

Mating in *M. natalensis* tends to occur at night (Cilliers, 1972), and in captivity it breeds freely (Willan, 1982). The low intensity, but prolonged courtship, in these murid rodents may imply monogamy and that pair - bonding occurs (Willan, 1982). However, polygamous associations have been observed both in captivity and in the field (Willan, 1982; Cilliers, 1972). Willan (1982) ascribed the low intensity of courtship to the colonial social organization which he presumed ensures frequent associations between potential mates.

Both females and males participate in taking care and protecting young until they are able to walk (Meester, 1960; Meester & Hallett, 1970), but the level of parental care is less than that in *R. pumilio* or *O. irroratus* (Willan & Meester, 1989). Choate (1972) and Meester (1960) pointed out that, if disturbed, females will eat their young - a common practice among rodents.

Being nocturnal (de Graaff, 1981; Haim & Fourie, 1980), visual and auditory communication is less important in *M. natalensis*, and it instead emphasizes olfactory and ultrasonic signals (Willan, 1982). Exchange of olfactory cues is particularly important during courtship, and may be important in maintenance of group cohesion (Willan, 1982). Tactile communication tends to involve allogrooming, and initial contact behaviour with conspecifics is direct and amicable (Willan, 1982).

1.4. AIMS AND APPROACH.

Cryptic species within the genus *Mastomys* can be diagnosed with confidence only through the application of molecular or biochemical techniques (Granjon *et al.* in press). However, museum specimens are not amenable to molecular or allozyme identification, and can be identified only by reliable qualitative and or quantitative morphological characters.

The study of Dippenaar *et al.* (1993) paved the way for morphometric discrimination of specimens of *M. natalensis s.l.* which lack cytogenetic or protein data. However, their study was based on samples from a limited geographic range in South Africa, and excluded juveniles and subadults.

The aims of this study, were :

1. To expand the applicability of the morphometric discrimination technique developed by Dippenaar *et al.* (1993) by including all available specimens from localities in southern Africa, and to involve all age groups.
2. To delineate the distribution ranges of the two cryptic species in southern Africa, as the current distribution ranges of the two species are based on a limited cytogenetic data (Keogh & Price, 1981; Green *et al.* 1980 ; Hallett, 1979 ; Gordon, 1978).

3. To examine the climatic and environmental correlates of the distribution patterns of the two species. Some authors have suggested that the distribution of the *M.natalensis s.l.* could be influenced by human interference (Gordon, 1978), but correlations between the current species distributions with the major biotic zones and rainfall areas, have also been noted (Gordon, 1984). The correspondence between environmental or climatic variables and the broader distributions of the two species, therefore, needs to be examined in detail.

4. To examine the correlation between historic outbreaks of bubonic plague and the distribution patterns of the two species. Many of the investigations on *Mastomys* in southern Africa were carried out because of the role the multimammate mice play in transmission of the plague bacillus, *Yersinia pestis* to humans (Davis, 1964). The discovery of two cytotypes within the *M. natalensis* species complex, therefore, had direct implications for epidemiological studies. Subsequent observations suggested that *M. coucha,* rather than *M. natalensis,* may be responsible for plague transmission to human. Isaacson *et al.* (1981) demonstrated that *M. coucha* was more susceptible to experimental infection with plague bacillus than *M. natalensis.* In addition, Taylor, Gordon & Isaacson (1981) also pointed out that *M. coucha* distribution range is positively correlated with the plague outbreak areas. The relationship between bubonic plague outbreak areas and the broader distribution of the two species needs to be examined, to understand the role of these rodent species in plague epidemiology.

The approach taken in this study follows the Biological Species Concept, which characterises species as reproductively isolated entities (Mayr & Ashlock, 1991; Mayr, 1963). Since this study was based on museum specimens, reproductive isolation was inferred from morphological evidence (Mayr & Ashlock, 1991; Wiley, 1981; Sneath & Sokal, 1973) indicated by numerical taxonomy (morphometrics) techniques.

Multivariate morphometric techniques can be used to examine patterns of allometry (Bookstein, Chernoff, Elder, Humphries, Smith & Strauss, 1985), and to identify patterns of isometric variability among characters (Chimimba & Dippenaar, 1995; Taylor & Meester, 1993). These techniques are commonly used as systematic tools to study variation within species (Chimimba & Dippenaar, 1994; Taylor & Meester, 1993; Patton & Rogers, 1983), and also among species (Dippenaar *et al.* 1993; Chapman, Cramer, Dippenaar & Robinson, 1992). While there are new developments in morphometric data acquisition methodology and analyses (Rohlf, 1990), the resources available for this study restricted application of this newer technology. Instead, the methodology approach taken in this study followed "traditional" morphometrics in which linear measurements are taken directly from specimens and then subjected to *a priori* and *a posteriori* analyses.

The geographic range covered in this study, is limited to southern Africa and includes all the countries south of Kunene and Zambezi rivers in which *M. natalensis s.l.* has been recorded to occur (see insert in Figure 1.1).

CHAPTER 2

GENERAL MATERIALS AND METHODS

This chapter describes general materials and methods used in analyses of intrapopulation and interpopulation variation.

2.1. MATERIAL EXAMINED.

Four hundred and seventy nine cytogenetically known specimens of *M. natalensis* and *M. coucha,* and 2388 specimens of *M. natalensis s.l.,* were examined (Appendix 1). Forty two of the cytogenetically known specimens included in the analyses were identified karyotypically during the course of the present study. The remainder of the known specimens were assigned to their respective species groups based on the diploid number of chromosomes or haemoglobin electromorphs, as indicated on Transvaal Museum catalogue cards. The procedure followed to obtain karyotypes is detailed in Section 2.6. All specimens examined are housed at the following museums: Durban Natural Science Museum, Durban (DM); Kaffrarian Museum, King William's Town (KM); Natural History Museum of Zimbabwe, Bulawayo (NHMZ); National Museum, Bloemfontein (NMB); Transvaal Museum, Pretoria (TM); the National Museum of Natural History, Smithsonian Institution, Washington D.C. (USNM); and the South African Museum, Cape Town (ZM).

2.2. MEASUREMENTS.

Seventeen cranial and dental measurements were taken, and are illustrated in Figure 2.1. Fourteen of these measurements were adopted directly from Dippenaar *et al* (1993).

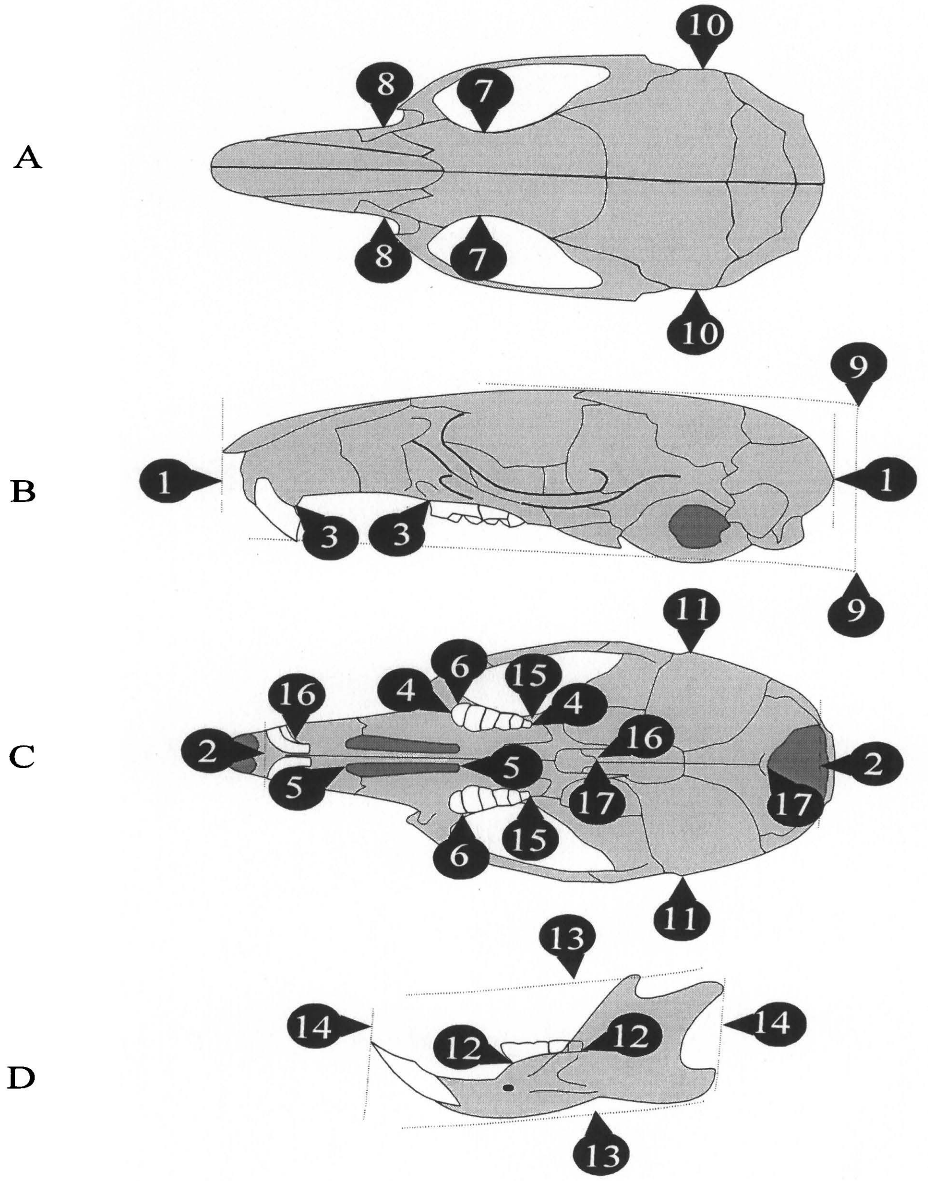


Figure 2.1 : Cranial and toothrow measurements as modified from Dippenaar *et al.* (1993) : 1. GLS, greatest length of skull; 2. CBL, condylobasal length; 3. DIAS, greatest length of the diastema; 4. MTR, greatest length of the maxillary toothrow at level of alveoli; 5. APF, greatest length of the palatal foramina; 6. GBP, greatest breadth of the palate; 7. IOB, interorbital breadth; 8. ROST, width of the rostrum; 9. DOB, greatest breadth of the braincase ; 10. BrBr, greatest length of braincase; 11. MAST, width of the braincase across mastoids; 12. MDT, greatest length of the mandibular toothrow at the level of alveoli; 13. MDH, greatest height of the mandible; 14. MDL, greatest length mandible including principal lower incisor; 15. PPW, post palatal width; 16. PAL, palatal length; 17. PPL, post palatal length.

Three additional measurements were taken from the palatal region of the skull (PPL, PPW, PAL), since measurements from this region loaded highly in the principal components analyses, indicating their importance in interspecific discrimination (Dippenaar *et al.* 1993). All measurements were recorded to the nearest 0,01mm with the Mitutoyo digital calliper (Model 500-231) linked directly to a customized DBase 3+ program (Ashton-Tate Software Inc, USA) by a Mitutoyo Interface (Model MRS 232) and Data- Q software programme developed by D. L. Schultz

2.3. MEASUREMENT ERROR.

Taylor, Meester & Rautenbach (1990) stated that the usefulness of continuous variables in describing biological processes depends on statistical properties such as resolution, precision, accuracy and conformity to a normal distribution.

Accuracy, defined as the correspondence between replicate pairs of measurements taken independently by two or more persons (DeBlase & Martin 1981), was quantified by computing Pearson's correlation coefficients (r) of the same fourteen measurements taken independently by Dippenaar *et al.* (1993) and myself. The correlation coefficients (r) ranged from 0.67 to 0.99, and twelve measurement indicated very high correlation ($r \geq 0.95$) between the two data sets (Table 2.1).

Precision was quantified by calculating the index of the measurement error (ME) following Taylor *et al.* (1990), using replicated data for the seventeen variables taken over a week period from eight specimens of both *M. coucha* and *M. natalensis.* The index of measurement error of all variables ranged from 1,27 - 9,80%. Measurement error accounting for less than 15% of the variable range indicates reliability of variables in measuring of biological trends (Taylor *et al.* 1990)

Table 2.1 : Table showing the level of precision and accuracy as quantified by computing (a) index of measuring error (ME) from replicated data of 17 measurements taken over a week period and (b) Pearson's correlation coefficients (*r*) between replicate data taken by Dippenaar *et al.* (1993) and myself. ME was calculated using the formula of Taylor *et al.* (1990) and is expressed as percentage of range of each measurement.

Measurement	Precision Measuring error (%)	Accuracy Pearson's correlation coefficient (r)
GLS	1,27	0,96
CBL	4,75	0,95
DOB	6,82	0,67
BrBr	7,26	0,98
MAST	8,68	0,85
IOB	6,60	0,98
ROST	6,71	0,98
APF	5,14	0,99
DIAS	3,11	0,99
PAL	7,08	----
GBP	8,33	0,98
PPW	7,58	----
MTR	9,34	0,97
MDT	9,80	0,98
MDH	1,92	0,99
MDL	2,08	0,99
PPL	3,06	-----

2.4. QUALITATIVE DENTAL MORPHOLOGY.

Qualitative dental characters, in particular cuspidation patterns of the occlusal surface of the dentition, have traditionally been used as diagnostic tools in rodents (Skinner & Smithers, 1990; de Graaf,1981). Despite the introduction of the new sophisticated taxonomical tools, identification of museum specimens, particularly of cryptic species (such as *M. coucha* and *M. natalensis*), is possible only through the use of morphometric procedures or reliable qualitative characters.

Pocock (1993) claimed that *M. natalensis* and *M. coucha* can be distinguished by the presence or absence of the t3 cusp on the third upper molar (Figure 2. 2 a), with at least 90% accuracy. He observed from positively identified specimens of both species that *M. coucha* has a t3 cusp, which is lacking in *M. natalensis*. The ostensible reliability of this diagnostic character has not, however, been independently tested.

Accordingly, all known *M. coucha* and *M. natalensis* specimens were given one of following scores: (i) 0 : absence of the t3 cusp (ii) 1: t3 cusp present but poorly developed (iii) 2: t3 cusp present and fully developed.

2.5. TOOTHWEAR CLASSES.

Age - related differences may interfere with geographic and interspecific patterns of morphometric variation, and thus need to be assessed. Several authors have shown age variation in morphometric data to be significant (Chimimba & Dippenaar, 1994; Dippenaar & Rautenbach, 1986; Patton & Rogers, 1983; Verheyen & Bracke, 1966). All specimens examined in this study were thus aged to asses the influence of age variation on craniometric data of both species.

Morris (1972) and Guilday (1957) questioned the reliability of toothwear as a measure of age, since toothwear is influenced by environmental factors (such as diet and habitat). However, toothwear is the only practical and simple criterion for ageing museum specimens, and has been widely applied in rodent taxonomy (Chimimba & Dippenaar, 1994; Dippenaar & Rautenbach, 1986; Verheyen & Bracke, 1966).

Cusp and dental terminology follows de Graaff (1981) and is illustrated in Figure 2. 2 a. Specimens were allocated to six toothwear (TW) classes according to the degree of wear on the cusps of the first upper molar (Fig 2. 2 b). The degree of toothwear was examined using a micrometer fitted to a Wild-Heerbrug microscope. The ageing criteria used were modified from Verheyen & Bracke (1966), and the following six toothwear classes were recognized :

TOOTHWEAR CLASS 1 (TW 1): M^1 unerupted or in the process of erupting. t1, t2, t3 high and well separated.

TOOTHWEAR CLASS 2 (TW 2): t1, t2, t3 connected by poorly developed dentine bridges.

TOOTHWEAR CLASS 3 (TW 3): t1, t2 and t3 in the first row of M^1 connected by well developed dentine bridges; lamellae 2 and 3 separated by a distinct groove.

TOOTHWEAR CLASS 4 (TW 4): the groove separating the 2nd and 3rd lamellae is worn on the lingual side of the molar, but dentine of the cusps are not continuous .

TOOTHWEAR CLASS 5 (TW 5) : the dentine of the 2nd and 3rd lamellae are in contact.

TOOTHWEAR CLASS 6 (TW 6): the bottom of the groove between lamel 1 and 2 is reached and the tooth is extremely worn, the layer of the enamel on the outer rim of the tooth is interrupted.

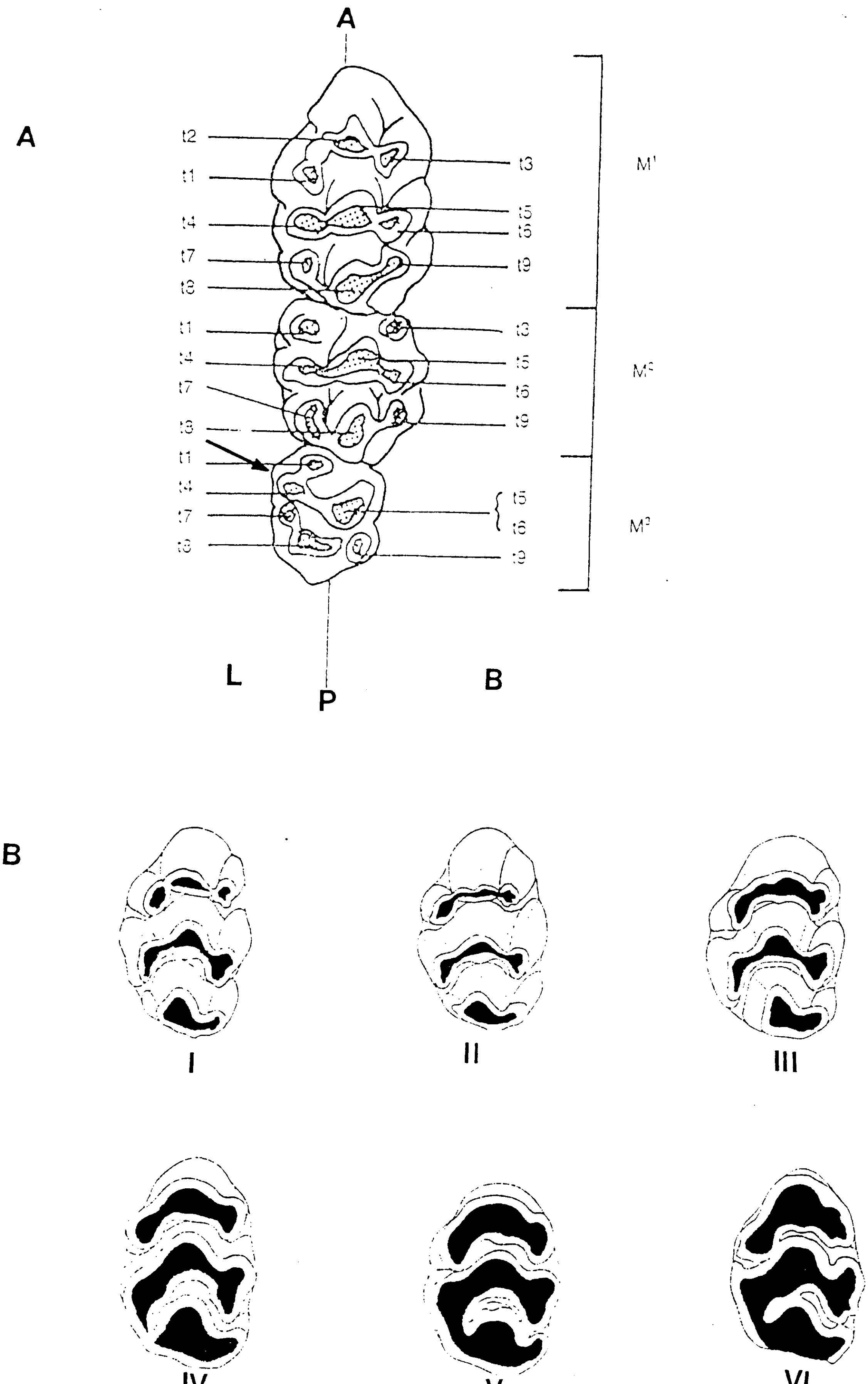


Figure 2.2 : Illustration of (a) the cusp and dental terminology following de Graaff (1981), A = anterior end, P = posterior end, L = lingual , B = buccal, and arrow indicates the position of the t3 cusp on the third upper molar (Pocock, 1993), (b) the degree of wear on the first upper molar in each of the six toothwear classes used to age specimens of *M. coucha, M. natalensis* and *M. natalensis (sensu lato)* as modified from Verheyen & Bracke (1966).

The toothwear classes in this study are considered arbitrary as there were no known-age *M. natalensis s.l.* available for calibration purposes. Their biological significance only serve to reflect the relative ages of specimens.

2.6. KARYOLOGY.

Forty two *M. natalensis* and *M. coucha* collected from five localities during the course of the present study were karyotyped. Somatic mitotic metaphase spreads were obtained from bone marrow preparations following the standard *in-vitro* method outlined by Green *et al.* (1980). Animals were yeast treated twenty - four hours before bone marrow preparations (Lee & Elder, 1980). Five spreads of each karyotyped specimen were analysed to determine the diploid number of chromosomes (2n). Slides of the spreads, and voucher specimens were deposited at the Transvaal Museum, Pretoria.

2.7. STATISTICAL ANALYSES.

All statistical analyses were performed using different subprograms of BIOΣTAT I and II (Pimentel & Smith, 1986; 1990) and NT-SYS PC - version 1.01 (Rohlf, 1986) on a DTK 286 microcomputer.

2.7.1. Univariate Procedures.

Univariate analyses included tests for outliers, and basic descriptive statistics which involved computation of arithmetic mean, standard deviation, and coefficient of variation. Kurtosis ($g1$), skewness ($g2$), and Kolmogorov-Smirnov D- statistic were calculated to assess normality, while the Serial Correlation C- statistic was used to test for randomness of the samples. Model I two-way analyses of variance (ANOVA) were used to simultaneously test for differences between factors, for each measurement independently. The former analyses were followed by *a posteriori* Student-Newman-Keul (SNK) multiple comparison tests to define maximally non-significant subsets ($p <$ 0,05 ; Sokal & Rohlf, 1981).

2.7.2. Multivariate Procedures.

A priori methods.

Principal components analyses (PCA):

There has been considerable debate on the merits and demerits of using single - group PCA (Airoldi & Flury, 1988; Thorpe, 1988). Thorpe (1988) recommended use of multi - group PCA (MPCA) on the grounds that within - group components and the between - group sources of variation may perturb one another in single - group analyses. Airoldi & Flury (1988), however, argued that MPCA requires stricter statistical assumptions than PCA. In their review of multivariate methods, James & McCulloch (1990) argued that there are several practical reasons why principal components analysis, whether PCA or MPCA, should never be used in a multi - sample situation. For exploratory purposes, both single - group PCA and MPCA were used in this study (Section 4.3). My results showed that the two methods were compatible in revealing patterns of geographic variation. However, single - group PCA was instrumental in separating cytogenetically known individuals according to species, whereas MPCA did not. For this reason, subsequent ordinations were based on single - group analyses rather than MPCA. An added advantage of single - group PCA is that it is a true *a priori* method which identifies phenetic groupings without predetermined assumptions of their group memberships (James & McCulloch, 1990).

Principal components analyses were based on among - character correlation matrices computed from single standardized data. Data were standardised (unit standard deviation, zero mean) by characters, as this equalizes the differences in magnitude of variables taken at different scales (James & McCulloch, 1990; Sneath & Sokal, 1973). Principal components analyses, which generates uncorrelated principal components from variables, is based on maximization of the variance of the components and variation is often summarized with only few components (James & McCulloch, 1990).

Unweighted pair - group method using averages (UPGMA) cluster analyses:

These analyses were aimed at identifying phenetically most similar individuals and to summarize *a priori* relationships between them (James & McCulloch, 1990; Sneath & Sokal, 1973). Cluster analyses (UPGMA) were based on average taxonomic distances (ATD) and product moment correlation coefficient (CORR) matrices computed from single standardized data. Cluster analyses (UPGMA) based on ATD and correlation matrices, reflect size and shape differences respectively (Sneath & Sokal, 1973).

Cluster analyses group operational taxonomic units (OTU's) according to a similarity measure and a grouping algorithm such as UPGMA (James & McCulloch, 1990, Sneath & Sokal, 1973). Distance - based morphometric studies often employ UPGMA algorithm (James & McCulloch, 1990; Sneath & Sokal, 1973), as this method computes average similarity or dissimilarity of each OTU to an existing cluster whilst weighing each OTU in that cluster equally, regardless of its structural subdivision (Sneath & Sokal, 1973).

A posteriori methods.

Multigroup discriminant functions analyses (MDA):

Multigroup discriminant analyses (MDA) were used to assess the authenticity of the *a priori* groups obtained. This technique maximizes variance among groups relative to within groups, and allows the identification of characters that are important in separating groups (Sneath & Sokal, 1973). Results of the analyses were used to classify individuals to actual groups, thus MDA were based on variance and covariance matrices (Sneath & Sokal, 1973) rather than on standardised matrices.

Multivariate analyses of variance (MANOVA):

Multivariate analyses of variance test for differences among group centroids, and were used to assess the statistical significance of differences between the groups delimited by *a priori* cluster and ordination analyses (James & McCulloch, 1990).

All these multivariate methods are discussed in detail by James & McCulloch (1990); Dippenaar & Rautenbach (1986) and Sneath & Sokal(1973).

CHAPTER 3

NONGEOGRAPHIC VARIATION

3.1. INTRODUCTION

Before examination of interspecific morphometric differences in organisms, the nature and extent of nongeographic variation within species should be assessed (Mayr & Ashlock, 1991). Failure to do so may lead to misinterpretation of results in subsequent analyses, as intrapopulation variation may be sufficiently pronounced to obscure intertaxon differences (Dippenaar & Rautenbach, 1986; Patton & Rogers, 1983).

Some authors view nongeographic variation as a composite of genetic and nongenetic compounds (Mayr & Ashlock, 1991), but others consider it in terms of age, sex, season, cohort or individual variation (Taylor & Meester, 1993; Van der Straeten & Dieterlen, 1992; Patton & Rogers, 1983, Robbins, 1973). Since sample sizes are often too small to allow examination of nongeographic variation at the levels of seasonal, cohort or individual related variation, the majority of practical applications are restricted to age and sexual dimorphism (Chimimba & Dippenaar, 1994; Taylor & Meester, 1993; Robinson & Dippenaar, 1987; Dippenaar & Rautenbach, 1986; Dippenaar, 1977). Similarly, in this study sample sizes were restrictive and consequently nongeographic variation was assessed only at the levels of age variation and sexual dimorphism.

Age variation is often an important factor, particularly in small rodents, which are known to continue growing cranially for some time after they start appearing in traps, and thus find their way into museum collections (Dippenaar & Rautenbach, 1986). Indeed, small rodents, like the Namaqua rock rat (*Aethomys namaquensis),* red veld rat (*Aethomys chrysophilus),* and the spiny rat (*Proechimys brevicauda),* show significant age variation

(Chimimba & Dippenaar, 1994, Patton & Rogers, 1983). Sexual dimorphism has also been documented as an important factor in other small mammals, such as the golden moles, slender mongoose *(Galerella sanguinea)*, and the black musk shrew (*Crocidura mariquensis)* (Bronner, 1995; Watson, 1988; Dippenaar, 1977).

Although univariate methods, such as two-way ANOVA (Robbins, 1973), and coefficients of variation (Genoways & Jones, 1972), have been used to test for nongeographic variation, these have been criticised by Leamy (1983) and Straney (1978). They suggested that since cell sizes are almost always unequal, separate factors in a multiple - way ANOVA are no longer independent. These authors instead proposed the use of percent contribution of sum of squares, and variance partitioning, respectively. Variance partitioning is computationally involved while percent sum of squares can be computed directly from a conventional two-way ANOVA table (Chimimba & Dippenaar, 1994; Taylor & Meester, 1993).

Other authors have used multivariate methods such as MANOVA (Willig, Owen & Colbert, 1986), regression analysis (Thorpe, 1976), and principal components analysis (Leamy & Thorpe, 1984), to search for patterns of nongeographic variation. Analyses in this study included both univariate and multivariate methods. These were performed on individual and pooled locality samples and were done to (i) test for age or sex related craniometric variation; and to (ii) delineate statistically similar groups (phena) for separate treatment in subsequent analyses.

3.2. MATERIALS AND METHODS

3.2.1. Material examined.

Two hundred and sixty - six cytogenetically identified specimens of *M. natalensis* and *M. coucha* were examined and aged following the criteria described in Section 2.5. Specimens examined, and a gazetteer, are listed in Appendix 1. In multivariate assessments, specimens with one or more missing measurements were excluded from analyses. Where necessary, some measurements were excluded to increase sample sizes. All multivariate analyses were based on specimens representing four populations with adequate samples sizes, namely: *M.coucha* - Groot Marico (North West Province), and Pretoria (Gauteng Province); *M.natalensis* - Barbeton (Mpumalanga Province) and Durban (KwaZulu-Natal Province) (Figure 4.1).

3.2.2. Qualitative dental character.

All cytogenetically identified specimens used in this study were also examined for the presence or absence of t3 cusp, and the variability of this character in sex and toothwear groups (Section 2.4).

3.2.3. Statistical analyses.

Data were initially screened for outliers, and individuals showing statistically significant ($p <$ 0,05) outlier values were included in the analyses after being re -measured or re - aged. All samples satisfied randomness tests, and none of the samples showed statistically - significant departures from normality when evaluated by kurtosis ($g1$), skewness ($g2$) and Kolmogorov - Smirnov D - statistics. In the two *M. coucha* samples, descriptive statistics of the 17 measurements in sex and TW groups were computed. Sexes were pooled in the *M. natalensis* samples as all toothwear groups, except TW 3, were represented by one or no specimen of each sex group.

The two *M. coucha* samples were subjected first, to two-way ANOVA to examine variation due to sex and age variation simultaneously. In *M. natalensis* OTU's, sample sizes were too small for simultaneous analysis of sexual dimorphism and age variation by two-way ANOVA. Consequently, sexual variation was assessed by one-way ANOVA of toothwear class 3 male and female specimens. The apparent lack of significant sexual differences between these samples justified pooling of sexes, thereby allowing subsequent one-way ANOVA of age groups. These analyses were followed by an *a posteriori* Student-Newman-Keul (SNK) multiple range comparison tests.

Univariate results of most samples indicated that variation due to sex was negligible (Table 3.2 - 3.5), and thus implying that the two sources of variation were not confounding each other. Samples were, therefore, subjected to single - group PCA and UPGMA cluster analyses, to identify *a priori* phenetic groups. These groups obtained were then subjected to MDA and their statistical integrity assessed by MANOVA. These multivariate methods allowed the inclusion of toothwear groups which had inadequate sample sizes (e.g. TW 1, 2 and 6 in the Pretoria sample) for analysis by two - way ANOVA.

3.3. RESULTS AND DISCUSSION.

3.3.1. Qualitative dental character.

Results showing the variability of the t3 cusp and its level of development in toothwear and sex groups of both *M. coucha* and *M. natalensis* are presented Tables 3.1.

In *M. coucha*, the t3 cusp frequency was high (> 75 % of the specimens) in TW 1 - 3 specimens but declined markedly with toothwear. In TW 4 specimens, the t3 cusp was present in only 48% of individuals, whilst in TW 5 - 6 specimens its incidence was less than 10%. The development of this cusp was about the same in TW 2 - 4, but was predominantly (57,14 % of the specimens) weakly developed in TW 1 specimens. Sample

Table 3.1 : Variability of the t3 cusp in each toothwear (TW) and sex group of the available *M. coucha and M. natalensis* specimens from southern Africa. + t3 cusp = present but poorly developed, ++ t3 cusp = present but well developed and - t3 cusp = absent. Relative percentages are given in parentheses.

TW group	- t3 cusp (%)	+ t3 cusp (%)	++ t3 cusp (%)	Sample size
M. coucha				
TW 1	2 (14,29)	8 (57,14)	4 (28,57)	14
TW 2	17 (22,97)	34 (45,95)	23 (31,08)	74
TW 3	30 (24,39)	44 (35,77)	49 (39,84)	123
TW 4	26 (52,00)	11 (22,00)	13 (26,00)	50
TW 5	17 (94,44)	1 (5,56)	0 (0,00)	18
TW 6	13 (92,86)	0 (0,00)	1 (7,14)	14
FEMALES	49 (34,03)	47 (32,64)	48 (33,33)	144
MALES	56 (36,36)	36 (23,38)	62 (40,46)	154
***M. coucha* Total**	**105 (33,55)**	**98 (31,31)**	**110 (35,14)**	**313**
M. natalensis				
TW 1	11 (91.67)	1 (8,33)	0 (0,00)	12
TW 2	21 (95,45)	1 (4,55)	0 (0,00)	22
TW 3	135 (95,74)	3 (2,13)	3 (2,13)	141
TW 4	46 (97,87)	1 (2,13)	0 (0,00)	47
TW 5	8 (88,89)	1 (11,11)	0 (0,00)	9
TW 6	8 (88,89)	1 (11,11)	0 (0,00)	9
FEMALES	128 (96,97)	3 (2,27)	1 (0,76)	132
MALES	101 (95,28)	3 (2,83)	2 (1,89)	106
***M. natalensis* Total**	**229 (95,42)**	**8 (3,33)**	**3 (1,25)**	**240**

sizes were too small in TW 5 and 6 groups to objectively assess the variability of this character. The variability of t3 cusp was generally comparable between the sex groups.

The frequency of t3 cusp in *M. natalensis* was much lower (< 5 % of the specimens) but the same pattern indicated in *M. coucha* was apparent in this species. Less than two percent of the individuals had a well developed t3 cusp, indicating that when present, t3 cusp in *M. natalensis* is poorly developed.

These results, therefore, partially support Pockock (1993), in that t3 cusp frequency is higher in *M. coucha* than in *M. natalensis.* However, even in *M. coucha,* t3 cusp tends to be poorly developed in most TW 1 - 2 individuals, but owing to quicker occlusal erosion its incidence is about the same as that of well - developed TW 3 - 4, and its frequency generally declines with increasing toothwear. Thus the variability of this character is influenced largely by the degree of occlusal attrition. This putative character, therefore, has limited diagnostic value. Furthermore the variability of this character is too high, even in toothwear groups where occlusal attrition is minimal, to warrant its use for taxonomic purposes. Pockock (1993) 's claim that this character can be used with 90% confidence must thus be rejected.

3.3.2. Statistical Analyses

Descriptive statistics

Descriptive statistics of the four samples of *M. coucha* and *M. natalensis* analysed are presented in Tables 3.2 - 3.5. In both *M. coucha* and *M. natalensis* samples mean values of most measurements generally increased with toothwear. Mean values for the two sexes were similar, except for the TW 5 specimens of the Pretoria sample where females were generally smaller than males (Table 3.3). But this may have been an artefact of small sample sizes, since only two specimens of either sex were available for the study. In toothwear or sex groups of all samples, most measurements had low standard deviations (< 1 sd). Coefficient of variation, which gives a rough measure of population variation (Sokal & Rohlf, 1981), was low in most measurements (< 10%), indicating that variation of these measurements within toothwear groups was minimal.

Mastomys coucha

Univariate patterns of variation.

Model 1 two - way ANOVA indicated highly significant ($p < 0{,}01$) age variation in 13 measurements of the Groot Marico sample (Table 3.2), and 11 measurements of the Pretoria sample (Table 3.3). Four measurements also differed significantly at the 0,05% probability level in the latter. In both populations, 13 measurements (excluding IOB PPW, MTR, and MDT in the Groot Marico sample, and IOB, PPW, and MDL in the Pretoria sample) differed significantly between age groups. There was no significant sexual size dimorphism in the Pretoria sample, and only two measurements (DOB, MAST) in the Groot Marico sample differed significantly ($p < 0{,}05$) between the sexes. In the Pretoria sample, however, significant sex - age interaction was indicated in nine measurements ($p <0{,}05$). Since ANOVA results indicated negligible sex differences in this sample, the sex - age interaction results may have reflected the smaller size of TW 5 females, which when considered independently were smaller than other TW groups (mean values; Table 3.3).

The SNK multiple range comparison tests for toothwear variation in the Groot Marico sample showed a consistent trend, whereby TW 4 specimens were significantly larger than TW 2 - 3 for most measurements, except for MDL which was significantly

Table 3. 2: Descriptive statistics and results of two-way ANOVA of 17 measurements of *M.coucha* males and females belonging to 3 toothwear classes from Groot Marico, Northwest Province, South Africa. X = arithmetic mean, SD = standard deviation, CV = coefficient of variation and n = sample size. ^{a} and ^{b} denote significance of F - values at $p < 0,05$ and $p < 0,01$ respectively.

						MEASUREMENTS												
OTU	STATS	GLS	CBL	DOB	BRBR	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
Toothwear Class 2 ♀	*X*	24,62	23,50	9,96	11,07	10,34	3,92	3,89	5,81	6,63	13,63	5,68	4,33	4,86	4,17	7,17	16,39	8,72.
	SD	0,67	0,81	0,16	0,32	0,28	0,12	0,13	0,35	0,23	0,49	0,37	0,21	0,21	0,11	0,26	0,13	0,47
	CV	2,73	3,43	1,61	2,88	2,72	3,17	3,30	6,05	3,50	3,60	6,54	4,76	4,26	2,53	3,64	2,16	5,36
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Toothwear Class 3 ♀	*X*	25,44	24,29	9,87	10,91	10,46	3,87	4,04	5,88	6,89	14,21	5,94	4,32	4,94	4,21	7,65	16,86	8,89
	SD	0,84	0,90	0,28	0,28	0,34	0,33	0,20	0,29	0,41	0,51	0,41	0,19	0,21	0,11	0,43	0,56	0,52
	CV	3,29	3,69	2,82	3,08	3,19	5,06	5,04	5,01	5,89	3,56	6,86	4,46	4,23	2,59	5,68	3,35	5,85
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Toothwear Class 4 ♀	*X*	28,24	27,06	10,37	11,85	11,27	3,92	4,59	6,60	8,06	16,08	6,19	4,47	5,01	4,22	8,74	18,83	10,48
	SD	0,91	1,17	0,26	0,37	0,37	0,21	0,25	0,14	0,32	0,54	0,05	0,31	0,12	0,06	0,46	0,72	0,47
	CV	3,23	4,32	2,49	3,16	2,18	5,22	5,45	2,07	3,90	3,35	0,83	6,88	2,30	1,36	5,27	3,80	4,52
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Toothwear Class 2 ♂	*X*	25,45	24,31	10,12	11,15	10,79	3,90	4,03	6,11	6,71	4,33	5,76	4,33	4,99	4,21	7,66	16,76	8,89
	SD	1,11	1,15	0,23	0,48	0,29	0,19	0,25	0,39	0,30	0,61	0,24	0,11	0,14	0,10	0,60	0,59	0,88
	CV	4,37	4,71	2,27	4,29	2,65	4,75	6,27	6,37	4,32	4,23	4,07	2,53	2,73	2,40	7,85	3,54	9,90
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Toothwear Class 3 ♂	*X*	25,67	24,54	10,06	11,40	10,76	3,89	4,01	6,07	6,98	14,22	5,88	4,32	4,94	4,18	7,67	17,06	9,25
	SD	1,02	1,01	0,25	0,37	0,38	0,11	0,14	0,39	0,43	0,63	0,23	0,13	0,16	0,09	0,49	0,76	0,31
	CV	3,97	4,10	2,52	3,28	3,56	2,84	3,52	6,45	6,10	4,40	3,95	2,98	3,27	2,24	6,45	4,47	3,35
	n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Toothwear Class 4 ♂	*X*	28,84	28,19	10,92	11,97	11,31	3,98	4,35	7,04	8,29	16,62	6,11	4,25	5,15	4,22	8,35	18,89	10,99
	SD	0,95	0,93	0,06	0,01	0,05	0,01	0,51	0,36	0,30	0,60	0,12	0,09	0,13	0,05	0,07	0,08	0,61
	CV	3,38	3,31	0,52	0,06	0,44	0,36	11,70	5,13	3,58	3,58	1,97	2,00	2,47	1,17	0,85	0,41	5,53
	n	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SEX	*F*	0,62	0,87	**4,53**^{a}	2,55	**5,16**^{a}	0,01	0,43	3,08	0,15	1,81	0,05	0,52	1,16	0,004	0,84	0,71	1,30
AGE	*F*	**36,24**^{b}	**33,56**^{b}	**14,54**^{b}	**11,59**^{b}	**12,26**^{b}	0,40	**21,10**^{b}	**14,86**^{b}	**41,06**^{b}	**27,65**^{b}	**5,86**^{b}	0,67	1,64	0,22	**14,68**^{b}	**32,92**^{b}	26, 86^{b}
SEX-AGE	*F*	1,08	1,18	2,25	1,92	2,56	0,21	2,37	0,71	0,88	2,06	0,35	0,96-	0,86	0,62	1,97	0,29	0,52

Table 3.3: Descriptive statistics and results of two-way ANOVA of 17 measurements of *M. coucha* males and females belonging to 3 toothwear classes from Pretoria, Gauteng Province, South Africa. *X* = arithmetic mean, *SD* = standard deviation, *CV* = coefficient of variation, *n* = sample size. ^{a} and ^{b} denote significance of *F* - values at $p < 0,05$ and $p < 0,01$ respectively.

OTU	STATS	MEASUREMENTS																
OTU	STATS	GLS	CBL	DOB	BRBR	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
Toothwear Class 3 ♀	*X*	26,64	25,34	10,32	10,98	10,78	3,97	4,43	6,30	7,11	14,72	5,92	4,43	5,07	4,30	7,83	17,49	9,41
	SD	1,70	1,80	0,37	0,43	0,42	0,25	0,32	0,34	0,60	1,07	0,50	0,12	0,18	0,05	0,59	1,08	1,04
	CV	6,38	7,11	3,56	3,93	3,86	6,21	7,26	5,41	8,50	7,24	8,48	2,67	3,60	1,15	7,56	19,1	1,09
	n	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Toothwear Class 4 ♀	*X*	29,01	27,64	10,76	11,26	11,20	4,06	4,69	6,75	8,16	16,38	6,73	4,38	5,55	4,29	8,21	19,14	10,07
	SD	0,71	0,90	0,19	0,06	0,10	0,09	0,09	0,22	0,47	0,67	0,33	0,18	0,21	0,11	0,.14	0,68	0,36
	CV	2,44	3,25	1,77	0,53	0,89	2,37	1,93	3,21	5,80	4,06	4,87	4,03	3,77	2,67	1,65	3,53	3,57
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Toothwear Class 5 ♀	*X*	26,99	25,51	10,61	10,75	10,66	3,99	4,38	6,58	7,14	14,95	6,56	4,11	5,38	4,39	7,31	17,34	9,45
	SD	1,17	0,73	0,13	0,11	0,10	0,07	0,13	0,19	0,06	0,26	0,15	0,09	0,05	0,09	0,51	0,67	0,42
	CV	2,44	3,25	1,77	0,53	0,89	2,37	1,93	3,21	5,80	4,06	4,87	4,03	3,77	2,67	1,65	3,53	4,49
	n	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Toothwear Class 3♂	*X*	26,53	25,33	10,17	10,97	10,63	4,00	4,33	6,14	7,12	14,70	5,98	4,37	5,06	4,24	7,81	17,40	9,36
	SD	1,39	1,66	0,56	0,49	0,39	0,15	0,30	0,45	0,63	1,15	0,41	0,11	0,21	0,11	0,38	1,16	0,69
	CV	5,24	0,55	5,46	4,47	3,64	3,81	6,82	7,34	8,89	7,83	6,78	2,56	4,17	2,60	4,86	6,67	7,39
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Toothwear Class 4 ♂	*X*	29,60	28,39	10,86	11,60	11,54	4,22	4,77	6,73	8,28	16,59	6,74	4,47	5,55	4,41	8,56	19,40	10,62
	SD	1,34	1,46	0,19	0,37	0,36	0,09	0,37	0,51	0,.53	0,85	0,47	0,10	0,22	0,08	0,57	0,66	1,04
	CV	4,52	5,14	1,72	3,14	3,15	2,12	7,73	7,50	6,41	5,10	6,99	2,12	3,87	1,80	6,61	3,38	9.76
	n	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Toothwear Class 5 ♂	*X*	31,17	30,07	10,94	11,79	11,68	4,09	5,10	7,29	8,85	17,72	6,87	4,50	5,66	4,58	9,02	20,34	11,35
	SD	0,21	0,32	0,05	0,26	0,26	0,10	0,48	0,29	0,06	0,11	0,26	0,09	0,27	0,16	0,42	0,36	0,16
	CV	0,66	1,06	0,45	2,22	2,24	2,42	9,43	3,98	0,72	0,60	3,71	1,89	4,75	3,40	4,63	1,77	1,43
	n	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
SEX	*F*	2,61	3,06	0,02	3,46	2,34	1,60	0,72	0,10	2,47	2,10	0,40	1,25	0,40	1,36	4,16	2,18	2,27
AGE	*F*	**12,59**^{b}	**10,12**^{b}	**7,35**^{b}	**3,87**^{a}	**9,86**^{b}	1,98	**4,46**^{a}	**8,20**^{b}	**12,17**^{b}	**11,35**^{b}	**11,38**^{b}	1,29	**18,89**^{b}	**7,93**^{b}	**3,74**^{a}	**10,92**^{b}	**4,58**^{a}
SEX -AGE	*F*	**3,91**^{a}	**3,67**^{a}	0,81	3,09	**4,76**^{a}	0,43	2,99	2,12	**3,88**^{a}	**3,56**^{a}	0,21	**5,16**^{a}	0,94	**4,12**^{a}	**5,28**^{a}	**4,27**^{a}	2,24

Table 3. 4: Descriptive statistics and results of one-way ANOVA of 17 measurements of *M. natalensis* belonging to six toothwear classes (sex groups pooled) from Barbeton, Mpumalanga Province, South Africa. *X*= arithmetic mean, *SD*= standard deviation, *CV*= coefficient of variation and n = sample size. ^{a}and ^{b} denote significance of *F* - values at $p <$ 0,05 and $p <$ 0,01 respectively.

		MEASUREMENTS																
OTU	STATISTICS	GLS	CBL	DOB	BRBR	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
Toothwear Class 2	*X*	25,32	23,58	10,45	11,24	10,78	4,04	3,90	5,93	6,62	13,52	5,54	4,54	5,02	4,17	7,11	15,94	8,70
	SD	0,70	0,85	0,18	0,14	0,17	0,07	0,14	0,22	0,40	0,63	0,22	0,13	0,39	0,09	0,07	0,60	0,28
	CV	2,80	3,61	1,67	1,20	1,57	1,62	3,47	3,64	6,06	4,67	3,92	2,88	7,84	2,10	1,01	3,79	3,16
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Toothwear Class 3	*X*	25,92	24,48	10,18	11,43	10,79	4,04	4,24	6,05	6,85	14,31	5,75	4,64	4,99	4,13	7,27	17,00	9,09
	SD	0,80	0,90	0,29	0,30	0,31	0,02	0,22	0,30	0,34	0,58	0,26	0,18	0,17	0,12	0,41	0,52	0,51
	CV	3,10	3,67	2,88	2,58	2,87	3,68	5,26	4,89	4,89	4,04	4,51	3,82	3,34	3,87	5,58	3,07	5,57
	n	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
Toothwear Class 4	*X*	29,52	28,10	10,89	11,54	11,28	4,20	4,86	6,95	8,25	16,21	6,24	4,76	5,32	4,29	8,63	19,46	10,74
	SD	0,88	1,12	0,39	0,28	0,33	0,31	0,47	0,47	0,57	0,86	0,25	0,16	0,17	0,24	0,45	0,81	0,69
	CV	2,98	4,00	3,59	2,40	2,96	7,43	9,73	6,77	6,85	5,31	3,93	3,35	3,10	5,65	5,17	4,15	6,47
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Toothwear Class 5	*X*	27,84	26,45	10,47	11,74	10,97	4,02	4,43	6,62	7,56	15,26	6,22	4,40	5,32	4,26	7,97	18,12	10,00
	SD	0,89	1,16	0,47	0,42	0,38	0,11	0,23	0,25	0,27	0,40	0,03	0,25	0,21	0,04	0,59	0,70	0,68
	CV	3,21	4,40	4,44	3,59	3,48	2,74	5,20	3,76	3,57	2,61	0,40	5,57	3,94	0,85	7,42	3,85	6,83
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Toothwear Class 6	*X*	28,79	27,27	10,49	11,64	11,22	4,01	4,69	6,91	7,92	16,03	6,35	4,44	5,08	4,32	8,06	18,70	10,47
	SD	1,87	2,07	0,27	0,36	0,51	0,06	0,42	0,45	0,80	1,21	0,52	0,23	0,20	0,12	0,71	1,36	1,15
	CV	6,51	7,59	2,56	3,09	4,54	1,41	9,03	6,55	10,07	7,55	8,23	5,09	4,01	2,76	8,83	7,28	10, 97
	n	4	5	3	4	3	5	5	5	5	5	5	5	5	5	5	5	5
SEX (TW 3)	*F*	0,13	0,03	0,27	4,81	1,49	0,29	0,27	0,61	0,11	1,00	1,50	0,19	0,004	0,004	0,03	0,002	0,51
AGE	*F*	**25,16**^{b}	**19,26**^{b}	**6,55**^{b}	1,51	**3,72**^{a}	1,35	**8,96**^{b}	**15,00**^{b}	**17,64**^{b}	**15,63**^{b}	**8,84**^{b}	**3,44**^{a}	**4,95**^{b}	0,07	**13,79**^{b}	**22,30**^{b}	**13, 08**^{b}

Table 3.5. Descriptive statistics and one-way ANOVA results of 17 measurements of *M. natalensis* belonging to four toothwear groups (sex groups pooled) from Durban, KwaZulu-Natal Province, South Africa. *X* = arithmetic mean, *SD* = standard deviation, *CV* = coefficient of variation and n = sample size. ^{a} and ^{b} denote significance of F - values at $p < 0{,}05$ and $p < 0{,}01$ respectively.

		MEASUREMENTS																
OTU	STATISTICS	GLS	CBL	DOB	BRBR	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
Toothwear Class 2	*X*	27,03	25,80	10,33	11,13	10,75	4,04	4,15	6,65	7,31	14,91	5,86	4,51	5,02	4,17	7,71	16,98	9,93
	SD	1,40	1,36	0,29	0,60	0,46	0,16	0,08	0,70	0,49	0,80	0,32	0,17	0,12	0,06	0,86	0,95	0,59
	CV	5,18	5,27	2,81	5,43	4,24	4,01	1,81	0,46	6,67	5,35	5,40	3,69	2,30	1,46	11,21	5,60	5,98
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Toothwear Class 3	X	27,54	26,08	10,54	11,45	10,97	4,12	4,47	6,63	7,59	15,25	5,94	4,69	5,17	4,22	7,80	17,83	10,03
	SD	0,93	0,99	0,23	0,26	0,26	0,16	0,22	0,32	0,45	0,71	0,36	0,29	0,16	0,11	0,48	1,20	0,58
	CV	3,36	3,79	2,21	2,24	2,41	3,91	5,00	4,80	5,90	4,67	6,05	6,20	3,13	2,53	6,17	6,71	5,76
	n	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Toothwear Class 4	*X*	30,01	28,84	10,86	11,71	11,49	4,31	5,10	7,24	8,74	17,05	6,32	4,92	5,42	4,54	8,62	19,93	9,13
	SD	1,53	1,77	0,21	0,22	0,27	0,08	0,34	0,47	0,59	0,96	0,21	0,18	0,17	0,15	0,48	1,14	4,57
	CV	5,09	6,13	1,97	1,91	2,38	1,88	6,57	6,43	6,73	5,65	3,39	3,67	3,19	3,34	5,52	5,70	--
	n	6	5	5	6	5	6	6	6	6	6	6	6	6	4	6	6	6
Toothwear Class 5	*X*	30,14	29,51	10,92	11,83	11,46	4,34	4,92	7,52	8,70	17,31	6,47	4,65	5,46	4,36	8,89	19,95	11,57
	SD	0,70	0,63	0,15	0,28	0,07	0,21	0,30	0,15	0,05	0,47	0,45	0,02	0,29	0,05	0,53	0,40	0,53
	CV	2,31	2,13	1,41	2,33	0,64	4,74	6,13	2,00	0,61	2,69	6,99	0,37	5,23	1,18	6,01	2,01	4,54
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SEX (TW 3)	*F*	1,01	1,86	0,02	0,34	0,59	1,81	0,76	0,17	1,83	0,60	0,22	1,32	1,29	0,55	1,99	0,88	1,08
AGE	*F*	**10,36**^{b}	**11,81**^{b}	**5,67**^{b}	**3,82**^{a}	**7,42**^{b}	**4,20**^{a}	**13,55**^{b}	**6,27**^{b}	**12,67**^{b}	**12,50**^{b}	**3,43**^{a}	2,27	**5,94**^{b}	**12,98**^{b}	**4,94**^{a}	**8,53**^{b}	0,70

Table 3.6: Results of the multiple range Student- Newman-Keul's comparison test for toothwear classes (TW 2 - 4) in *M. coucha* from Groot Marico, Northwest Province. Vertical lines denote significant subsets ($p < 0,05$), ASD = all means are significantly different, TW = toothwear class, n = sample sizes.

Measurement	TW (n)	Mean (sorted)
GLS	2 (17)	24.96│
	3 (21)	25.57│
	4 (7)	28.41
CBL	2 (17)	23.83│
	3 (21)	24.43│
	4 (7)	27.38
DOB	3 (21)	9.98│
	2 (16)	10.03│
	4 (7)	10.53
BRBR	2 (16)	11.10│
	3 (20)	11.20│
	4 (7)	11.88
MAST	2 (15)	10.52│
	3 (21)	10.63│
	4 (8)	11.20
ROST	2 (17)	3.95│
	3 (21)	4.02│
	4 (7)	4.52
APF	2 (16)	5.92│
	3 (21)	5.99│
	4 (7)	6.73
PAL	2 (17)	13.92│
	3 (20)	14.25│
	4 (7)	15.96
DIAS	2 (17)	6.73│
	3 (21)	6.94│
	4 (7)	8.13
GBP	2 (17)	5.71│
	3 (21)	5.90│ │
	4 (7)	6.17 │
MDH	2 (12)	7.33│
	3 (18)	7.66│
	4 (5)	8.59
MDL	2 (16)	16.53
	3 (21)	16.98 ASD
	4 (6)	18.85
PPL	2 (14)	8.80│
	3 (20)	9.08│
	4 (6)	10.65

Table 3.7 : Results of the multiple range Student - Newman - Keuls's comparison test for toothwear classes (TW 3 - 5) in *M. coucha* from Pretoria, Gauteng Province. Vertical line denotes non - significant subsets ($p < 0,05$), between toothwear class means and ASD = all means are significantly different, TW = toothwear class, n = sample size.

Measurement	TW (n)	Mean (ranked)		
GLS	3 (14)	26.59		
	5 (4)	29.08	│	
	4 (9)	29.27	│	
CBL	3 (14)	25.33		
	5 (4)	27.79	│	
	4 (9)	27.97	│	
DOB	3 (14)	10.26	│	
	5 (4)	10.77	│	│
	4 (9)	10.80		│
BRBR	3 (14)	10.97		
	5 (4)	11.27	│	
	4 (9)	11.41	│	
MAST	3 (14)	10.72		
	5 (4)	11.17	│	
	4 (9)	11.35	│	
APF	3 (14)	6.23		
	4 (9)	6.74	│	
	5 (4)	6.93	│	
DIAS	3 (14)	7.11		
	5 (4)	7.99	│	
	4 (9)	8.21	│	
PAL	3 (14)	14.71		
	5 (4)	16.33	│	
	4 (9)	16.48	│	
GBP	3 (14)	5.95		
	5 (4)	6.71	│	
	4 (9)	6.73	│	
MTR	3 (14)	5.06		
	5 (4)	5.52	│	
	4 (9)	5.55	│	
MDT	3 (14)	4.28		
	4 (9)	4.34 ASD		
	5 (4)	4.48		
MDH	3 (14)	7.82	│	
	5 (4)	8.16	│	│
	4 (9)	8.36		│
MDL	3 (14)	17.45		
	5 (4)	18.83	│	
	4 (9)	19.26	│	

Table 3.8 : Results of the SNK multiple range comparison test for sex - age interaction in nine measurements of *M. coucha* from Pretoria, Gauteng Province. Non - significant subsets ($p < 0,05$) are indicated in vertical lines, TW = toothwear, n = sample sizes.

Measurement	Age (TW) - sex group	Means (ranked)
GLS	3 ♂	26.53
	3 ♀	26.64
	5 ♀	26.99
	4 ♀	29.01
	4 ♂	29.90
	5 ♂	31.17
CBL	3 ♂	25.33
	3 ♀	25.34
	5 ♀	25.51
	4 ♀	27.64
	4 ♂	28.39
	5 ♂	30.07
MAST	3 ♂	10.63
	5 ♀	10.66
	3 ♀	10.78
	4 ♀	11.20
	4 ♂	11.53
	5 ♂	11.67
DIAS	3 ♀	7.11
	3 ♂	7.12
	5 ♀	7.14
	4 ♀	8.16
	4 ♂	8.28
	5 ♂	8.85
PAL	3 ♂	14.70
	3 ♀	14.72
	5 ♀	14.94
	4 ♂	16.38
	4 ♀	16.59
	5 ♂	17.72
PPW	5 ♀	4.11
	3 ♂	4.37
	4 ♀	4.38
	3 ♀	4.43
	4 ♂	4.47
	5 ♂	4.50
MDT	3 ♂	4.24
	4 ♀	4.29
	3 ♀	4.30
	5 ♀	4.39
	4 ♂	4.41
	5 ♂	4.58
MDH	5 ♀	7.31
	3 ♂	7.81
	3 ♀	7.83
	4 ♀	8.21
	4 ♂	8.55
	5 ♂	9.02
MDL	5 ♀	17.33
	3 ♀	17.40
	3 ♂	17.49
	4 ♂	19.14
	4 ♀	19.40
	5 ♂	20.33

significantly different in all toothwear groups (Table 3. 6). Similarly, in the Pretoria sample TW 4 - 5 individuals were significantly larger than TW 3 individuals for most measurements, except for MDT which was different in all age groups (Table 3.7). The SNK multiple range comparison tests for the sex - age interaction in nine measurements of the Pretoria sample indicated no single pattern of grouping, except in five measurements (GLS, CBL, DIAS, PAL, MDH, MDL) where TW 5 females consistently grouped with smaller TW 3 individuals and or with TW 4 specimens (Table 3.8). The disparity in size between males and females of TW 5, may have reflected small sample sizes used (n = 4; ♂ = 2, ♀ = 2) or an artefact of chance since statistical differences were probable at only 0,05%.

Multivariate patterns of variation.

Principal components analyses (PCA) based on standardized data resulted in some separation of the toothwear groups along PC axis I in both samples of *M. coucha* (Figure 3.1). In the Groot Marico sample, TW 4 specimens plotted well to the left of TW 1 - 3 specimens . In the Pretoria sample toothwear separation along PC I was characterised by broader overlap of TW groups, indicating that size differences were intergraded. There was, however, a tendency of specimens of smaller TW 1- 3 to group apart from those of older TW 4 - 6. Comparison of subsequent axes of both samples revealed no apparent separation of specimens according to sex groupings. Principal component axis I was a general size vector, as indicated by high and negative values (Table 3.9) for most variables. Separation of specimens and toothwear groups along this axis thus reflected mainly differences in overall size, which decreased from left to right. Few variables (IOB, PPW and MTR in the Groot Marico sample and IOB, GBP, PPW, and MDT in the Pretoria sample) loaded relatively low on this axis and thus participated only weakly in toothwear group

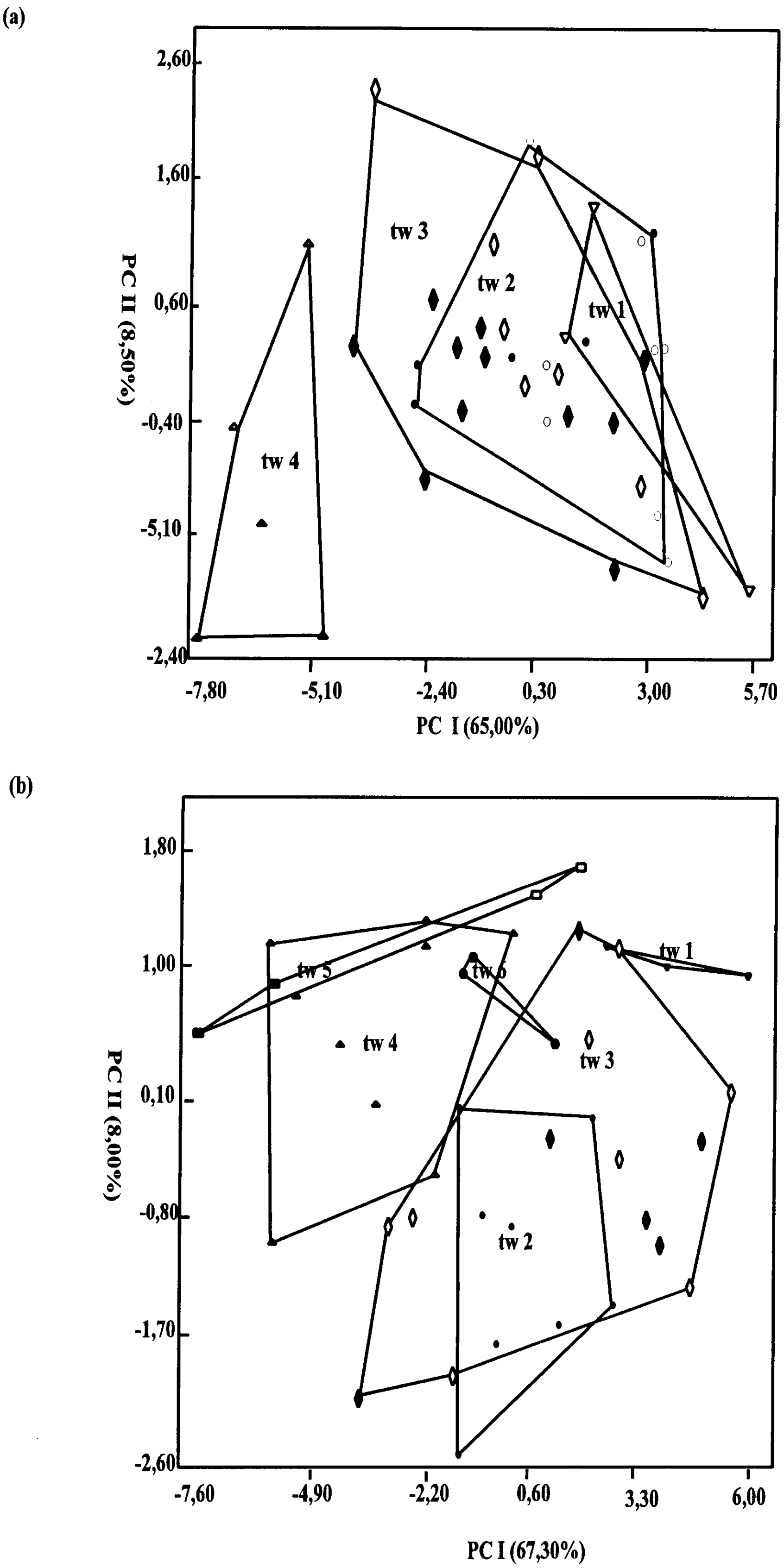


Figure 3.1: The first two axes from principal components analyses of *M. coucha* from (a) Groot Marico, Northwest Province and (b) Pretoria, Gauteng Province, South Africa. Minimum convex polygons enclose individuals of each toothwear group. Closed and open symbols represent females and males respectively. TW denotes toothwear class.

Table 3.9: Loadings for the first two axes from the principal components analyses of *M. coucha* from (a) Groot Marico, Northwest Province and (b) Pretoria, Gauteng Province. The percent variance of each variable contribution is given in parentheses. Variables that loaded relatively high on either axis are indicated in bold.

Measurements	Axis I	Axis II
(a) Groot Marico		
GLS	**-0,30 (92,32)**	-0.12 (1,88)
CBL	**-0,30 (94,03)**	-0,13 (2,34)
DOB	**-0,27 (76,06)**	-0,07 (0,61)
BRBR	**-0,26 (70,41)**	-0,09 (0,61)
MAST	**-0,27 (73,78)**	0,05 (0,33)
IOB	-0,16 (26,07)	**0,24 (7,72)**
ROST	**-0,28 (81,91)**	-0,12 (1,88
APF	**-0,26 (70,01)**	-0,07 (0,70)
DIAS	**-0,29 (88,96)**	-0,13 (2,43)
PAL	**-0,29 (89,83)**	-0,09 (1,10)
GBP	**-0,22 (49,11)**	**0,25 (8,67)**
PPW	-0,10 (10,67)	**0,58 (45,31)**
MTR	-0,12 (14,74)	**0,51 (35,65)**
MDT	**-0,29 (86,61)**	-0,07 (0,69)
PPL	**-0,26 (68,63)**	**-0,20 (5,30)**
% trace	64,95 %	8,46%
(b) Pretoria		
GLS	**-0,29 (92,71)**	-0,04 (0,17)
CBL	**-0,29 (96,36)**	-0,06 (0,56)
DOB	**-0,25 (69,88)**	0,03 (0,14)
BRBR	**-0,24 (67,35)**	0,03 (0,10)
MAST	**-0,28 (86,43)**	0,06 (0,42)
IOB	-0,19 (40,85)	0,05 (0,30)
ROST	**-0,27 (82,46)**	0,03 (0,16)
APF	**-0,25 (70,95)**	-0,16 (3,62)
DIAS	**-0,28 (92,57)**	-0,08 (0,60)
PAL	**-0,29 (92,98)**	-0,07 (0,58)
GBP	-0,17 (32,30)	**0,55 (41,15)**
PPW	-0,13 (18,89)	**0,22 (6,79)**
MTR	**-0,21 (48,35)**	**0,44 (26,72)**
MDT	-0,08 (6,92)	**0,52 (36,36)**
MDH	**-0,24 (67,87)**	**-0,33 (14,99)**
MDL	**-0,29 (92,70)**	-0,06 (0,45)
PPL	**-0,27 (85,02)**	-0,14 (2,71)
% trace	67,33%	7,99%

separation. Thus toothwear variation was significant and involved mainly changes in overall size. Like univariate analysis, sexual dimorphism or shape differences between toothwear or sex groups were negligible.

A phenogram based on ATD matrix showed a similar pattern of separation as univariate and PCA results and divided specimens into two major clusters. In the Groot Marico sample (Figure 3.2), cluster A contained the majority of the smaller TW 1 - 3 specimens (100% TW 1 - 3), and cluster B comprised of all specimens of toothwear 4. A similar pattern was reflected in the Pretoria sample (Figure 3.4), with cluster A containing the majority of specimens smaller TW 1 - 3 (85 % TW 1 - 3), while the majority of older TW 4 - 6 (62 % TW 4 - 6) grouped in cluster B. The overall pattern that emerged from these results again suggested that TW 1 - 3 specimens are significantly smaller individuals than TW 4 - 6 individuals. Shape differences in both samples were negligible, as evidenced by the lack of structure on the phenograms derived from the correlation matrix (Figure 3.3; Figure 3.5). Both the distance and correlation based phenograms showed no clear evidence of pronounced sexual dimorphism in either shape or size in either of the two populations analysed.

Multigroup discriminant function analyses (MDA) of the Groot Marico and Pretoria samples resulted in 80% and 85% overall *a posteriori* classifications of toothwear groups, respectively. Toothwear groups were thus craniometrically well - differentiated. MDA results in both samples (Figure 3.6), agreed with PCA and cluster analysis results in indicating significant differences among toothwear subgroups. Most mis -identified specimens of TW 1 - 3 or TW 4 - 6 were within each of these major phena. In the Groot Marico sample TW 1 - 3 individuals separated from TW 4 along canonical variate I (CV I). Similarly, in

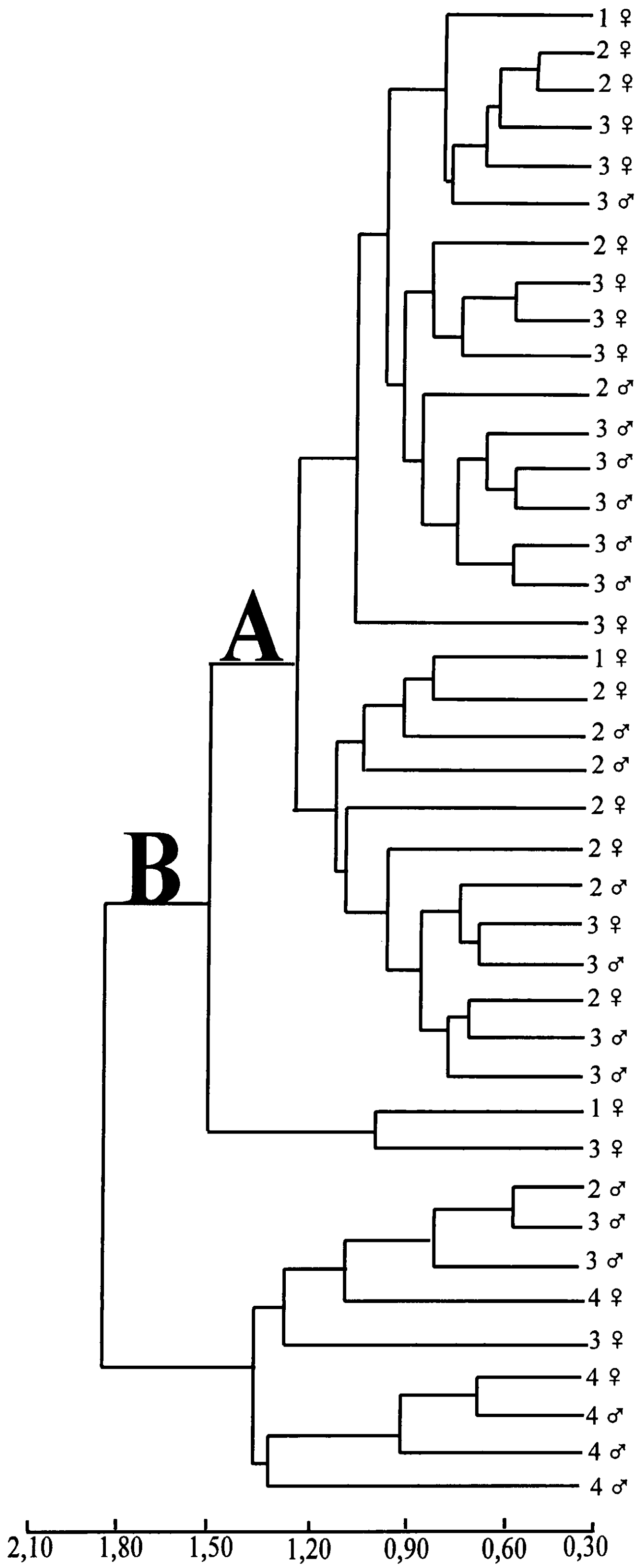


Figure 3.2 : Average taxonomic distance phenogram from cluster analysis of sex and toothwear groups of *M. coucha* from Groot Marico, Northwest Province, South Africa. Toothwear groups are denoted in numbers, and females and males are denoted as ♀ and ♂ respectively. Cophenetic correlation coefficient = 0,72.

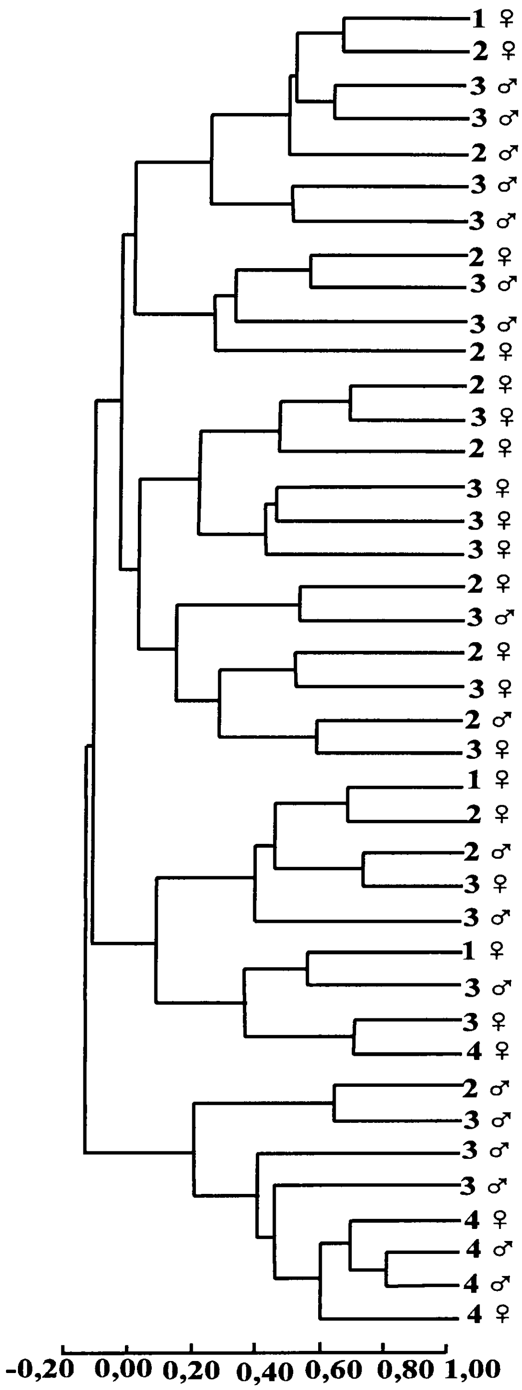


Figure 3.3 : Correlation phenogram from cluster analysis of sex and toothwear groups of *M. coucha* from Groot Marico, Northwest Province, South Africa. Toothwear groups are denoted with numbers and ♂ and ♀ denote males and females respectively. Cophenetic correlation coefficient = 0,57.

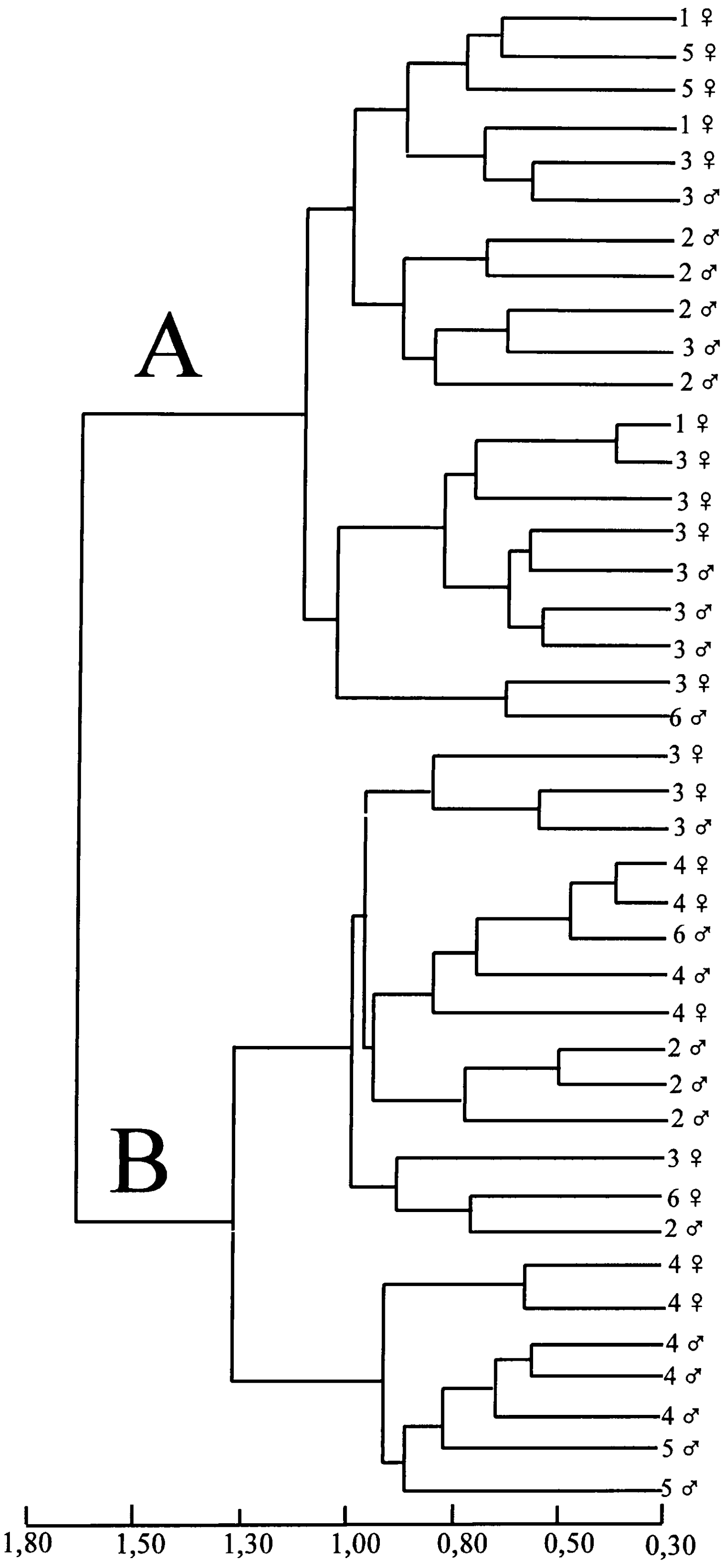


Figure 3.4 : Average taxonomic distance phenogram from cluster analysis of sex and toothwear groups of *M. coucha* from Pretoria, Gauteng Province, South Africa. Toothwear groups are indicated with numbers. Males and females are denoted as ♂ and ♀ respectively. Cophenetic correlation coefficient = 0,63.

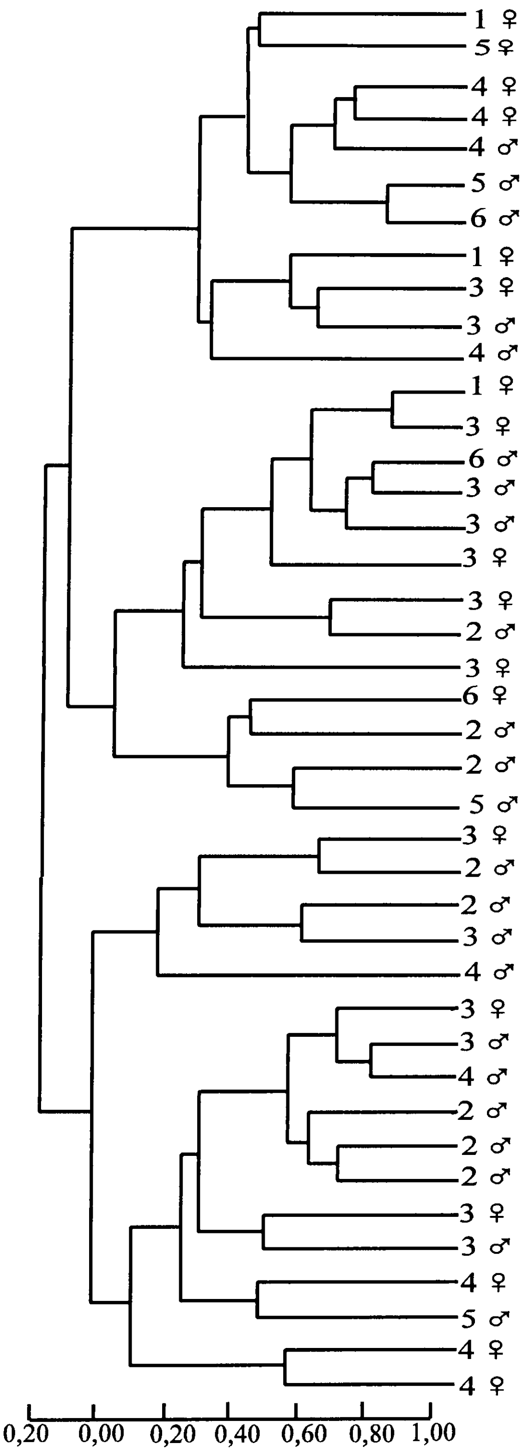


Figure 3.5: Correlation phenogram from cluster analysis of toothwear and sex groups of *M. coucha* from Pretoria, Gauteng Province. Toothwear groups are indicated numbers and ♂ and ♀ denote males and females respectively. Cophenetic correlation coefficient = 0,66.

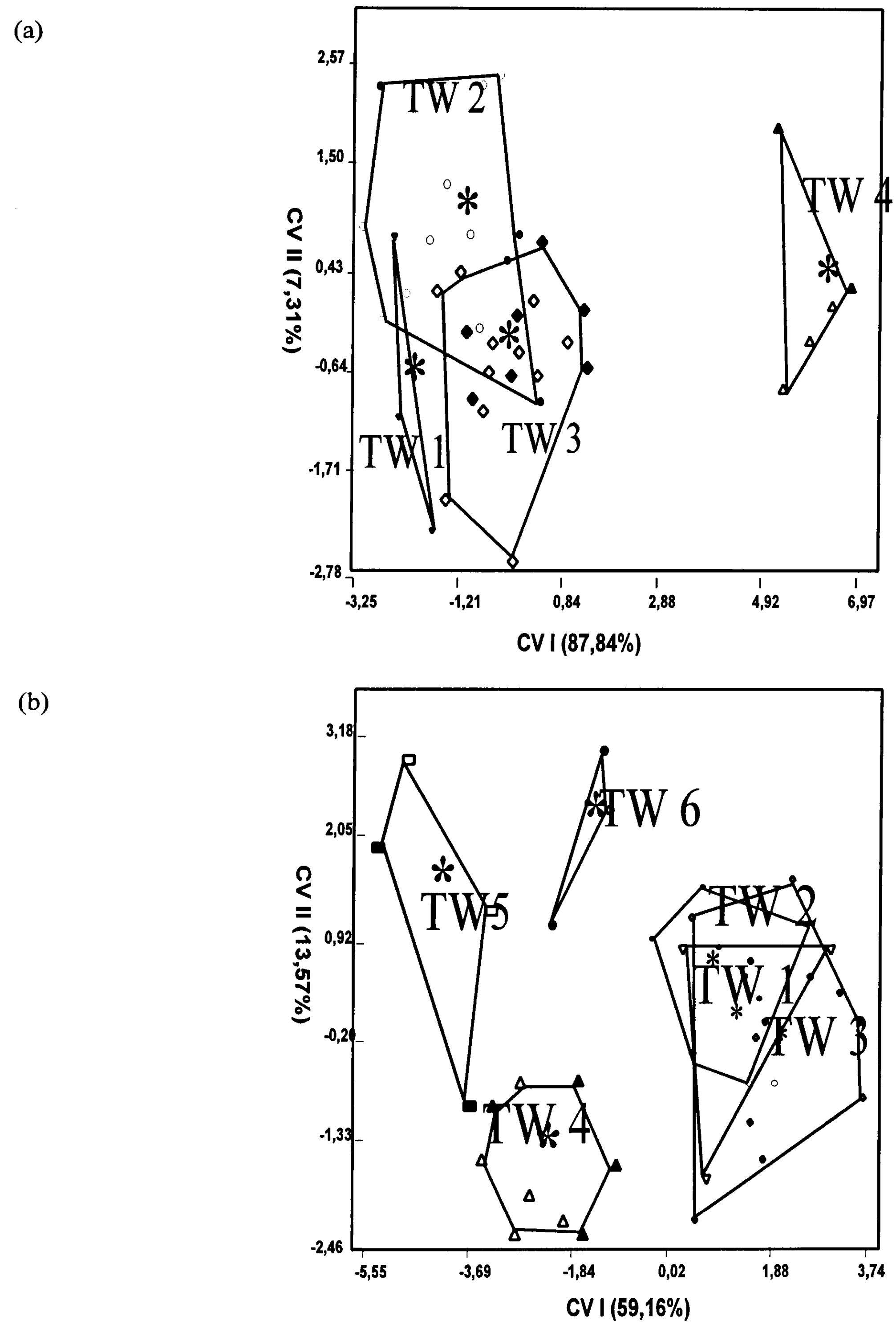


Figure 3.6 : Pairwise comparison of the first two canonical variates axes from multi - group discriminant function analyses of toothwear and sex groups from (a) Groot Marico, Northwest Province and (b) Pretoria, Gauteng Province, South Africa. Closed and open symbols denote males and females respectively. Group centroids are indicated by asterisks.

the Pretoria sample TW 1 - 3 clearly separated from TW 4 - 6, but there was also separation between TW 4, 5, and 6 individuals along CV II.

Multivariate analysis of variance (MANOVA) indicated that centroids of toothwear groups differed significantly in both samples (Groot Marico: $F = 1{,}95_{(48,63)}$ $p < 0{,}01$; Pretoria : $F = 1{,}77_{(85,96)}$, $p < 0{,}01$). Owing to small sample sizes, only the Groot Marico sample (8 ♀ and 10 ♂ of TW 3) was subjected to MANOVA test for sexual dimorphism. This indicated that sex groups did not differ significantly from each other ($F = 2{,}31_{(16,1)}$, $p > 0{,}05$)

Mastomys natalensis

Univariate patterns of variation

Model 1 one - way ANOVA indicated highly significant differences among toothwear groups ($p < 0{,}01$) in 11 measurements for the Durban sample (Table 3.5) and 12 measurements for the Barbeton sample (Table 3.4). Four measurements differed significantly at the 0,05 % probability level in the Durban sample, while only two measurements were significant at this level in the Barbeton sample. Measurements that showed significant age variation were generally comparable in both samples, and there were only a few measurements (PPW, PPL in the Durban sample and BRBR, IOB, MDT in the Barbeton sample) that did not differ significantly between age groups. None of the measurements in either sample differed significantly between sexes. The SNK multiple range comparison tests (Table 3.10) of the Durban sample indicated a trend congruous with the ANOVA results. In 10 measurements the smaller TW 2 - 3 individuals consistently grouped apart from older TW 4 - 5 in different subsets. In the Barbeton sample, however, separation between TW 2 - 3 and TW 4 - 6 on the basis of size was less pronounced, and only six measurements differed

Table 3.10 :Results of the multiple range Student- Newman-Keul's comparison tests for toothwear classes (TW 2 - 5) in *M. natalensis* from Durban, KwaZulu- Natal Province. Non significant subsets ($p < 0,05$) are indicated in vertical lines, NSD = means not significantly different, TW = toothwear class, n = sample size.

Measurement	TW (n)	Means (ranked)
GLS	2 (3)	27.03
	3 (13)	27.54
	4 (6)	30.00
	5 (3)	30.14
CBL	2 (3)	25.80
	3 (13)	26.08
	4 (5)	28.84
	5 (3)	29.51
DOB	2 (3)	10.33
	3 (13)	10.54
	4 (5)	10.86
	5 (3)	10.92
BRBR	2 (3)	11.13
	3 (13)	11.45
	4 (6)	11.71
	5 (3)	11.83
MAST	2 (3)	10.75
	3 (13)	10.97
	5 (3)	11.46
	4 (5)	11.49
IOB	2 (3)	4.04
	3 (13)	4.12 NSD
	4 (6)	4.31
	5 (3)	4.34
ROST	2 (3)	4.15
	3 (13)	4.47
	5 (3)	4.92
	4 (6)	5.10
APF	3 (13)	6.63
	2 (3)	6.65
	4 (6)	7.23
	5 (3)	7.52
DIAS	2 (3)	7.31
	3 (13)	7.58
	5 (3)	8.70
	4 (6)	8.74
PAL	2 (3)	14.91
	3 (13)	15.25
	4 (6)	17.05
	5 (3)	17.31
GBP	2 (3)	5.86
	3 (13)	5.94 NSD
	4 (6)	6.32
	5 (3)	6.47
MTR	2 (3)	5.02
	3 (13)	5.17
	4 (6)	5.42
	5 (3)	5.46
MDT	2 (3)	4.17
	3 (13)	4.22
	4 (3)	4.36 NSD
	5 (6)	4.54
MDH	2 (3)	7.71
	3 (11)	7.80 NSD
	4 (4)	8.61
	5 (3)	8.89
MDL	2 (3)	16.98
	3 (13)	17.83
	4 (6)	19.92
	5 (3)	19.95

Table 3.11 : Results of the SNK multiple range comparison test for toothwear classes (TW - 6) in *M. natalensis* from Barbeton, Mpumalanga Province. Non - significant subsets ($p < 0,05$) are indicated in vertical lines, NSD = means not significantly different, TW = toothwear classes, n = sample sizes.

Measurement	TW (n)	Means (ranked)
GLS	2 (3)	25.32
	3 (26)	25.92
	5 (3)	27.84
	6 (4)	28.80
	4 (6)	29.52
CBL	2 (3)	23.58
	3 (26)	24.48
	5 (3)	26.45
	6 (5)	27.27
	4 (6)	28.10
DOB	3 (26)	10.18
	2 (3)	10.45
	5 (3)	10.47
	6 (3)	10.49
	4 (6)	10.89
MAST	3 (25)	10.78
	2 (3)	10.78
	5 (3)	10.97
	6 (3)	11.22
	4 (6)	11.28
ROST	2 (3)	3.90
	3 (26)	4.24
	5 (3)	4.43
	6 (5)	4.69
	4 (6)	4.83
APF	2 (3)	5.93
	3 (26)	6.05
	5 (3)	6.62.
	6 (5)	6.91
	4 (6)	6.95
DIAS	2 (3)	6.62
	3 (26)	6.85
	5 (3)	7.56
	6 (5)	7.92
	4 (6)	8.25
PAL	2 (3)	13.52
	3 (26)	14.31
	5 (3)	15.26
	6 (5)	16.03
	4 (6)	16.20
GBP	2 (3)	5.54
	3 (26)	5.75
	5 (3)	6.22
	6 (6)	6.24
	4 (5)	6.35
PPW	5 (3)	4.40
	6 (5)	4.44
	2 (3)	4.54 NSD
	3 (22)	4.64
	4 (10)	4.70
MTR	3 (22)	4.98
	2 (3)	5.02
	6 (5)	5.08 NSD
	4 (10)	5.20
	5 (3)	5.32
MDH	2 (3)	7.11
	3 (26)	7.27
	5 (3)	7.97
	6 (5)	8.06
	4 (6)	8.63
MDL	2 (3)	15.94
	3 (26)	17.00
	5 (3)	18.12
	6 (5)	18.70
	4 (6)	19.46
PPL	2 (3)	8.70
	3 (26)	9.09
	5 (3)	10.00
	6 (5)	10.47
	3 (6)	10.74

significantly, whilst others showed overlapping subsets thus indicating the continuous nature of size variation (Table 3.11).

Multivariate patterns of variation.

In scatterplots of the first two principal components, TW groups tended to separate along PC I (Figure 3.7). In the Durban sample, separation of toothwear groups was not clear but there was a tendency for TW 1 - 3 individuals to plot apart from those of TW 4 - 5. Similarly, in the Barbeton sample there was some overlap between toothwear groups but specimens of TW 2 - 3 had a tendency to plot separately from those TW 4 - 6. These results, therefore, concur with those for *M. coucha* in showing that the smaller TW 1 - 3 individuals are significantly smaller than TW 4 - 6. As in *M. coucha*, the overlap between toothwear groups of both *M.natalensis* samples, implies that size variation is gradual rather than discontinuous. There was no evidence of separation according to sex groups along either of the two PC axes. The loadings of most measurements along PC axis I were all high and negative in both samples, indicating that PC axis I was a general size vector (Table 3.12). Separation along this axis therefore, reflected a decrease in size from left to right. Only PPW in the Durban sample, and three measurements (IOB, PPW, MDT) in the Barbeton sample loaded relatively low on PC I axis, and thus participated minimally in toothwear separation.

Distance based cluster analyses indicated clear age - related separation between the two subsets of toothwear groups in both samples. The majority of TW 2 - 3 specimens in the Durban sample grouped in cluster A (87 % TW 2 - 3) well apart from the majority of TW 4 - 5, which all fell in cluster B (71 % TW 4 - 5) (Figure 3.8). Similarly, in the Barbeton sample the majority of TW 2 - 3 specimens grouped separately in cluster A (100% TW 2 - 3), whereas most TW 4 - 6 specimens (75 % TW 4 - 6) grouped in cluster B (Figure 3.10).

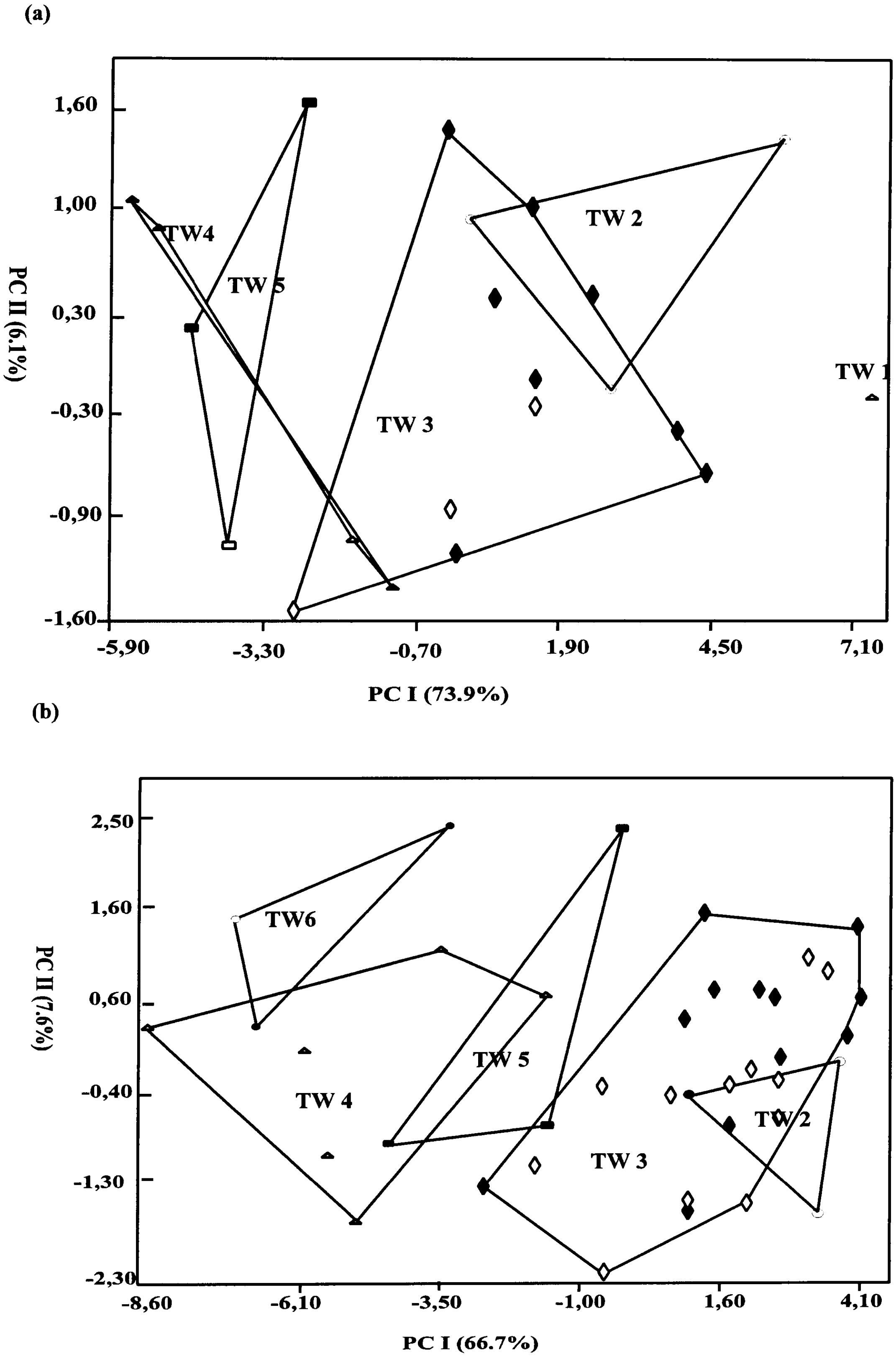


Figure 3.7 : The first two axes from principal components analyses of *M. natalensis* from (a) Durban, KwaZulu - Natal Province and (b) Barbeton, Mpumalanga Province, South Africa. Minimum convex polygons enclose individuals of each toothwear group. Closed and open symbols represent females and males respectively. TW denotes toothwear classes.

Table 3.12 : Loadings of the first two axes from principal components analyses of *M. natalensis* from (a) Durban , KwaZulu - Natal Province and (b) Barbeton, Mpumalanga Province. The percent variance contribution is given in parentheses.

Measurement	Axis I	Axis II
(a) Durban		
GLS	**-0,28 (94,44)**	0,14 (1,85)
CBL	**-0,28 (94.53)**	0,17 (2,67)
DOB	**-0,25 (73,14)**	0,08 (0,66)
BRBR	**-0,20 (47,51)**	**-0,33 (10,65)**
MAST	**-0,27 (87,16)**	-0,01 (0,001)
IOB	**-0,21 (53,35)**	**-0,58 (32,38)**
ROST	**-0,26 (79,48)**	-0,03 (0,09)
APF	**-0,27 (84,76)**	0,14 (2,01)
DIAS	**-0,28 (94,02)**	0,14 (1,82)
PAL	**-0,28 (95,03)**	0,13 (1,55)
GBP	**-0,22 (57,59)**	**0,25 (6,14)**
PPW	-0,16 (30,07)	**-0,31 (9,22)**
MTR	**-0,22 (55,06)**	**-0,48 (22,35)**
MDT	**-0,23 (62,65)**	-0,02 (0,02)
MDH	**-0,26 (79,52)**	**0,23 (5,28)**
MDL	**-0,28 (93,39)**	0,07 (0,44)
% trace	73,86%	6,07%
(b) Barbeton.		
GLS	**-0,29 (94,59)**	0,12 (1,91)
CBL	**-0,29 (96,55)**	0,10 (1,39)
DOB	**-0,22 (52,44)**	**-0,40 (2,11)**
BRBR	-0,16 (28,90)	**-0,47 (29,13)**
MAST	**-0,23 (61,57)**	**-0,44 (25,27)**
IOB	-0,15 (26,32)	**-0,37 (17,96)**
ROST	**-0,27 (82,72)**	0,09 (1,01)
APF	**-0,27 (82,60)**	0,19 (4,55)
DIAS	**-0,28 (89,39)**	0,14 (2,60)
PAL	**-0,28 (90,41)**	0,16 (3,18)
GBP	**-0,25 (68,85)**	**0,28 (9,83)**
PPW	-0,06 (4,47)	**-0,22 (6,30)**
MTR	**-0,21 (48,57)**	-0,13 (2,22)
MDT	**-0,19 (40,31)**	0,03 (0,31)
MDH	**-0,28 (88,08)**	0,05 (0,31)
MDL	**-0,29 (92,31)**	0,13 (2,07)
PPL	**-0,28 (86,42)**	0,06 (0,44)
% trace	67,33%	7,99%

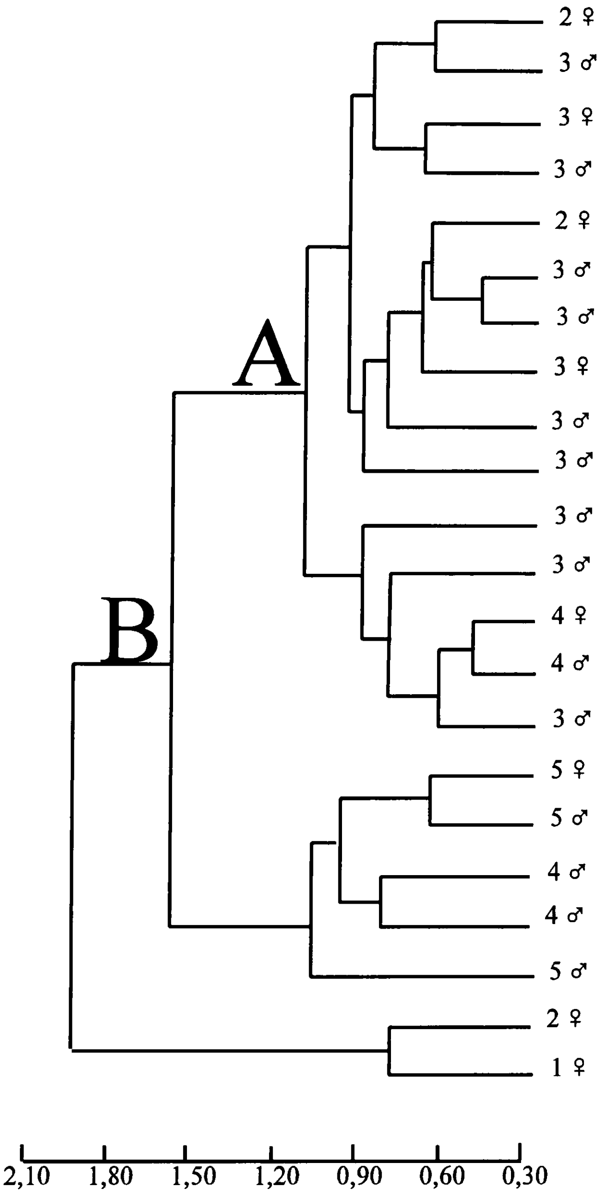


Figure 3.8 : Average taxonomic distance phenogram from cluster analysis of sex and toothwear group of *M. natalensis* from Durban, KwaZulu- Natal Province, South Africa. Toothwear groups are denoted with numbers, and males and females are represented with ♂ and ♀ respectively. Cophenetic correlation coefficient = 0,74.

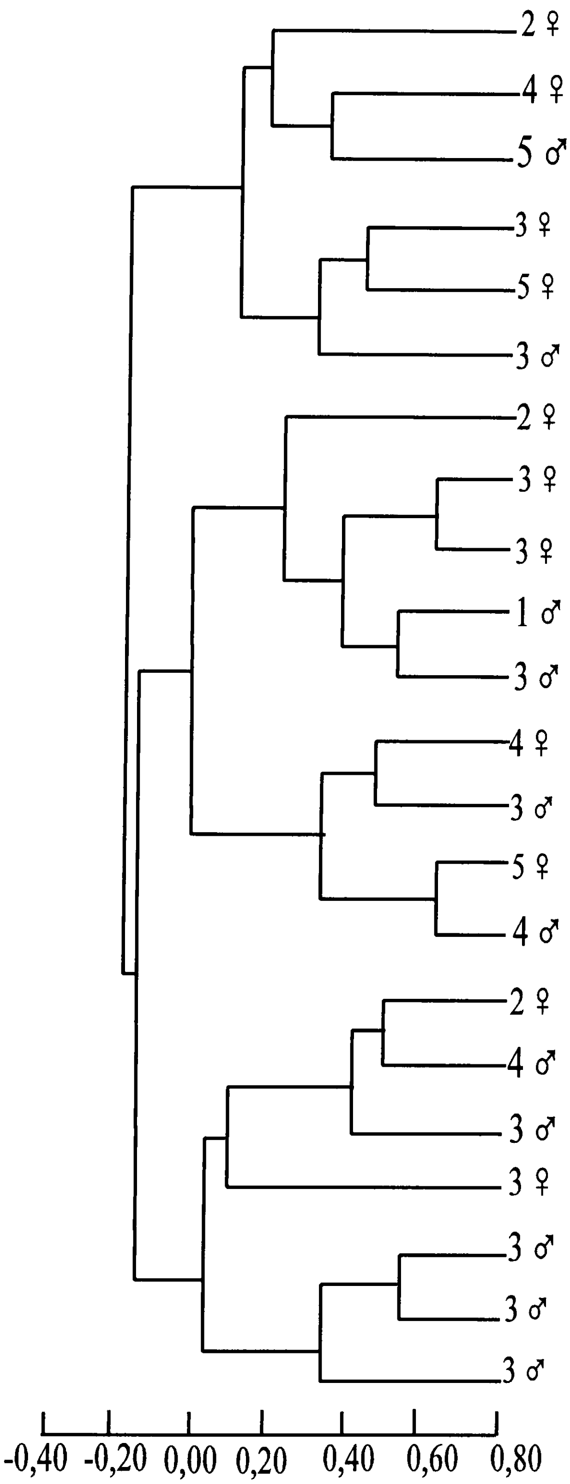


Figure 3.9: Correlation phenogram from cluster analysis of sex and toothwear groups of M. natalensis from Durban, KwaZulu - Natal Province, South Africa. Toothwear groups are indicated with numbers, and ♂ and ♀ denote males and females respectively. Cophenetic correlation coefficient = 0,68.

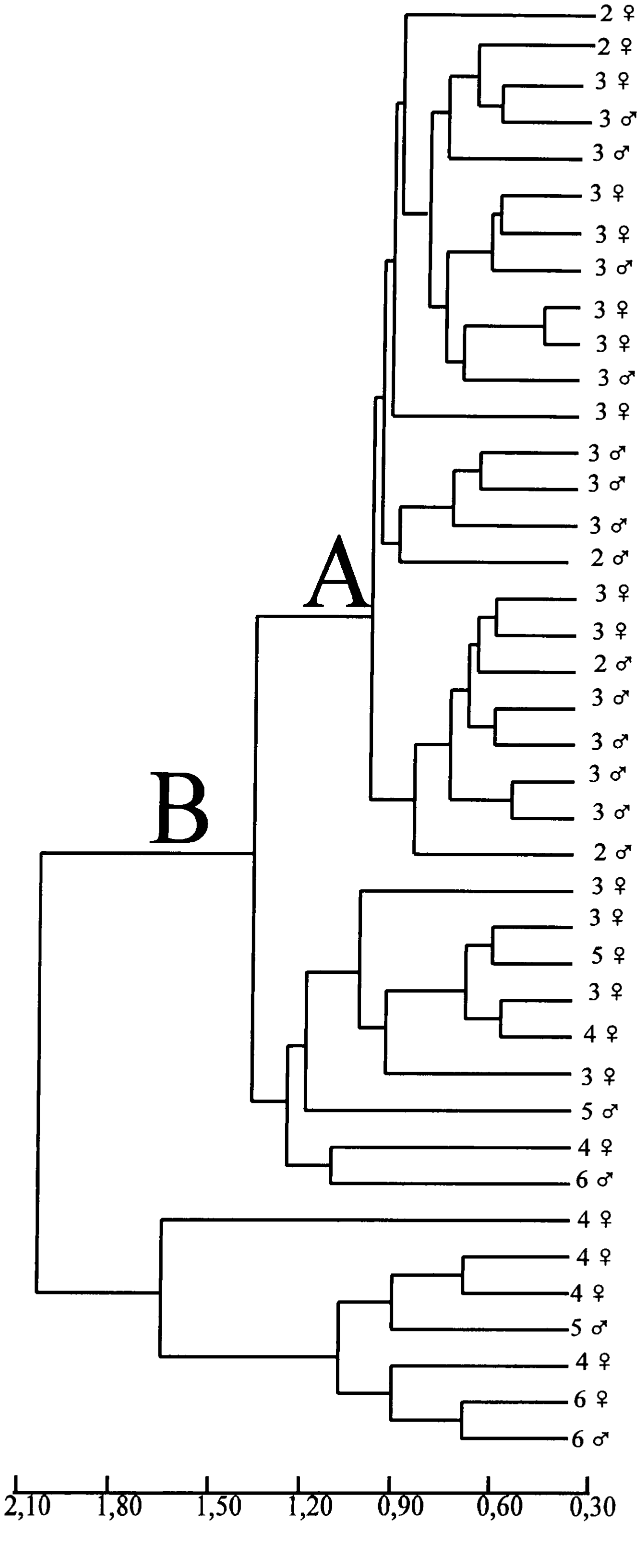


Figure 3.10: Average taxonomic distance phenogram from cluster analysis of sex and toothwear groups of *M. natalensis* from Barbeton, Mpumalanga Province, South Africa. Toothwear groups are denoted with numbers. ♂ and ♀ denote males and females respectively.

Cophenetic correlation coefficient = 0,82.

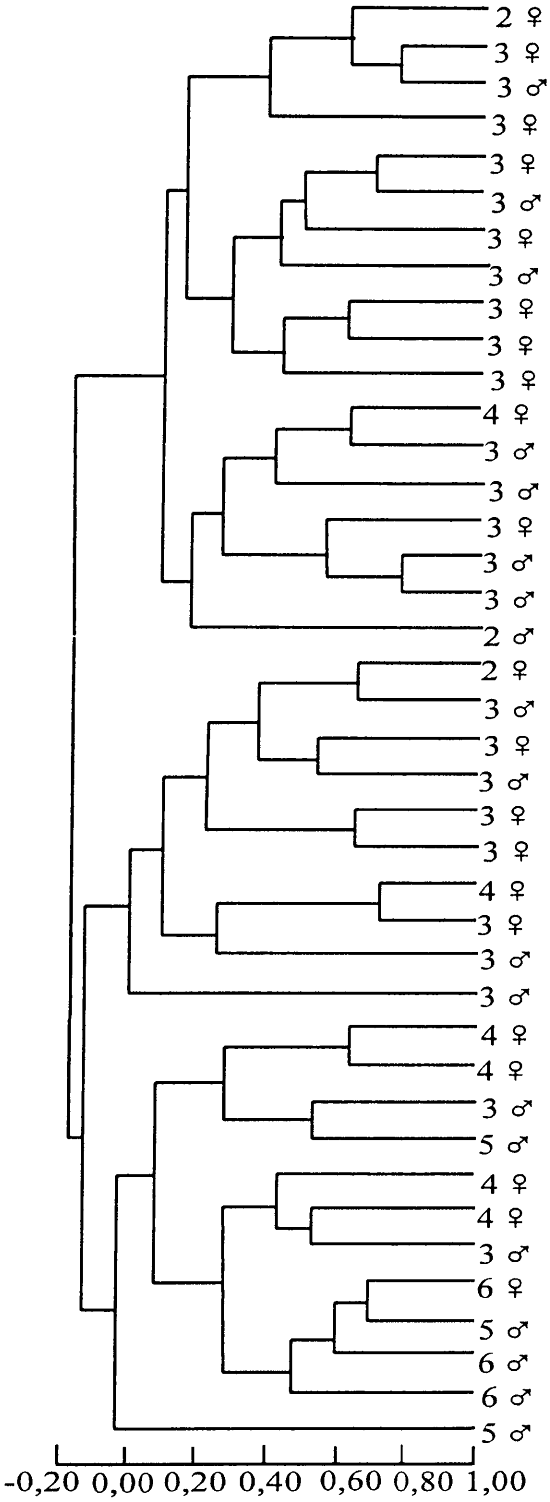


Figure 3.11 : Correlation phenogram from cluster analysis of sex and toothwear groups of *M. natalensis* from Barbeton, Mpumalanga Province, South Africa. Toothwear groups are indicated with numbers and ♂ and ♀ represent males and females respectively. Cophenetic correlation coefficient = 0,59.

In the latter sample there was, however, some overlap involving specimens of TW 3 which grouped with TW 4 - 6 individuals in cluster B, which further illustrated that size variation is intergraded. In both samples there was no apparent separation of toothwear groupings in the correlation phenogram (Figure 3.9 and 3.11). Both distance and correlation phenograms indicated a lack of substantial sexual dimorphism in both samples. Cluster analyses results, therefore, were in general agreement with results of univariate and PCA analyses, in showing that significant shape differences, OTU's representing the two species tended to group apart (Figure 4.11).

Multigroup discriminant functions analyses (MDA) produced a 100% *a posteriori* classification of toothwear groups in both samples. In both samples, MDA resulted in maximum separation of toothwear groups along both canonical variate (CV) axes I and II (Figure 3.12). In the Durban sample , all toothwear groups separated widely along CV II but TW 3 - 5 also separated from TW 2 along CV I . Similarly in the Barbeton sample, toothwear groups separated apart along CV II, and TW 2 - 3 separated from TW 4 -6 along CV I.

MANOVA indicated significant differences between toothwear groups in both samples (Barbeton : $F = 2{,}62_{(68,76)}$, $p < 0{,}01$; Durban: $F = 4{,}43_{(48,6)}$, $p < 0{,}05$). Because of sample size limitations, only the Barbeton sample (12 ♀ and 13 ♂ of TW 3) was subjected to MANOVA for sexual dimorphism, but no significant differences were detected ($F = 0{,}54_{(17,7)}$, $p > 0{,}05$).

3.4. CONCLUSION

More novel univariate methods of assessing non -geographic variation have been proposed (Leamy, 1983; Straney, 1978). However, two - way ANOVA results which can be used to calculate %SSQ were available only for *M. coucha* samples but not for

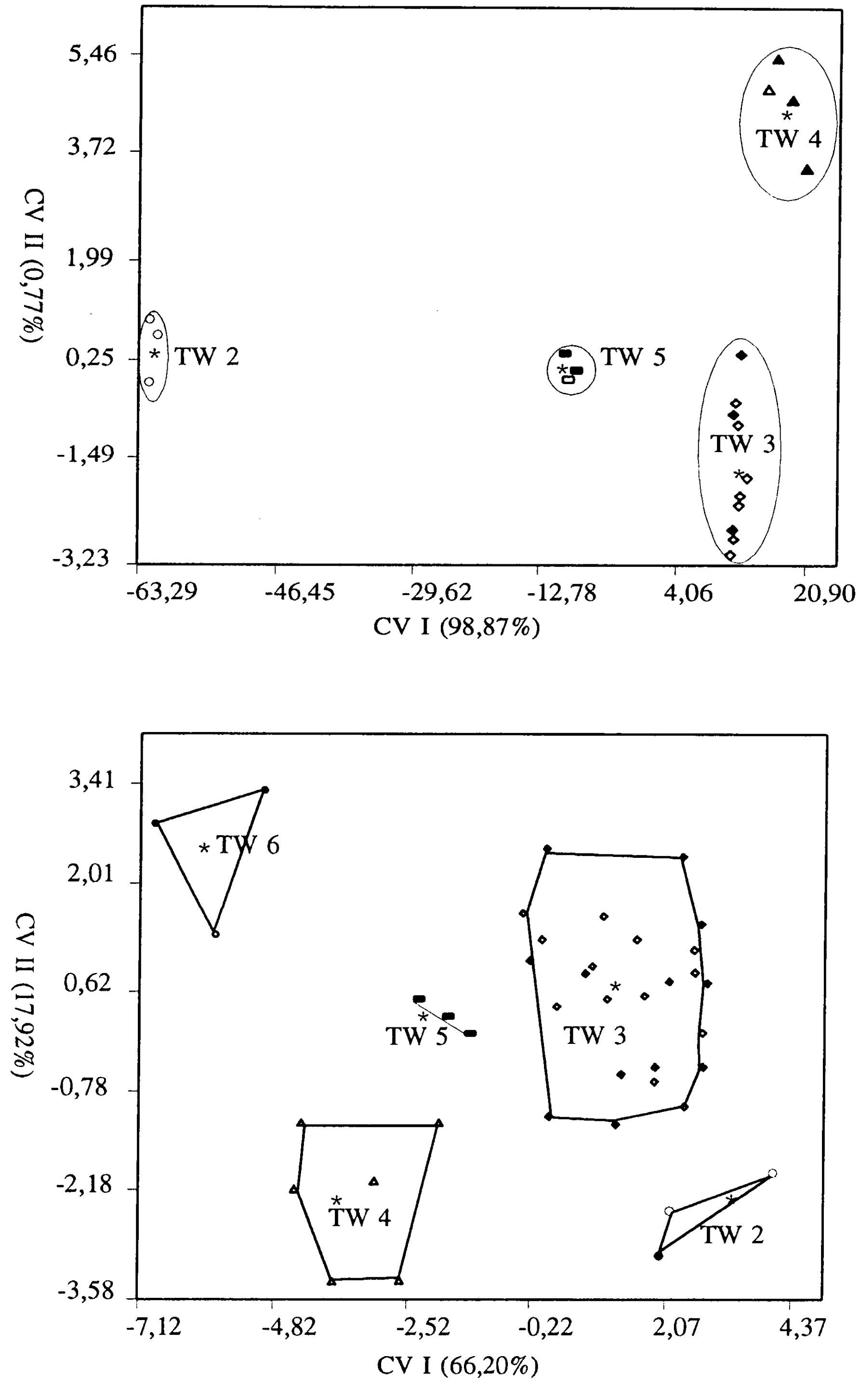


Figure 3.12 : Pairwise comparison of the first two canonical variates axes from multi - group discriminant functions analyses of toothwear and sex groups from (a) Durban, KwaZulu - Natal Province and (b) Barbeton, Mpumalanga Province. Closed and open symbols denote males and females respectively. Group centroids are indicated by asteriks.

M. natalensis. Consequently, non -geographic variation in this study could not be evaluated using this method as patterns across species could not be compared. Since other studies have demonstrated the congruence between the new methods and the conventional two - way ANOVA (Chimimba & Dippenaar, 1994; Taylor & Meester, 1993), the current results are considered reliable.

Results of analyses of *M. coucha* and *M. natalensis* were largely compatible and indicated similar patterns of nongeographic variation, including a lack of significant sexual dimorphism. Age - related variation , however, was marked and significant and involved mainly size - related differences between toothwear groups in both species. Lack of sexual dimorphism but pronounced age differences, has also been shown in other rodent species, such as *Aethomys chrysophilus*; *A. namaquensis* (Chimimba & Dippenaar, 1994); *Proechimys brevicauda* (Patton & Rogers, 1983); and *Taterillus gracilis* (Robbins, 1973). In both *M. coucha* and *M. natalensis* species, roughly the same set of variables participated in toothwear differentiation, thereby indicating that the nature of nongeographic variation was largely similar in both species. The degree of age variation was, however, more pronounced in *M. natalensis* than in *M. coucha*, and this was indicated by differing levels of separation when samples were subjected to MDA.

Multivariate analyses, when considered independently showed that size variation is intergraded, so that toothwear groups were not unequivocally delineated. Only when SNK multiple range comparison tests were also considered, did it became apparent that TW 1 - 3 individuals are significantly smaller than TW 4 - 6 individuals, and thus the two toothwear subgroups should be treated separately in subsequent analyses. Despite small sample sizes the current results provide a useful baseline pertaining to treatment of sex and toothwear groups in both *M. coucha* and *M. natalensis*. While it would have been ideal to assess the nature and extent of nongeographic variation throughout the southern African geographic range of the two species, it was practically impossible to do so, owing to inadequate sample size. Since sample sizes are often restrictive, studies assessing variation within populations often assume that nongeographic patterns are largely invariant geographically (Dippenaar & Rautenbach, 1986). Similarly in this study, the patterns of nongeographic variation are considered to be true throughout the southern African distribution range of the two species.

CHAPTER 4

INTERPOPULATIONAL VARIATION

4.1. INTRODUCTION.

Molecular differences, as evidenced by cytogenetic and electrophoretic variation within the *M. natalensis* species complex and the absence of hybrids in nature, show that *M. coucha* and *M. natalensis* are indeed distinct biological species. However, specimens of *Mastomys* comprise a large proportion of small mammal museum collections in southern Africa, but these are not amenable to karyotypic or electrophoretic identification (Gordon, 1984). There is, therefore, a clear need to identify morphometric correlates of the molecular and biochemical data, as the two species are virtually indistinguishable by other external morphological means.

Dippenaar *et al.* (1993) showed that the two southern African species within *M. natalensis s.l.* can be distinguished craniometrically. Their study is important in that it paves the way for identification of specimens of *M. natalensis s.l.* from museums, as well as from past studies for which voucher specimens are available. Such information may also allow the distribution and biogeography of the two species to be clarified.

The study of Dippenaar *et al.* (1993), however, was based on only cytogenetically known adult specimens from ten pooled locality samples in South Africa. Furthermore, juveniles and subadults which, represent a large portion of the species at certain times of the year, and which often constitute large portions of museum collection, were not included in their analyses. They also emphasized that since the extent of geographic variation in each species is not known, their results are not necessarily suitable for identification of specimens from far outside the geographic limits of their study. The discrimination technique developed by them, therefore, needs to be expanded in scope, to make it geographically more representative, and to ensure that it can be confidently used to identify juveniles and subadults.

The main aim of this chapter, therefore, is to : (i) identify morphometric correlates of the two species throughout southern Africa, (ii) develop a morphometric discrimination technique that will be applicable to all age groups and as much of southern Africa as possible, and (iii) apply this technique to identify cytogenetically unknown specimens from other localities in southern Africa.

4.2. MATERIALS AND METHODS

4.2.1. Material examined

Four hundred and seventy nine specimens of *M. coucha* and *M. natalensis*, identified on the basis of diploid number of chromosomes or haemoglobin electromorph, were used to develop the morphometric discrimination technique. This technique was then applied to craniometric data for 2388 *M. natalensis s.l.* from various localities in southern Africa to facilitate identification of cytogenetically unknown specimens, and establish distributional limits of the two species. Seventeen measurements were taken on each of these specimens (Section 2.2), and all specimens were aged following the criteria described in Section 2.5. Specimens missing one or more of the seventeen measurements were excluded from the multivariate analyses.

Localities represented by less than four specimens were pooled with geographically adjacent localities. Caution was taken not to pool localities separated by known biogeographical and vegetational barriers. Geographically isolated localities (e.g. OTU's 10, 13, 14, and 22, Table 4.1) were treated as independent localities, even though represented by inadequate sample sizes. This pooling approach resulted in recognition of 37 operational taxonomic units (OTU's) from southern Africa (Figure 4.1 and Table 4.1).

4.2.2. Qualitative dental character

All cytogenetically known specimens used in this study were examined for the presence or absence of the t3 cusp following the criteria described in Section 2.4, to assess geographic and interspecific variability of the character.

4.2.3. Statistical analyses.

Preliminary analyses based on single specimens.

A series of preliminary analyses carried out to assess the homogeneity of samples, included PCA and cluster analyses, and were based on individual specimens rather than mean values. Analyses carried out within species were based on individuals from all localities considered (Figure 4.2), whilst analyses between species were performed on individuals from either individual localities (Figures 4.3; 4.4; 4.5) or pooled localities (not shown here).

Geographic variation

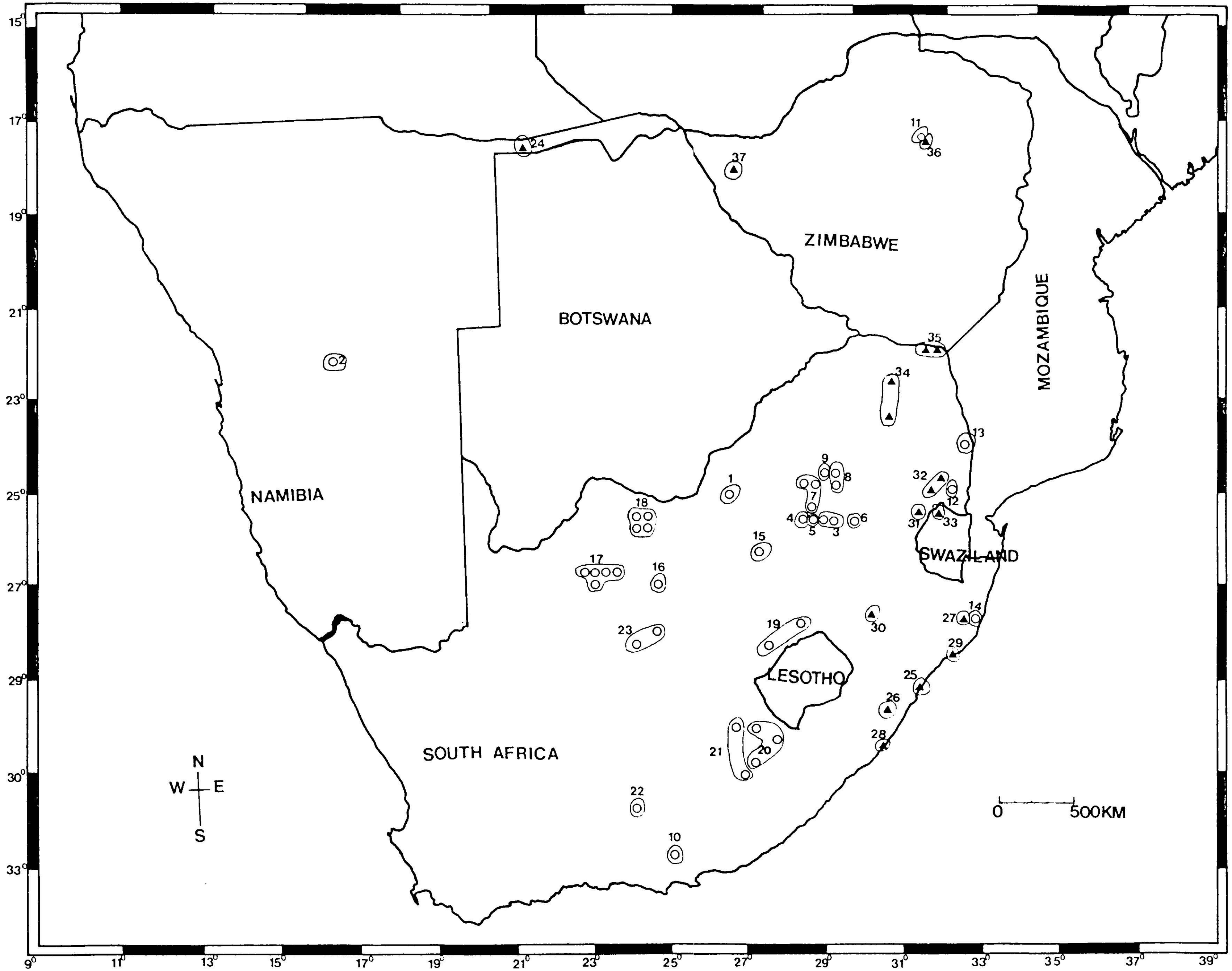
Preliminary analyses based on individual specimens (Section 4.3.2), showed that it was necessary to examine geographic variation prior to interspecific variation analyses. For each of the 23 *M. coucha* OTU's and the 14 *M. natalensis* OTU's, descriptive statistics (mean value, standard deviation and coefficient of variation) were thus computed.

Ideal statistical techniques for analysing geographic variation are those that are able to elucidate all types of geographic variation (e.g. categorical, clinal, nature and position of hybrid zones) (Thorpe, 1976). Use of more than one technique is, therefore, recommended. .

Table 4. 1 : Pooled and unpooled localities of specimens of *M.coucha* and *M.natalensis* (Appendix 1) used as operational taxonomic units (OTU'S) in ordination analysis and cluster analysis. Sample sizes are indicated in parentheses.

OTU number	OTU name	Pooled localities and sample sizes
M.coucha		
1	Groot Marico	Groot Marico Nursery Farm (31)
2	Windhoek	Windhoek (5)
3	Delmas	Delmas Farm 225 (6), Delmas (3)
4	Rietfontein	Rietfontein (7)
5	Springs	Springs Municipal Bird Sanctuary (2) , Suikerbosrand Nature Reserve, Brakpan (1)
6	Bethal	Komati Power Station, Bethal (10)
7	Fontana	Fontana Military area (3), Soutpan Experimental Farm (8), Wapadrand x 5 (ERF 318) (3)
8	Pretoria	Rietvlei Dam, Irene(5),University Experimental Farm, Pretoria(16), Verwoedburg, Cambridge Rd (2)
9	Hartebeeshoek	Hartebeeshoek Plot 30 (11)
10	Glenconner	Glenconner (2)
11	Norton	Norton, near Harare (30)
12	Hectorspruit	Lodwichs Lust Farm, Hectorspruit (6)
13	Satara camp	Satara Camp, Kruger National Park (2)
14	Nyekai	Nyekai (3)
15	Potchefstroom	Farm Nooitgedacht, 14 KM from Potchefstroom (7)
16	Vaalharts Establishment	Vaalharts Establishment (15)
17	Kuruman	Crofton Slabbert (1), Kuruman (9),Farm Vleiplaas (1),Selsden, ERF 97 (3),Moffat Church (2), Ulster (3)
18	Vryburg	De Hoop (2),Deeplagte (3), Hoogmoed (1), Kolhaar Farm (3 , Molopo river (3)
19	Ficksburg	Ficksburg (2), Farm Sandvlei, Bethlehem (1)
20	Dordrecht	Dordrecht (2), Barkly East (4), Lady Grey (1)
21	Sterkstroom	Sterkstroom (9), Aliwal North (1)
22	Aberdeen	Aberdeen (3)
23	Kimberly	Backhouse (2), De Bad (1), Gelukpoint (1), Kimberly (1)
M. natalensis		
24	Mahango	Mahango (13), Popa Caprivi (3)
25	Durban	Bluff Nature Reserve (3), Durban (17)
26	Richmond	Farm Hilltop, Richmond (4)
27	Hluhluwe	Hluhluwe Game Reserve (8), Research Camp (10)
28	Port Edward	Port Edward (13)
29	Umlalazi	Umlalazi Nature Reserve (5)
30	Van Reenen	Wyford Farm, Van Reenen (10)
31	Badplaas	Groenvaly, Badplaas (9)
32	Chamberie Nursery,Barbeton	Chamberie Nursery, Barbeton (28)
33	Barbeton Military area	Barbeton Military area (7), Pretorius Kop (1)
34	De hoek	De hoek (2), Entabeni (1)
35	Madimbo Military area	Madimbo Military area (12)
36	Norton	Norton, Harare (47)
37	Sinamotella	Sinamotella, Hwange National Park (35)

Figure 4.1 : Map of southern Africa, showing the 37 OTU's used in ordination and cluster analyses. OTU constituents are defined in Table 4.1. ○ = *M. coucha,* and ▲ = *M. natalensis.*



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Consequently, both univariate and multivariate methods were used in these analyses Model I one - way ANOVA of all OTU's within each species was used to assess the significance of geographic variation in each of the 17 variables independently. These analyses were followed by *a posteriori* SNK multiple range comparison tests (MCT). Multiple range tests are particularly useful in elucidating categorical geographic variation (Sneath & Sokal, 1973) and have been used widely in studies of geographic variation (Thorpe, 1976 ; Gabriel & Sokal, 1969).

Multivariate analyses included single - grouped PCA based on single - standardised product moment correlation coefficients among characters, and UPGMA cluster analyses based on both among - OTU average taxonomic distance (ATD) and product moment correlation coefficients, computed from single standardised data.

Interspecific variation.

Delimitation of species was based on multivariate statistical procedures and these were performed on arithmetic mean values, with the exception of MDA which were based on individual specimens.

Since use of single - group PCA or MPCA has been subject of some debate (Airoldi & Flury, 1988; Thorpe, 1988; Section 2.7.2) both techniques were used to explore data. The results of the analyses are presented in Section 4.3.4, and justified the identification of *a priori* phenetic groupings among the thirty seven OTU's by single - group PCA. Cluster analyses (UPGMA) based on average taxonomic distances (ATD) and product moment correlation (CORR) matrices were also computed.

Discriminant function analyses (MDA) were then used to assess the authenticity of these *a priori* groupings, while MANOVA was used to test statistical differences between group centroids. Standardized coefficients and overall means generated from MDA were then used to calculate discriminant scores to identify unknown specimens using the technique explained in detail by Taylor, Rautenbach, Gordon, Sink & Lotter (1995); Dippenaar *et al.* (1993); Chapman *et al.* (1992) and Dippenaar & Rautenbach (1986).

4.3. RESULTS AND DISCUSSION

4.3.1 Qualitative dental character.

Variability in the presence or absence of t3, and its incidence geographically and between species is summarised in Table 4.2. In general, the frequency of t3 cusp was considerably higher in *M. coucha* OTU's (67,13%) than in *M. natalensis* OTU's (3,46 %). A few *M. coucha* OTU's (OTU's 5, 8, 12, 15) lacked t3 completely. This may have been reflecting the variability of t3 with toothwear (Section 3.3.2), as all these OTU's were represented predominantly by older TW 4 - 6 individuals (% TW 4 - 6 : OTU 5 - 100%, OTU 8 - 60,87%, OTU 12 - 90%, OTU 15 - 57,14%). The Norton population (OTU 11) represented largely by smaller TW 1 -3 specimens (82,35% TW 1-3) was also characterised by a low t3 presence indicating that t3 may vary geographically regardless of toothwear .

In *M. coucha* the incidence of well developed t3 cusp was high (38,06%), whereas 29,1 % of individuals had only poor development of t3. The variability of the development of t3 followed no clear geographical pattern. Nine *M. coucha* OTU's (OTU 's 1, 5, 8, 9, 10, 13, 14, 20, 22) were characterised by high incidence of poorly developed t3 (Table 4.2),

Table 4.2 : Variability of the t3 cusp the 37 OTU's of *M. coucha* and *M. natalensis* from southern Africa. OTU constituents are defined in Table 4.1. + t3 = present but poorly developed, ++ t3 = present and well developed, - t3 = absent. Relative percentages are given in parentheses.

OTU number\ name	++ t3 cusp	+ t3 cusp	- t3 cusp	Total specimens
M. coucha				
1. Groot Marico	16 (32,65)	20 (40,82)	13 (26,53)	49
2. Windhoek	3 (42,86)	1 (14,29)	3 (42,86)	7
3. Delmas	5 (41,67)	2 (16,67)	5 (41,67)	12
4. Rietfontein	4 (57,14)	2 (28,57)	1 (14,29)	7
5. Springs	0 (0,00)	1 (33,33)	2 (66,67)	3
6. Bethal	5 (55,56)	2 (22,22)	2 (22,22)	9
7. Fontana	5 (35,71)	4 (28,57)	5 (35,71)	14
8. Pretoria	1 (4,35)	7 (30,43)	15 (65,22)	23
9. Hartebeeshoek	4 (36,36)	5 (45,45)	2 (8,18)	11
10.Glenconner	0 (0,00)	2 (66,67)	1 (33,33)	3
11.Norton, Harare	9 (26,47)	3 (8,82)	22 (64,71)	34
12.Hectorspruit	0 (0,00)	0 (0,00)	10 (100)	10
13.Satara camp	0 (0,00)	1 (50,00)	1 (50,00)	2
14.Nyekai	1 (33,33)	2 (66,67)	0 (0,00)	3
15.Potchefstroom	1 (14,29)	1 (14,29)	5 (71,43)	7
16.Vaalharts Establishment	12 (60,00)	7 (35,00)	1 (7,00)	20
17.Kuruman	14 (66,67)	4 (19,05)	3 (18,18)	21
18.Vryburg	9 (60,00)	5 (33,33)	1 (0,08)	15
19.Ficksburg	5 (71,43)	2 (28,57)	0 (0,00)	7
20.Dordrecht	4 (36,36)	5 (45,46)	2 (18,18)	11
21.Sterkstroom	9 (69,23)	3 (23,08)	1 (0,08)	13
22.Aberdeen	0 (0,00)	3 (60,00)	2 (40,00)	5
23.Kimberly	3 (50,00)	2 (33,33)	1 (16,67)	6
Total	**110 (38,06)**	**84 (29,07)**	**95 (32,87)**	**289**
M. natalensis				
24.Mahango	0 (0,00)	0 (0,00)	25 (100)	25
25.Durban	1 (4,00)	3 (12,00)	21 (84,00)	25
26.Richmond	0 (0,00)	1 (20,00)	4 (80,00)	5
27.Hluhluwe	0 (0,00)	0 (0,00)	22 (100)	22
28.Port Edward	1 (6,25)	1 (6,25)	14 (87,50)	16
29.Umlalazi	0 (0,00)	0 (0,00)	7 (100)	7
30.Van Reenen	0 (0,00)	0 (0,00)	10 (100)	10
31.Badplaas	0 (0,00)	0 (0,00)	10 (100)	10
32.Chamberie Nursery	0 (0,00)	0 (0,00)	27 (100)	27
33.Barbeton Military area	0 (0,00)	0 (0,00)	7 (100)	7
34.De hoek	0 (0,00)	0 (0,00)	3 (100)	3
35.Madimbo Military area	0 (0,00)	1 (6,25)	15 (93,75)	16
36.Norton, Harare	0 (0,00)	0 (0,00)	50 (100)	50
37.Sinamatella	1 (2,70)	0 (0,00)	36 (97,30)	37
Total	**6 (2,31)**	**3 (1,15)**	**251 (96,54)**	**260**

but this may have been a reflection of occlusal attrition, as most of these OTU's were represented predominantly by smaller (TW 1 - 3) specimens (% TW 1 -3 : OTU 1 - 79,59%, OTU 5 - 0 % , OTU 8 - 39,19%, OTU 9 - 100%, OTU 10 - 66,67%, OTU 13 - 50%, OTU 14 - 100%, OTU 20 - 72,73%, OTU 22 - 84,62%).

When present in *M. natalensis*, the t3 cusp was generally poorly developed (66,67 %), and the variability of its development did not particularly follow any apparent geographic pattern. These results partially support the findings of Pocock (1993), in that t3 frequency is higher in *M. coucha* than in *M. natalensis*. However, the incidence and development of t3 clearly varies with toothwear, and in *M. coucha* may also vary geographically. This character cannot, therefore, be used to reliably to diagnose *M. coucha* and *M. natalensis* specimens which lack molecular or biochemical data.

4.3.2. Preliminary analyses based on single specimens.

Geographic variation.

Principal components analyses of *M. coucha* individuals from all localities (Figure 4.2 a), indicated no clear geographical separation along PC axis I. There was, however, apparent age differentiation along this axis, as older toothwear groups (TW 4 - 6) tended to group toward the left of the scattergram. Principal components axis II, however, clearly differentiated specimens from the only OTU in Zimbabwe (OTU 11, Table 4.1) from those in South Africa and Namibia. Examination of eigenvector coefficients for PC I (Table 4.3) showed most variables to be high and negative, suggesting that toothwear differentiation was size based. Principal components axis II was a bipolar vector reflecting mainly differences in cranial shape. Five measurements (BrBr, IOB, GBP, PPW, MDT), dominated this shape axis and thus influenced differentiation of the Zimbabwe OTU from

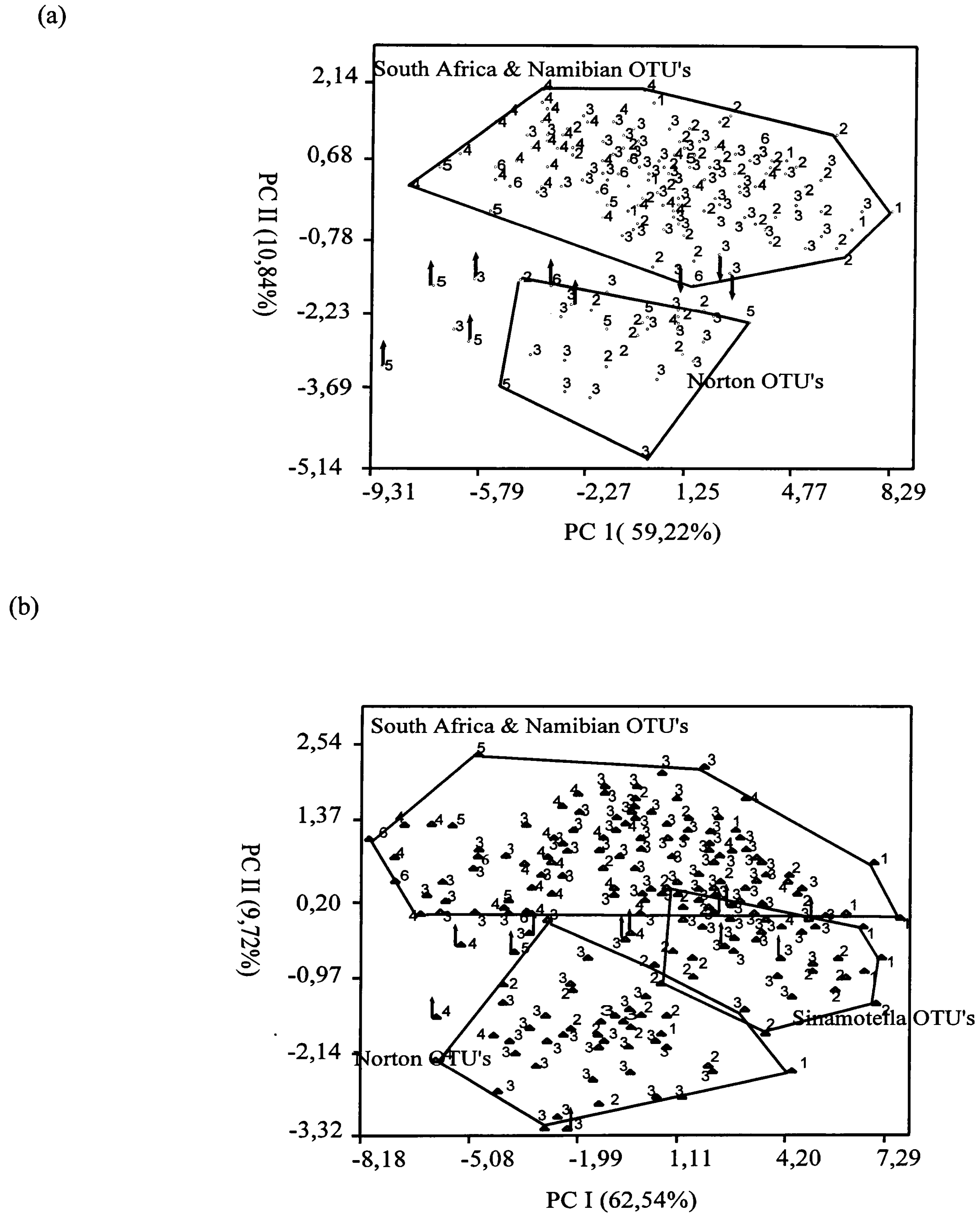


Figure 4.2 : Scatterplot of PC I and PC II from single group PCA of individual specimens of (a) *M. coucha* (b) *M. natalensis* from southern Africa. Arrows indicate outlying specimens and toothwear groups are denoted by numbers.

Table 4.3 : Variable loadings of the first two PC axes from the preliminary PCA results of infraspecific and intraspecific variation of *M. coucha* and *M. natalensis* individual specimens from southern Africa. Values indicated bold loaded relatively on the corresponding axis.

	Infraspecific variation				Interspecific variation	
	M. coucha		*M. natalensis*			
	PC I	PC II	PC I	PC II	PC I	PC II
GLS	**-0,30 (89,66)**	0,15 (4,35)	**-0,30 (93,38)**	0,01 (0,001)	**-0,31 (87,70)**	-0,12 (2,50)
CBL	**-0,31** (94,24)	0,(2,03)	**-0,30** (95,26)	-0,01 (0,06)	**-0,31 (93,14)**	-0,06 (0,67)
DOB	**-0,24 (51,69)**	-0,16 (4,21)	**-0,24** (58,62)	-0,07 (0,19)	**-0,24** (53,04)	0,06 (0,69)
BrBr	**-0,20** (45,30)	**0,33** (12,84)	-0,16 (11,81)	**0,48** (51,19)	**-0,26** (65,35)	0,06 (0,59)
MAST	**-0,26 (68,78)**	0,09 (0,65)	**-0,26** (61,79)	0,23 (12,76)	**-0,27** (68,81)	0,07 (0,82)
IOB	-0,13 (19,49)	**0,30 (12,99)**	-0,15 (15,19)	**0,45** (35,92)	-0,12 (13,92)	**-0,36 (21,08)**
ROST	**-0,27 (77,30)**	0,10 (0,43)	**-0,28** (78,15)	0,10 (1,98)	**-0,27** (70,42)	-0,004 (0,003)
APF	**-0,27 (68,01)**	-0,01 (0,04)	**-0,27** (76,85)	0,04 (0,06)	**-0,26** (63,41)	-0,02 (0,04)
DIAS	**-0,29 (84,39)**	0,05 (0,37)	**-0,29** (90,39)	-0,05 (0,57)	**-0,31** (88,18)	-0,08 (1,01)
PAL	**-0,30 (87,43)**	-0,01 (0,03)	**-0,29** (92,68)	-0,03 (0,39)	**-0,31** (90,37)	0,06 (0,57)
GBP	-0,16 (17,84)	**-0,51** (49,66)	-0,18 (35,61)	**-0,51** (35,95)	**-0,22** (45,24)	0,16 (4,32)
PPW	-0,11 (8,99)	**-0,44** (49,37)	-0,16 (16,03)	-0,15 (6,40)	-0,05 (2,48)	0,08 (1,15)
MTR	-0,14 (18,90)	0,05 (5,18)	-0,09 (13,11)	**0,39** (42,63)	-0,04 (1,17)	**0,53 (46,07)**
MDT	-0,11 (9,02)	**-0,53** (52,03)	-0,19 (32,75)	-0,14 (1,18)	-0,01 (0,03)	**0,66 (72,84)**
MDH	**-0,27 (72,19)**	-0,04 (0,42)	**-0,27** (81,91)	-0,12 (3,01)	**-0,27** (66,43)	0,13 (3,00)
MDL	**-0,30 (89,12)**	-0,03 (0,21)	**-0,29** (91,33)	-0,07 (1,27)	**-0,30** (84,01)	0,08 (1,08)
PPL	**-0,29 (83,84)**	-0,02 (0,020	**-0,27** (73,460	-0,14 (3,93)	-0,23 (50,82)	-0,26 (10,85)

South African and Namibian OTU's.

In *M. natalensis* (Figure 4.2 b) specimens from South Africa and Namibia plotted apart from those from Zimbabwe along PC II. Within the South Africa\ Namibia group , specimens tended to plot apart along PC I according to toothwear. Specimens from the two Zimbabwe localities tended to separate along PC I. Specimens from Norton (OTU 36) clearly separated from those from Sinamotella (OTU 37), irrespective of toothwear differences. Along PC axis II, the two Zimbabwe OTU's (OTU 36, 37, Table 4.1) separated apart from the majority of South African and Namibian OTU's. Loadings for most variables along PC I were mainly high and negative, suggesting that this reflected mainly size variation (Table 4.3). Principal component II, however, was bipolar vector apparently influenced mainly by differences in cranial shape, with BrBr, IOB, GBP and MTR loading high on this axis.

The variability of BrBr, IOB, GBP, in both species suggest that these may be "evolutionary active" characters in *M. natalensis* species complex, as these are equally important in interspecific variation (Section 4.3.4, Dippenaar *et al.* 1993,). In *M. coucha* characters from the palatal region (GBP, PPW) loaded high on PC II (Table 4.3), indicating that they are susceptible to geographic variation. Mandibular toothrow length (MDT) was also important and varied geographically in *M. coucha*, but maxillary toothrow length (MTR) varied geographically in *M. natalensis*.

While the main aim of these analyses was to assess the homogeneity of samples, the pattern that emerged from the results suggested that shape - related geographic variation is significant in both species. It was not clear, however, if size - related geographic variation is just as important, as age - related differences may have confounded any potential pattern.

Results of the study by Dippenaar *et al.* (1993), which were based only on adult specimens from a limited geographic area, suggested that size - related geographic variation in *M. coucha*.

Toothwear groups were, however, pooled in subsequent analyses of geographic variation, since the main aim of these analyses was to assess the extent of shape - related variation between geographic groupings, and to determine similar groups for pooling in analyses of interspecific variation. Furthermore, treatment of toothwear groups separately would have reduced the number of OTU's considerably, as some localities were represented by few or no specimens of either old (TW 4 -6) or smaller toothwear (TW 1 - 3) groups.

Interspecific variation.

Interspecific differences based on individual specimens were exemplified by the results of the Norton locality and these are presented in Figures 4.3; 4.4; and 4.5. Similar results were also evident in other pooled localities that were examined. No separation of the two species was evident along PC I, indicating that they are similar in overall size (Figure 4.3). The two species tended to plot apart along PC II. Principal components axis I was a unipolar size vector since most variables were high and negative (Table 4.3). Principal component axis II was bipolar shape vector influenced mainly by four measurements (IOB, MTR, MDT and PPL). Interorbital breadth (IOB) was also found to be significant in distinguishing the two species in the study by Dippenaar *et al.* (1993). While GBP was also influential in interspecific variation in the Dippenaar *et al.* (1993), in this study post palatal length was more significant (Table 4.3).

A correlation based UPGMA cluster analysis (which tends to reflect shape variation - Sneath & Sokal, 1973) similarly differentiated species into two subclusters (Figure 4.4).

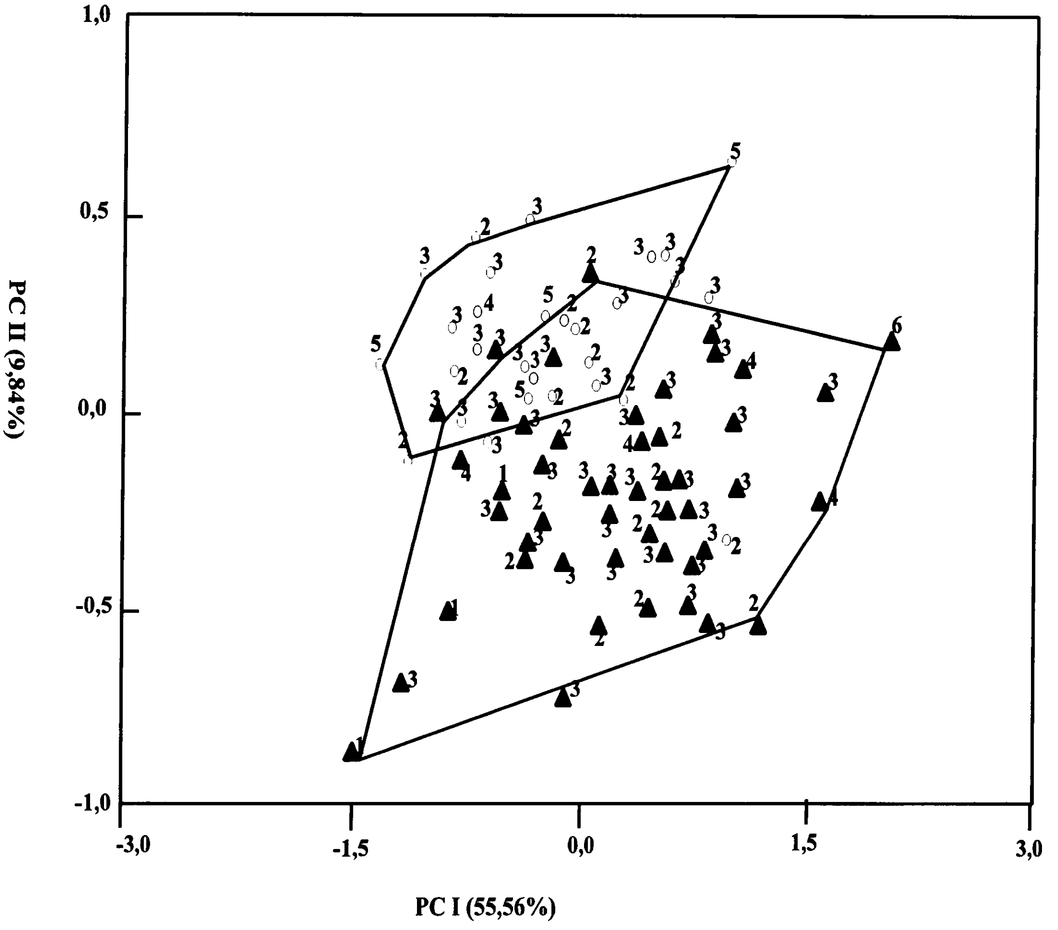


Figure 4.3 : Scattergram of principal components axes I and II from PCA based on *M. natalensis* and *M. coucha* individuals from Norton, Zimbabwe. Circles denote *M. coucha* OTU's and triangles denote *M. natalensis* OTU's. Numbers 1 - 6 denote toothwear groups.

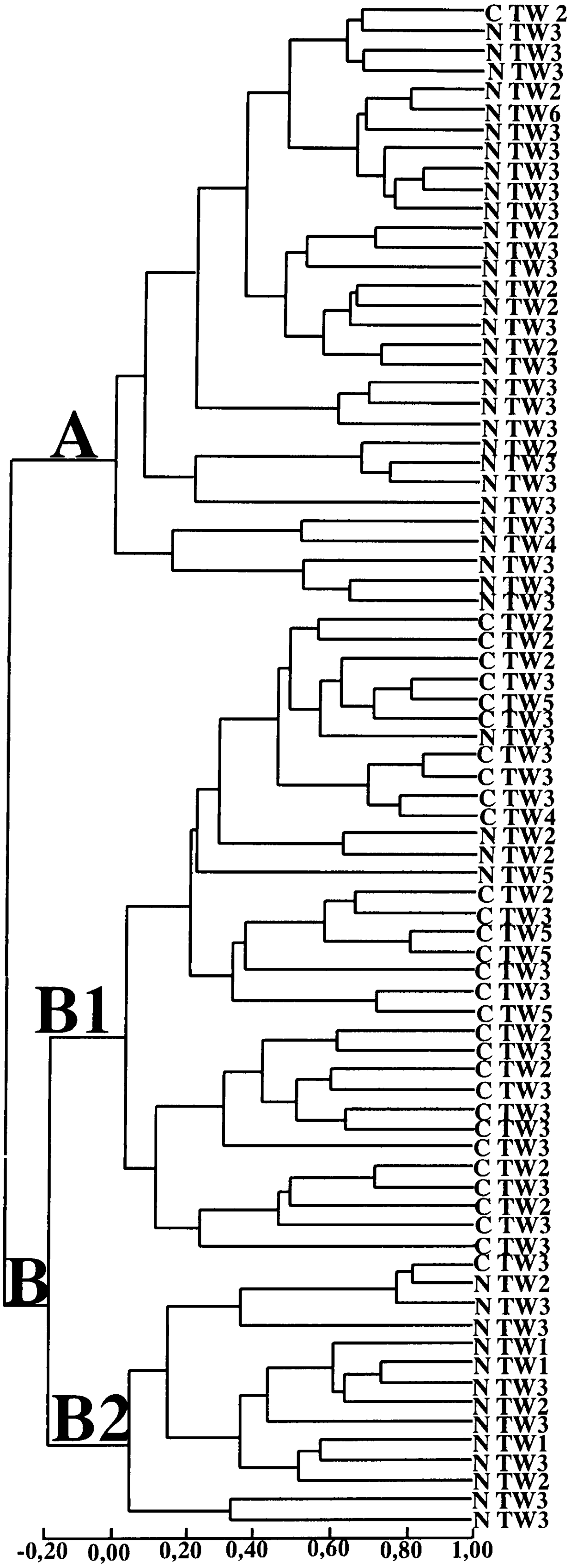


Figure 4.4 : Correlation phenogram from cluster analysis of *M. coucha* and *M. natalensis* individuals from Norton, Zimbabwe. TW denotes toothwear. Cophenetic correlation coefficient = 0,68.

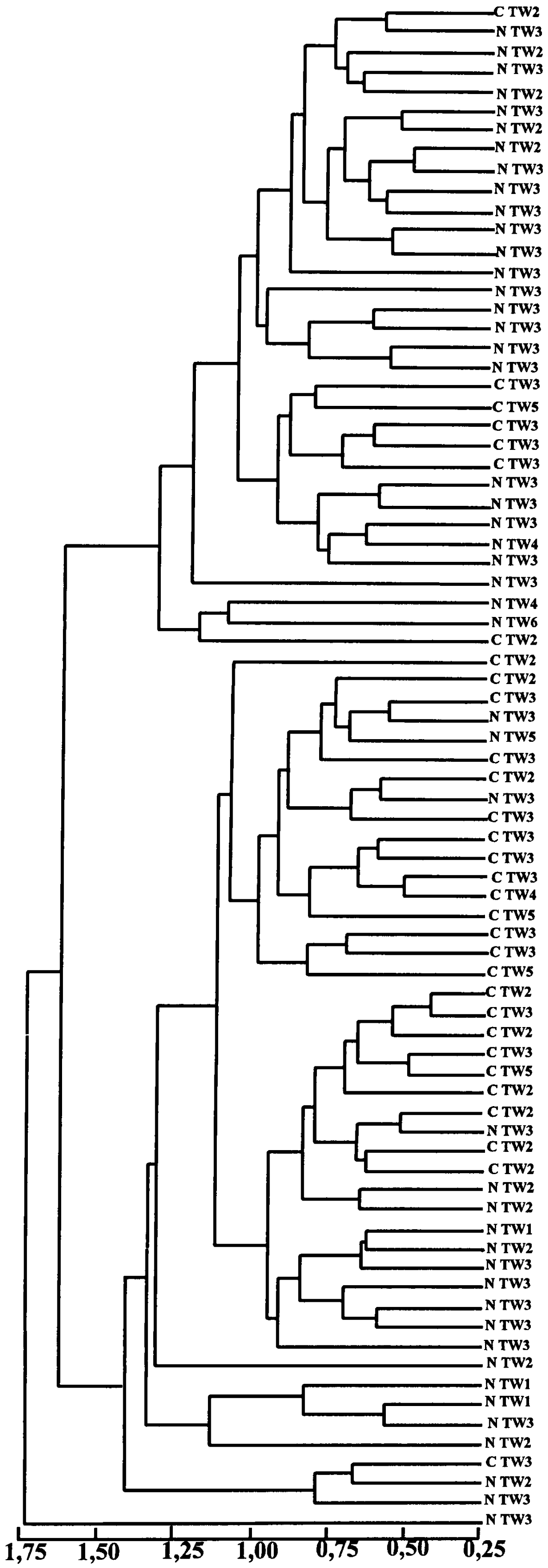


Figure 4.5: Average taxonomic distance phenogram from cluster analysis of *M. coucha* and *M. natalensis* individuals from Norton, Zimbabwe. TW denotes toothwear. Cophenetic correlation coefficient = 0,62.

Mastomys natalensis grouped in cluster A and B2, and apart from *M. coucha* specimens which instead grouped together in B1. The results of the UPGMA cluster analysis based on ATD, revealed no meaningful pattern (Figure 4.5).

These results show that while the two species do not differ markedly in size, shape - related differences between them are sufficiently pronounced for craniometric diagnosis. Although the results of nongeographic variation (Chapter 3) suggested craniometric disparity in size between TW 1 - 3 groups and TW 4 - 6, these preliminary analyses showed that age -related differences in size are not sufficiently marked to obscure patterns of geographic and interspecific variation in the *Mastomys natalensis* species complex. Pooling of toothwear groups was thus justified to increase samples sizes of OTU’s.

4.3.3. Geographic variation.

M. coucha.

Univariate analyses.

Descriptive statistics are presented in Table 4.4. Mean values of most variables showed no clear variation on a geographic basis. Greatest breadth of the palate (GBP), however, was relatively greater in the only Zimbabwe OTU (OTU 11) available than in the majority of South Africa and Namibia OTU's. Most OTU's showed relatively high standard deviation values (> 1 sd), especially for six measurements (GLS, CBL, PAL, DIAS, MDH, and PPL). This may be attributed to age -related variation introduced by pooling of TW groups. Standard deviation estimates the amount of variation in a population, and weighs each item of a distribution by its distance from the centre of the distribution (Sokal & Rohlf, 1981). Coefficient of variation values, which tend to reflect variation within a population

Table 4.4: Descriptive statistics and one - way ANOVA results of the 17 measurements of the 23 *M.coucha* OTU's from southern Africa. *X* = arithmetic mean, *SD* = standard deviation, *CV* = coefficient of variation, and *n* = sample size. ^{a} and ^{b} denote significance of *F* - values at $p < 0,05$ and $p < 0,01$ respectively. OTU constituents are defined in Table 4.1.

OTU	STATS	GLS	CBL	DOB	BrBr	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
1	X	25,67	24,55	10,06	11,23	10,69	3,89	4,06	6,05	7,00	14,32	5,85	4,35	4,97	4,19	7,62	17,00	9,17
	SD	1,48	1,56	0,33	0,49	0,43	0,15	0,29	0,46	0,60	1,03	0,33	0,20	0,37	0,10	0,63	0,95	0,81
	CV	5,78	6,34	3,29	4,33	3,98	3,91	7,11	7,52	8,56	7,21	5,60	4,65	7,45	2,38	8,21	5,56	8,79
	n	49	49	48	47	47	49	48	49	49	49	49	49	49	48	37	47	44
2	X	27,79	26,67	10,49	11,50	10,88	3,89	4,52	6,44	7,49	15,19	5,84	4,43	5,02	4,25	8,26	18,14	10,39
	SD	1,96	1,81	0,62	0,46	0,64	0,17	0,37	0,57	0,60	1,04	0,34	0,19	0,13	0,11	0,67	1,32	1,00
	CV	7,05	6,80	5,93	4,02	5,88	4,39	8,13	8,92	7,94	6,84	5,86	4,27	2,52	2,63	8,14	7,28	9,65
	n	6	6	6	6	6	9	9	9	9	9	9	9	8	9	8	9	4
3	X	29,21	27,93	10,39	11,82	11,39	3,97	4,82	6,63	8,00	16,31	6,06	4,35	5,09	4,27	8,62	19,11	10,48
	SD	1,01	1,05	0,38	0,31	0,29	0,13	0,28	0,34	0,32	0,67	0,21	0,26	0,17	0,12	0,35	0,68	0,66
	CV	3,46	3,77	3,63	2,59	2,51	3,24	5,81	5,19	3,97	4,09	3,46	5,98	3,36	2,80	4,09	3,56	6,29
	n	12	12	10	11	9	12	12	12	12	12	12	12	12	12	12	12	12
4	X	28,86	27,68	10,37	11,80	11,09	4,03	4,74	6,67	7,95	16,52	5,99	4,42	5,21	4,30	8,77	19,25	10,48
	SD	0,57	0,68	0,17	0,19	0,26	0,12	0,10	0,45	0,34	0,87	0,11	0,35	0,18	0,10	0,22	0,31	0,34
	CV	1,96	2,44	1,65	1,60	2,35	3,02	2,04	6,74	4,31	5,27	1,90	7,86	3,39	2,27	2,45	1,62	3,28
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
5	X	29,15	27,99	10,88	11,55	11,32	3,99	4,74	6,89	8,01	16,37	6,28	4,41	5,51	4,30	8,72	19,05	10,74
	SD	0,86	0,81	0,14	0,10	0,05	0,06	0,28	0,15	0,35	0,86	0,23	0,20	0,12	0,01	0,16	0,91	0,39
	CV	2,95	2,88	1,25	0,90	0,42	1,51	5,95	2,17	4,41	5,26	3,62	4,50	2,20	0,13	1,83	4,80	3,63
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
6	X	27,03	25,63	10,33	11,42	10,93	3,85	4,28	6,33	7,40	15,08	5,89	4,42	5,21	4,28	7,46	17,88	9,38
	SD	0,81	0,78	0,32	0,31	0,23	0,05	0,12	0,35	0,38	0,53	0,29	0,14	0,16	0,09	0,22	0,59	0,40
	CV	3,01	3,05	3,08	2,68	2,08	1,23	2,73	5,55	5,13	3,49	5,00	3,18	2,99	2,07	2,94	3,30	4,31
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10
7	X	26,41	24,96	10,25	10,95	10,71	3,98	4,31	6,11	6,96	14,37	5,91	4,35	5,18	4,29	7,75	17,19	9,27
	SD	1,46	1,50	0,37	0,41	0,34	0,21	0,27	0,43	0,55	0,79	0,41	0,15	0,22	0,12	0,45	1,01	0,81
	CV	5,53	6,02	3,57	3,78	3,20	5,21	6,26	7,03	7,94	5,47	6,98	3,39	4,17	2,73	5,77	5,86	8,74
	n	13	13	13	13	13	14	14	15	14	14	14	14	14	14	14	14	13

Table 4.4 cont'd

OTU	STATS	GLS	CBL	DOB	BrBr	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
8	X	27,41	26,19	10,55	10,67	10,94	4,02	4,57	6,51	7,61	15,55	6,37	4,40	5,33	4,38	8,03	18,21	9,79
	SD	2,99	2,97	0,42	2,28	0,67	0,20	0,43	0,55	0,92	1,48	0,63	0,21	0,29	0,14	0,66	1,55	1,06
	CV	10,91	11,34	4,01	21,32	6,14	4,94	9,61	8,38	12,04	9,54	9,84	4,71	5,52	3,12	8,27	8,50	10,82
	n	26	26	23	23	22	26	25	23	24	24	24	24	24	24	23	24	23
9	X	27,85	26,76	10,40	11,02	10,87	3,93	4,43	6,33	7,32	14,96	5,74	4,38	5,11	4,15	8.05	17,66	9,74
	SD	0,98	1,25	0,76	0,42	0,30	0,16	0,38	0,91	1,12	2.04	0,49	0,18	0,18	0,23	1,09	2,25	1,26
	CV	3,52	1,25	7,28	3,85	2,79	4,11	8,49	14,43	15,29	13,62	8,46	4,00	3,55	5,46	13,48	12,72	12,91
	n	9	9	9	10	8	8	9	11	11	11	11	11	10	11	11	11	11
10	X	28,34	27,30	10,60	11,94	11,23	4,09	4,53	6,56	7,42	15,16	5,85	4,54	5,28	4,49	8,63	18,72	10,25
	SD	0,95	1,21	0,16	0,34	0,52	0,09	0,21	0,54	0,49	0,44	0,26	0,16	0,43	0,32	0,32	1,07	0,20
	CV	3,34	4,43	1,46	2,88	4,66	2,23	4,68	8,26	6,55	2,91	4,50	3,47	8,16	7,14	3,75	5,73	1,95
	n	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	3
11	X	26,81	25,94	10,58	11,09	10,90	3,83	4,46	6,53	7,47	15,59	7,16	4,84	4,88	4,66	8,39	18,58	10,03
	SD	1,12	1,21	0,27	0,21	0,28	0,13	0,26	0,32	0,47	0,77	0,50	0,38	0,27	0,23	0,47	0,82	0,67
	CV	4,17	4,67	2,55	1,88	2,55	3,49	5,74	4,85	6,31	4,91	6,96	7,93	5,49	5,00	5,63	4,43	6,67
	n	32	32	33	33	33	34	34	34	34	34	34	34	34	34	34	34	32
12	X	28,09	26,76	10,31	11,47	10,97	4,03	4,58	6,73	7,68	15,59	6,22	4,38	5,08	4,27	8,02	18,35	10,18
	SD	1,63	1,78	0,45	0,48	0,47	0,14	0,38	0,44	0,64	1,06	0,44	0,21	0,26	0,11	0,57	1,15	1,03
	n	5,81	6,66	4,39	4,22	4,33	3,50	8,21	6,51	8,37	6,81	6,99	4,74	5,03	2,47	7,10	6,26	10,14
13	X	30,46	29,46	10,73	11,83	11,52	4,12	4,91	7,12	8,59	17,35	6,40	4,58	5,28	4,30	9,15	19,84	11,10
	SD	1,27	1,66	0,41	0,19	0,08	0,24	0,24	0,48	0,72	1,21	0,86	0,22	0,30	0,18	0,70	1,11	0,97
	CV	4,16	5,62	3,82	1,62	0,68	5,80	4,82	6,76	8,42	7,00	13,38	4,70	5,69	4,16	7,68	5,61	8,73
	n	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	2
14	X	28,11	26,81	10,42	11,54	10,91	4,21	4,70	6,32	7,40	15,36	6,05	4,32	5,02	4,22	8,71	18,37	9,75
	SD	0,85	1,03	0,06	0,22	0,17	0,08	0,22	0,52	0,22	0,53	0,38	0,12	0,08	0,06	0,42	0,57	0,48
	CV	3,01	3,85	0,53	1,88	1,56	1,79	4,69	8,25	2,99	3,42	6,31	2,81	1,61	1,30	4,80	3,09	4,96
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
15	X	30,15	28,99	11,10	11,55	11,47	3,99	4,74	7,19	8,54	17,33	7,91	4,76	5,70	4,78	9,26	20,35	11,84
	SD	0,90	0,98	0,31	0,30	0,29	0,18	0,21	0,38	0,50	0,86	0,49	0,21	0,29	0,29	0,51	0,60	0,70
	CV	2,98	3,39	2,83	2,56	2,53	4,37	4,35	5,22	5,85	4,96	6,23	4,33	5,15	6.07	5,56	2,94	5,92
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

Table 4.4 cont'd

OTU	STAT	GLS	CBL	DOB	BrBr	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
16	X	28,02	26,82	10,23	11,60	10,97	4,03	4,62	6,55	7,35	15,56	5,91	4,52	4,85	4,49	8,33	18,41	10,24
	SD	1,16	1,30	0,23	0,34	0,33	0,16	0,32	0,36	0,43	0,79	0,32	0,27	0,25	0,17	0,50	0,71	0,82
	CV	4,15	4,86	2,21	2,93	3,04	4,00	6,83	5,45	5,79	5,07	5,43	5,96	5,16	3,84	6,02	3,84	7,96
	n	16	16	15	15	15	18	18	18	18	18	18	18	18	13	18	18	16
17	X	26,93	25,86	10,22	11,45	10,37	3,93	4,36	6,42	7,13	15,03	5,86	4,38	4,95	4,29	8,04	17,62	9,62
	SD	1,32	1,56	0,27	0,31	2,54	0,14	0,28	0,43	0,48	0,87	0,20	0,26	0,22	0,17	0,62	0,82	0,80
	CV	4,15	4,86	2,21	2,93	3,04	4,00	6,83	5,45	5,79	5,07	5,43	5,96	5,16	3,84	6,02	3,84	7,96
	n	21	21	19	19	19	21	21	21	21	21	21	21	21	21	21	21	21
18	X	27,51	26,03	10,29	11,28	10,82	3,94	4,46	6,36	7,11	15,03	5,92	4,51	4,90	4,11	7,91	17,99	9,78
	SD	1,60	1,60	0,21	0,48	0,46	0,08	0,25	0,48	0,11	1,24	0,40	0,05	0,29	1,16	0,67	1,07	0,83
	CV	5,81	6,14	2,00	4,23	4,28	1,94	5,61	7,60	10,03	8,27	6,66	4,75	5,90	28,16	8,44	5,94	8,45
	n	14	14	14	14	14	10	15	15	15	15	15	15	13	15	14	15	14
19	X	27,45	26,34	10,24	11,87	10,97	4,05	4,57	6,54	7,22	15,41	5,84	4,57	5,19	4,41	8,30	18,36	9,76
	SD	0,96	1,07	0,15	0,27	0,44	0,14	0,29	0,34	0,41	0,62	0,21	0,22	0,18	0,20	0,41	0,74	0,48
	CV	3,49	4,07	1,44	2,31	3,99	3,49	6,27	5,18	5,68	4,03	3,67	4,77	3,51	4,64	4,96	4,05	4,95
	n	7	7	4	5	4	7	7	7	7	7	7	7	7	7	7	7	6
20	X	27,50	26,33	10,18	11,46	10,82	3,95	4,56	6,29	7,28	15,18	5,83	4,38	5,11	4,30	8,32	18,33	10,07
	SD	1,32	1,39	0,32	0,47	0,38	0,17	0,26	0,40	0,42	0,67	0,13	0,27	0,15	0,10	0,47	0,73	0,61
	CV	4,80	5,28	3,11	4,07	3,46	4,30	5,72	6,29	5,73	4,40	2,21	6,19	2,96	2,21	5,70	4,00	6,10
	n	8	8	6	5	6	9	9	9	9	9	9	9	9	9	9	9	5
21	X	26,87	25,88	10,05	11,37	10,75	3,92	4,43	6,27	7,01	14,81	5,80	4,36	4,98	4,35	8,30	18,12	7,98
	SD	1,32	1,31	0,25	0,46	0,30	0,18	0,23	0,26	0,51	0,89	0,23	0,20	0,34	0,14	0,33	1,06	3,78
	CV	4,91	5,07	2,43	4,02	2,79	4,70	5,24	4,11	7,20	5,98	4,01	4,51	6,91	3,27	3,98	5,83	47,34
	n	11	10	11	11	10	13	13	13	13	13	13	13	13	13	13	13	12
22	X	28,12	26,99	10,35	11,75	11,25	4,01	4,65	6,74	7,55	15,61	5,93	4,63	4,98	4,33	8,68	18,56	9,89
	SD	1,06	1,21	0,15	0,33	0,44	0,16	0,53	0,20	0,45	0,66	0,24	0,29	0,31	0,07	0,68	0,73	0,85
	CV	3,76	4,49	1,47	2,77	3,95	4,00	11,47	3,02	5,96	4,20	3,97	6,16	6,13	1,51	7,83	3,93	8,61
	n	5	5	4	3	3	5	5	5	5	5	5	5	5	5	5	5	4

Table 4.4 cont'd

OTU	STAT	GLS	CBL	DOB	BrBr	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
23	X	28,67	27,42	10,52	11,65	1,29	3,98	4,74	6,78	7,60	15,64	6,06	4,50	5,01	4,38	8,47	18,80	10,48
	SD	1,32	1,18	0,12	0,28	0,29	0,22	0,41	0,29	0,44	0,41	0,48	0,13	0,29	0,16	0,77	0,86	0,42
	CV	4,59	4,31	1,14	2,38	2,58	5,51	8,65	4,29	5,85	2,64	7,89	2,95	5,86	3,57	9,06	4,59	3,96
	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
F - values		**6,47**^{b}	**5,78**^{b}	**5,53**^{b}	**1,87**^{a}	1,09	**3,19**^{b}	**6,76**^{b}	**4,32**^{b}	**5,00**^{b}	**6,26**^{b}	**21,97**^{b}	**5,76**^{b}	**5,55**^{b}	**3,76**^{b}	**6,03**^{b}	**6,76**^{b}	**4,25**^{b}

relative to other similar populations (Sokal & Rohlf, 1981) were relatively low (< 10%) in . most measurements

F - values from Model one - way ANOVA (Table 4.4) were highly significant at 0,01% level, indicating that all measurements except MAST varied significantly on a geographic basis. Among the 23 OTU's, only one measurement (BrBr) differed significantly at 0,05% level. SNK multiple range comparison tests of the 23 OTU's showed broadly overlapping non -significant subsets ($p < 0{,}05$) in most measurements, and did not categorize groups according to any meaningful geographic trend (Table 4.5). Greatest breadth of the palate was the only variable which separated the OTU's into two subsets, one with the Norton (OTU 11) and Potchefstroom (OTU 15) OTU's and the other OTU's from South Africa and Namibia.

Multivariate analyses.

Principal components analysis results based on mean values for the 23 OTU's, are presented in Figure 4.6 a. Pairwise comparison of PC axes I and II indicated no meaningful separation according to geography along PC axis I. Two discrete groupings were, however, evident along PC axis II, as the majority of South African and Namibian OTU's clustered below the only OTU from Zimbabwe (OTU 11) and that from Potchefstroom (OTU 15). A correlation matrix based minimum spanning tree connected South Africa\ Namibia OTU's to the Norton OTU (OTU 11) which further connected to the Potchefstroon OTU (OTU 15) (Figure 4.6 a). The grouping of Potchefstroom specimens with those from Zimbabwe is puzzling. These specimens were identified karyotypically during the course of this study to *M. coucha.* However, it is possible that a transcription error was made at the Transvaal museum whilst cataloguing specimens and thus their identity may have been confused..

Table 4.5: Results of SNK multiple range comparison tests of the 16 measurements of the 23 *M. coucha* OTU's from southern Africa. Non - significant subsets (*p* < 0,005) are indicated by vertical lines, ASD = all means significantly different, n = sample sizes. OTU constituents are defined in Table 4.1.

GLS		CBL		DOB		BrBr		IOB	
OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean
1 (49)	25,67	1 (49)	24,55	21(11)	10,05	8 (25)	10,67	11 (34)	3,83
7 (13)	26,40	7 (13)	24,96	1 (48)	10,06	7 (13)	10,95	6 (10)	3,85
11 (32)	26,81	6 (10)	25,63	20(6)	10,18	9 (10)	11,02	2 (9)	3,89
21 (11)	26,87	17 (21)	25,86	17(19)	10,22	11(33)	11,09	1 (49)	3,89
17 (21)	26,93	21 (10)	25,88	16(15)	10,23	1 (47)	11,23	11 (13)	3,92
6 (10)	27,02	11 (32)	25,94	19(4)	10,24	18(14)	11,28	17 (21)	3,93
8 (26)	27,41	18 (14)	26,02	7 (13)	10,25	21(11)	11,37	9 (9)	3,93
19 (7)	27,45	8 (26)	26,19	18(14)	10,29	6 (10)	11,41	18(10)	3,94
20 (8)	27,50	20 (8)	26,33	12 (7)	10,31	17(19)	11,45	20(9)	3,95
18 (14)	27,51	19 (7)	26,34	6 (10)	10,33	20 (5)	11,46	3 (12)	3,97
2 (6)	27,78	2 (6)	26,67	22(4)	10,35	12 (8)	11,47	7 (14)	3,98
9 (9)	27,85	9 (9)	26,76	4 (4)	10,37	2 (6)	11,50	23(5)	3,98
16 (16)	28,02	12 (9)	26,76	3 (10)	10,39	14(3)	11,54	15(7)	3,99
12 (8)	28,09	14 (3)	26,81	9 (9)	10,40	15(7)	11,55	5 (3)	3,99
14 (3)	28,11	16 (16)	26,82	14(3)	10,42	5 (3)	11,55	22(5)	4,01
22 (5)	28,12	22 (5)	26,99	2 (6)	10,49	16(15)	11,60	4 (7)	4,03
10 (3)	28,34	10 (3)	27,30	23(5)	10,52	23(5)	11,65	16(18)	4,03
23 (5)	28,67	23 (5)	27,42	8 (23)	10,55	22(3)	11,75	8 (26)	4,03
4 (7)	28,67	4 (7)	27,68	11(33)	10,58	4 (7)	11,80	12(9)	4,03
5 (3)	29,15	3 (12)	27,93	10(3)	10,60	3 (11)	11,82	19(7)	4,05
3 (12)	29,21	5 (3)	27,99	13(2)	10,73	13(2)	11,82	10(4)	4,09
15 (7)	30,15	15(7)	28,99	5 (3)	10,88	19(5)	11,87	13(3)	4,12
13 (2)	30,44	13(2)	29,46	15(7)	11,10	10(3)	11,94	14(3)	4,21

Table 4.5 cont'd.

ROST		APF		DIAS		PAL		GBP	
OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean
1 (49)	4,06	1 (48)	6,05	7 (14)	6,96	1 (49)	14,32	9 (11)	5,74
6 (10)	4,28	7 (15)	6,11	1 (49)	7,00	7 (14)	14,37	21 (13)	5,80
7 (14)	4,30	21 (13)	6,27	21 (13)	7,01	21 (13)	14,81	20 (9)	5,83
17 (21)	4,36	20 (9)	6,29	18 (15)	7,11	9 (11)	14,96	19 (7)	5,84
21 (13)	4,42	14 (3)	6,32	17 (21)	7,13	18 (15)	15,03	2 (9)	5,84
9 (9)	4,43	9 (11)	6,33	19 (7)	7,22	17 (21)	15,03	10 (4)	5,85
18 (15)	4,46	6 (10)	6,33	20 (9)	7,28	6 (10)	15,08	1 (49)	5,85
11 (34)	4,46	18 (15)	6,36	9 (11)	7,32	10 (4)	15,16	17 (21)	5,86
8 (25)	4,51	17 (21)	6,42	16 (18)	7,35	20 (9)	15,18	6 (10)	5,89
2 (9)	4,52	2 (9)	6,44	14 (3)	7,40	2 (9)	15,19	7 (14)	5,91
10 (4)	4,53	8 (23)	6,51	6 (10)	7,40	14 (3)	15,36	16 (18)	5,91
20 (9)	4,56	11 (34)	6,53	10 (4)	7,41	19 (3)	15,41	18 (15)	5,92
19 (7)	4,57	19 (7)	6,54	11 (34)	7,47	8 (24)	15,55	22 (5)	5,93
12 (9)	4,58	16 (18)	6,55	2 (9)	7,49	16 (18)	15,56	4 (7)	5,99
16 (18)	4,62	10 (4)	6,56	22 (5)	7,55	11 (34)	15,59	14 (3)	6,05
22 (5)	4,65	3 (12)	6,63	23 (5)	7,60	12 (9)	15,59	3 (12)	6,06
14 (3)	4,70	4 (7)	6,67	8 (24)	7,61	22 (5)	15,61	23 (5)	6,06
4 (7)	4,74	12 (9)	6,73	12 (9)	7,68	23 (5)	15,64	12 (9)	6,22
15 (7)	4,74	22 (5)	6,74	4 (7)	7,95	3 (12)	16,31	5 (3)	6,28
23 (5)	4,74	23 (5)	6,78	3 (12)	8,00	5 (3)	16,37	8 (24)	6,37
5 (3)	4,74	5 (3)	6,89	5 (3)	8,01	4 (7)	16,52	13 (3)	6,40
3 (12)	4,82	13 (3)	7,12	15 (7)	8,54	15 (7)	17,33	11 (34)	7,16
13 (3)	4,91	15 (7)	7,19	13 (3)	8,59	13 (3)	17,35	15 (7)	7,91

Table 4.5 cont'd.

PPW		MTR		MDT		MDH			MDL
OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean	OTU (n)	Mean
14 (3)	4,32	16 (18)	4,85	18 (15)	4,11	6 (9)	7,46	1 (47)	17,00
1 (49)	4.35	11 (34)	4,88	9 (11)	4,15	1 (37)	7,62	7 (14)	17,19
7 (14)	4.35	18 (13)	4,90	1 (48)	4,19	7 (14)	7,75	17 (21)	17,62
3 (12	4.35	17 (21)	4,95	14 (3)	4,22	18 (14)	7,91	9 (11)	17,66
21 (13)	4.36	1 (49)	4,97	2 (9)	4,25	12 (9)	8,02	6 (10)	17,88
17 (21)	4.37	22 (5)	4,98	12 (9)	4,27	8 (23)	8,03	18 (15)	17,99
12 (9)	4.38	21 (13)	4,98	3 (12)	4,27	17 (21)	8,04	21 (13)	18,12
9 (11)	4.38	23 (5)	5,01	6 (10)	4,28	9 (11)	8,05	2 (9)	18,14
20 (9)	4.38	2 (8)	5,02	17 (21)	4,29	2 (8)	8,26	8 (24)	18,21
8 (24)	4.39	14 (3)	5,02	7 (14)	4,29	19 (7)	8,30	19 (7)	18,26
5 (3)	4.41	12 (9)	5,09	4 (7)	4,30	21 (13)	8,30	20 (9)	18,33
6 (10	4.42	3 (12)	5,09	20 (9)	4,30	20 (9)	8,32	12 (9)	18,35
4 (7)	4.42	20 (9)	5,11	13 (3)	4,30	16 (18)	8,33	14 (3)	18,37
2 (9)	4.43	9 (10)	5,11	5 (3)	4,30	11 (34)	8,39	16 (18)	18,41
23 (5)	4.50	7 (14)	5,18	22 (5)	4,33	23 (5)	8,47	22 (5)	18,56
18 (15)	4.51	19 (7)	5,19	21 (13)	4,35	3 (12)	8,62	11 (34)	18,58
16 (18)	4.52	6 (10)	5,21	8 (24)	4,38	10 (4)	8,63	10 (4)	18,72
10 (4)	4.53	4 (7)	5,21	23 (5)	4,38	22 (5)	8,68	23 (5)	18,80
19 (7)	4.57	10 (4)	5,28	19 (7)	4,41	14 (3)	8,71	5 (3)	19,05
13 (3)	4.58	13 (3)	5,28	16 (13)	4,49	5 (3)	8,72	3 (12)	19,11
22 (5)	4.63	8 (24)	5,33	10 (4)	4,49	4 (7)	8,77	4 (7)	19,25
15 (7)	4.76	5 (3)	5,51	11 (34)	4,66	13 (3)	9,15	13 (3)	19,84
11 (34)	4.84	15 (7)	5,70	15 (7)	4,78	15 (7)	9,26	15 (7)	20,35

Table 4.5 cont'd

PPL	
OTU (n)	Mean
21 (12)	7,97
1 (44)	9,17
7 (13)	9,27
6 (10)	9,38
17 (21)	9,62
9 (11)	9,74
14 (3)	9,75
19 (6)	9,76
18 (14)	9,78
8 (23)	9,79
2 (4)	9,89
11 (32)	10,03
20 (5)	10,07
12 (9)	10,18
16 (16)	10,24
10 (3)	10,25
2 (4)	10,39
3 (12)	10,47
4 (7)	10,48
23 (5)	10,48
5 (3)	10,74
13 (2)	11,09
15 (7)	11,84

Figure 4.6 : Results of mean - based geographic variation analyses of the 23 *M. coucha* ` OTU's by (a) principal components analysis (b) correlation based cluster analysis and (c) ATD based cluster analysis. OTU's are defined and shown in Table 4.1 and Figure 4.1.

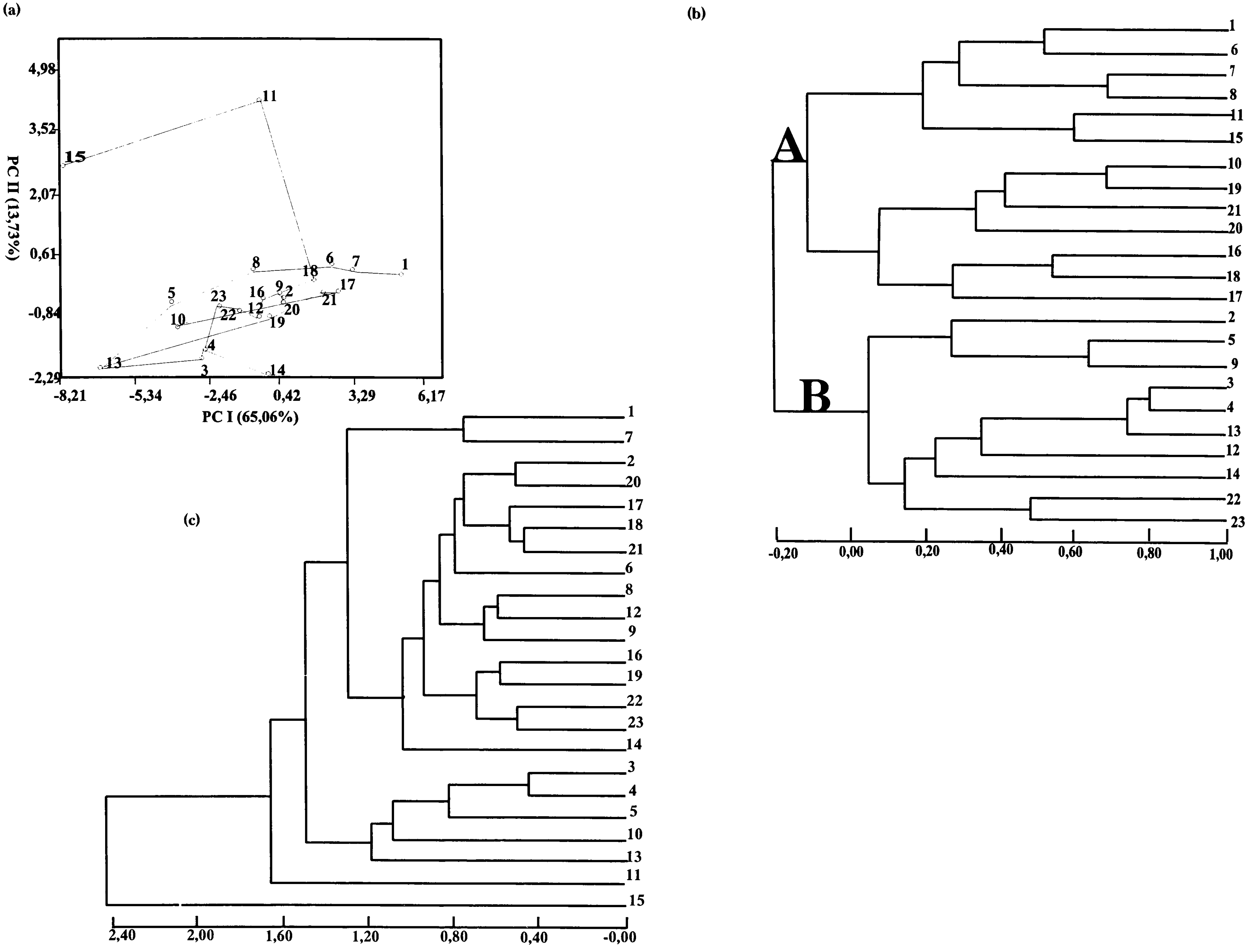

(a)
PC II (13,73%)
PC I (65,06%)
4,98
3,52
2,07
0,61
-0,84
-2,29
-8,21
-5,34
-2,46
0,42
3,29
6,17
11
15
8
18
6
7
1
5
23
16
9
2
17
21
10
22
12
20
19
4
13
3
14
(b)
A
B
1
6
7
8
11
15
10
19
21
20
16
18
17
2
5
9
3
4
13
12
14
22
23
-0,20
0,00
0,20
0,40
0,60
0,80
1,00
(c)
1
7
2
20
17
18
21
6
8
12
9
16
19
22
23
14
3
4
5
10
13
11
15
2,40
2,00
1,60
1,20
0,80
0,40
-0,00

Table 4.6 : Variable loadings of the first two axes from PCA results of infraspecific variation in *M. coucha* and *M. natalensis* OTU's from southern Africa. Analyses were performed on mean values. Values indicated in bold loaded relatively high on the particular axis. Percentage contribution of variables are indicated in parentheses.

	M. coucha		*M. natalensis*	
	PC I	PC II	PC I	PC II
GLS	**-0.29** (95,83)	-0.15 (0,97)	**-0,30** (90,93)	0,07 (5,53)
CBL	**-0.29** (97,00)	-0.13 (0,16)	**-0,30** (92,29)	0,03 (3,74)
DOB	**-0.25** (10,14)	0.18 (14,40)	-0,10 (68,61)	**-0,25** (7,90)
BrBr	-0.17 (16,94)	**-0.34** (53,16)	-0,13 (32,86)	**0,48** (27,54)
MAST	**-0.27** (68,09)	-0.09 (0,83)	**-0,25** (77,79)	0,06 (1,85)
IOB	-0.13 (14,30)	**-0.40** (67,06)	-0,12 (18,25)	**0,54** (36,99)
ROST	**-0.26** (83,25)	**-0.22** (10,36)	**-0,28** (75,11)	**0,21** (11,10)
APF	**-0.28** (80,14)	0.03 (2,23)	**-0,27** (85,29)	0,10 (0,26)
DIAS	**-0.28** (89,73)	-0.003(0,14)	**-0,29** (83,41)	-0,03 (0,002)
PAL	**-0.29** (93,57)	-0.002(0,45)	**-0,30** (90,37)	-0,04 (0,001)
GBP	-0.18 (26,74)	**0.46** (63,79)	-0,16 (35,38)	**-0,53** (48,46)
PPW	-0.16 (75,99)	0.39 (0,07)	**-0,27** (28,78)	0,02 (35,70)
MTR	-0.19 (11,13)	0.04 (0,99)	-0,10 (41,23)	0,07 (0,45)
MDT	-0.15 (48,14)	**0.47** (2,47)	**-0,21** (23,91)	-0,10 (51,44)
MDH	**-0.27** (70,63)	-0.08 (8,04)	**-0,26** (78,25)	-0,19 (1,61)
MDL	**-0.29** (94,93)	0.004 (1,77)	**-0,30 (95,58)**	-0,09 (0,004)
PPL	**-0.28** (89,66)	0.06 (4,12)	**-0,29** (87,91)	-0,13 (0,79)

Scrutiny of variable loadings (Table 4.6) indicated high and negative values for most measurements along PC I. This axis, therefore, reflected size variation. Principal components axis II was influenced mainly by six measurements (BrBr, IOB, ROST, GBP, PPW, MDT). Separation between the Zimbabwe OTU and those from South Africa and , Namibia along this axis, thus reflected mainly differences in cranial shape. These characters which tended to load relatively low on PC axis I, were also important in separating geographic groupings in the PCA of individual specimens (Section 4.3.2). Only width of the rostrum (ROST) did not load high in the PCA of individuals, whereas in this analysis it was significant in contributing to size variation.

Cluster analyses (UPGMA) phenograms based on correlation and distance coefficients are presented in Figure 4. 6 (b) and Figure 4. 6 (c) respectively. Examination of both phenograms, showed a lack of any apparent subclustering on a geographical basis. Although preliminary analyses and PCA results suggested that the OTU from Zimbabwe (OTU 11) differs in cranial shape from those in South Africa and Namibia OTU's, in the correlation phenogram this OTU grouped in subcluster A1 together with the Groot Marico (OTU 1), Bethal (OTU 6), Fontana (OTU 7), Pretoria (OTU 8) and Potchefstroom (OTU 15) OTU's. While this suggested that these OTU's were very similar, no geographic pattern was evident when these were plotted on a map.

M. natalensis.

Univariate analyses.

Descriptive statistics of the 14 *M. natalensis* OTU's are presented in Table 4. 7. Mean values of all measurements showed no explicit increase or decrease on a geographic basis. Most OTU’s showed relatively high standard deviation values for GLS, CBL, PAL

Table 4.7: Descriptive statistics and results of one -way ANOVA of the 17 measurements for 14 *M. natalensis* OTU's from southern Africa. X = arithmetic mean, SD = standard deviation, CV = coefficient of variation and n = sample size. [a] and [b] denote $p <$ 0,05 and $p <$ 0,01 respectively. OTU constituents are defined in Table 4.1.

OTU	STAT	GLS	CBL	DOB	BrBr	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
24	X	28,86	27,30	10,45	11,43	11,03	4,07	4,73	6,95	8,03	16,18	6,28	4,78	5,41	4,27	8,42	19,24	10,36
	SD	1,50	1,60	0,30	0,32	0,37	0,18	0,37	0,41	0,57	0,99	0,37	0,23	0,16	0,14	0,75	1,13	0,75
	CV	5,20	5,86	3,66	2,60	2,90	4,46	7,89	5,85	7,06	6,11	5,95	4,89	3,02	3,24	8,87	5,86	7,26
	n	19	19	18	22	20	27	27	27	27	27	27	27	27	27	26	27	18
25	X	28,22	26,88	10,61	11,50	11,07	4,17	4,62	6,84	7,89	15,78	6,06	4,71	5,24	4,29	8,04	18,35	10,35
	SD	1,83	2,01	0,31	0,36	0,42	0,19	0,41	0,56	0,81	1,31	0,39	0,26	0,23	0,19	0,73	1,65	0,98
	CV	6,48	7,47	2,91	3,11	3,76	4,49	8,91	8,18	10,26	8,29	6,50	5,53	4,33	4,46	9,13	9,00	9,45
	n	26	25	25	26	25	26	26	26	26	26	26	26	26	26	26	26	23
26	X	28,38	27,04	10,21	11,51	11,29	4,19	4,84	6,83	7,59	15,68	6,06	4,85	5,04	4,65	7,79	18,76	10,67
	SD	2,29	1,92	0,42	0,25	0,16	0,17	0,38	0,60	0,55	1,10	0,27	0,10	0,10	0,30	0,75	1,41	0,85
	CV	8,06	7,10	4,12	2,17	1,44	4,03	7,89	8,81	7,24	6,99	4,41	2,08	1,95	6,51	9,62	6,34	7,92
	n	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
27	X	27,75	26,04	10,54	11,66	10,63	4,20	4,54	6,50	7,40	15,29	5,99	4,62	5,14	4,23	7,82	17,56	9,71
	SD	1,30	1,44	0,33	0,30	2,40	0,17	0,31	0,51	0,67	1,08	0,24	0,20	0,23	0,16	0,51	0,87	0,69
	CV	4,70	5,54	3,17	2,55	22,57	3,65	6,89	7,85	9,08	7,08	3,93	4,81	4,44	3,87	6,51	4,95	7,13
	n	22	22	22	22	22	24	24	24	24	24	24	24	24	24	23	21	21
28	X	28,72	27,46	9,84	11,44	11,13	4,15	4,66	6,96	8,03	15,98	6,13	4,75	5,22	4,24	8,24	18,65	10,84
	SD	1,69	1,75	2,76	0,30	0,47	0,29	0,32	0,55	0,68	1,12	0,45	0,24	0,25	0,18	0,60	1,29	0,73
	CV	5,90	6,37	28,06	2,58	4,26	5,88	6,88	7,97	8,45	6,99	7,39	6,00	4,76	4,22	7,25	6,92	6,75
	n	15	15	15	15	15	15	15	15	15	15	15	15	15	15	14	15	14
29	X	27,98	26,54	10,67	11,71	11,17	4,73	4,64	6,57	7,67	15,17	6,09	4,22	5,24	4,20	8,44	18,16	10,31
	SD	2,07	2,09	0,45	0,37	0,36	0,17	0,38	0,55	0,63	1,25	0,41	0,29	0,34	0,17	0,69	1,23	0,94
	CV	7,39	7,87	4,20	3,13	3,20	3,99	8,19	8,36	8,14	8,27	6,79	6,07	6,41	4,10	8,17	6,76	9,11
	n	7	7	7	7	7	7	7	7	7	7	7	7	7	7	5	7	7
30	X	28,05	26,62	10,66	11,53	11,17	4,21	4,74	6,58	7,56	15,41	6,12	4,62	5,26	4,31	8,07	18,06	10,03
	SD	2,38	2,48	0,45	0,33	0,42	0,14	0,43	0,79	0,96	1,41	0,40	0,34	0,26	0,18	0,85	1,69	1,21
	CV	8,47	9,30	4,21	2,85	3,73	3,43	9,02	12,02	12,93	9,15	6,52	7,26	4,89	4,06	10,52	9,36	12,08
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table 4.7 cont'd

OTU	STATS	GLS	CBL	DOB	BRBR	MAST	IOB	ROST	APF	DIAS	PAL	GBP	PPW	MTR	MDT	MDH	MDL	PPL
31	X	28,70	27,48	10,87	11,41	11,27	3,99	4,62	6,65	8,03	16,36	7,44	4,81	5,45	4,64	8,70	19,35	11,40
	SD	1,50	1,49	0,26	0,46	0,48	0,23	0,30	0,53	0,58	1,35	0,35	0,37	0,32	0,30	0,81	1,28	0,64
	CV	8,47	9,30	4,21	2,85	3,73	3,43	9,02	12,02	12,93	9,15	6,52	7,26	4,89	4,06	10,52	9,36	12,08
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
32	X	26,23	24,73	10,24	11,46	10,81	4,02	4,25	6,15	6,98	14,45	5,77	4,67	5,07	4,15	7,34	17,18	9,16
	SD	1,41	1,35	0,36	0,26	0,34	0,14	0,25	0,39	0,55	0,85	0,29	0,16	0,22	0,15	0,51	0,94	0,67
	CV	5,37	5,46	3,54	2,31	3,15	3,59	5,82	6,35	7,82	5,90	5,02	3,51	4,41	3,48	6,90	5,47	7,27
	n	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
33	X	26,46	24,92	10,57	11,44	10,99	4,17	4,22	6,01	7,00	14,27	5,82	4,56	4,98	4,12	7,67	17,10	9,54
	SD	2,07	2,35	0,45	0,33	0,43	0,27	0,43	0,49	0,86	1,21	0,36	0,20	0,22	0,11	0,94	1,61	1,22
	CV	7,83	9,44	4,24	2,92	3,90	6,47	10,20	8,11	12,26	8,48	6,26	4,33	4,34	2,57	12,21	9,42	12,82
	n	7	7	7	7	7	8	7	7	7	7	7	7	7	7	7	7	7
34	X	29,35	27,94	10,58	11,41	11,14	4,17	4,77	6,95	8,30	16,04	6,19	4,79	5,25	4,24	8,74	19,34	11,24
	SD	1,71	1,64	0,31	0,22	0,08	0,07	0,11	0,46	0,61	0,85	0,39	0,35	0,20	0,23	0,55	1,11	0,85
	CV	5,83	5,88	2,97	1,90	0,69	1,60	2,31	6,59	7,30	5,31	6,27	7,28	3,85	5,34	6,29	5,76	7,53
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
35	X	26,54	25,26	10,16	11,35	10,76	3,98	4,24	6,29	7,19	14,14	5,61	4,54	5,04	4,09	7,78	17,18	9,44
	SD	1,76	1,73	0,32	0,37	0,38	0,15	0,46	0,67	0,59	1,62	0,29	0,16	0,12	0,21	0,70	1,15	0,80
	CV	6,62	6,84	3,18	3,30	3,56	3,64	10,78	10,72	8,26	11,46	5,14	3,62	2,45	5,04	9,04	6,71	8,48
	n	16	16	16	16	16	15	16	16	16	16	16	16	16	16	16	16	16
36	X	28,39	27,10	10,77	11,22	11,04	3,99	4,46	6,68	7,91	15,90	7,28	4,84	4,74	4,34	8,46	18,86	10,83
	SD	1,48	1,57	0,29	0,24	0,25	0.18	0,29	0,51	0,61	0,92	0,34	0,40	0,34	0,23	0,63	0,98	1,06
	CV	5,21	5,78	2,66	2,18	2,22	4,54	6,29	7,63	7,76	5,76	4,72	8,16	7,15	5,23	7,49	5,17	9,82
	n	47	47	48	48	48	48	48	48	48	48	48	48	48	48	48	48	47
37	X	25,63	24,30	10,35	10,85	10,62	3,95	4,12	6,18	6,79	14,00	6,30	4,36	5,20	4,07	7,63	16,70	9,22
	SD	1,52	1,50	0,31	0,33	0,31	0,18	0,25	0,41	0,42	1,09	0,36	0,19	0,29	0,16	0,66	1,10	0,71
	CV	5,94	6,18	3,00	3,01	2,96	4,51	6,17	6,68	6,23	7,76	5,66	4,24	5,56	3,89	8,62	6,61	7,65
	n	35	35	35	35	35	35	35-	35	35	35	35	35	35	35	35	35	35
F - values		9,55^{b}	9,15^{b}	2,29^{b}	11,27^{b}	1,31	3,12^{a}	8,34^{b}	6,84^{b}	10,93^{b}	10,53^{b}	50,73^{b}	6,48^{b}	13,43^{b}	9,51^{b}	7,26^{b}	11,35^{b}	12,25^{b}

Table 4.8: Results of the SNK multiple range comparison test of 16 measurements for 14 *M. natalensis* OTU's. Non significant subsets are indicated by vertical lines and n = sample size. OTU constituents are defined in Table 4.1.

GLS		CBL		DOB		BrBr		IOB	
OTU (n)	Means	OTU (n)	Means	OTU (n)	Means	OTU (n)	Means	OTU (n)	Means
37 (35)	25,63	37 (35)	24,30	28 (15)	9,83	37 (35)	10,85	37 (35)	4,12
32 (28)	26,23	32 (28)	24,73	35 (16)	10,16	36 (48)	11,22	33 (7)	4,22
33 (7)	26,46	33 (7)	24,92	26 (4)	10,21	35 (16)	11,35	35 (16)	4,24
35 (16)	26,54	35 (16)	25,26	32 (28)	10,24	31 (9)	11,41	32 (28)	4,25
27 (22)	27,75	27 (22)	26,04	37 (35)	10,35	34 (3)	11,41	27 (24)	4,54
29 (7)	27,98	29 (7)	26,54	24 (18)	10,45	24 (22)	11,43	36 (48)	4,56
30 (10)	28,05	30 (10)	26,62	27 (22)	10,54	33 (7)	11,44	25 (26)	4,62
25 (26)	28,22	25 (25)	26,88	33 (7)	10,57	28 (15)	11,44	31 (9)	4,62
26 (4)	28,38	26 (4)	27,04	34 (3)	10,58	32 (28)	11,46	29 (7)	4,64
36 (47)	28,39	36 (47)	27,10	25 (25)	10,61	25 (26)	11,50	28 (15)	4,66
31 (9)	28,70	24 (19)	27,30	30 (10)	10,66	26 (4)	11,51	24 (27)	4,73
28 (15)	28,72	28 (15)	27,46	29 (7)	10,67	30 (10)	11,53	30 (10)	4,74
24 (19)	28,86	31 (9)	27,48	36 (48)	10,77	27 (22)	11,66	34 (3)	4,77
34 (3)	29,35	34 (3)	27,94	31 (9)	10,87	29 (7)	11,71	26 (4)	4,84

Table 4.8 cont'd

DIAS		APF		PAL		GBP		PPW	
OTU (n)	Means	OTU (n)	Means	OTU (n)	Means	OTU (n)	Mean	OTU (n)	Mean
33 (7)	6,01	37 (35)	6,79	37 (35)	14,00	35 (16)	5,61	37 (35)	4,36
32 (28)	6,15	32 (28)	6,98	35 (16)	14,14	32 (28)	5,77	35 (16)	4,54
37 (35)	6,18	33 (7)	7,00	33 (7)	14,27	33 (7)	5,82	33 (7)	4,56
35 (16)	6,29	35 (16)	7,19	32 (28)	14,45	27 (24)	5,99	30 (10)	4,61
27 (24)	6,50	27 (24)	7,39	29 (7)	15,17	26 (4)	6,05	27 (24)	4,62
29 (7)	6,57	30 (10)	7,45	27 (24)	15,29	25 (26)	6,06	32 (28)	4,67
30 (10)	6,58	26 (4)	7,59	30 (10)	15,40	29 (7)	6,09	25 (26)	4,71
31 (9)	6,65	29 (7)	7,67	26 (4)	15,68	30 (10)	6,12	29 (7)	4,73
36 (48)	6,68	25 (26)	7,89	25 (26)	15,78	28 (15)	6,13	28 (15)	4,75
26 (4)	6,83	36 (48)	7,91	36 (48)	15,90	34 (3)	6,19	24 (27)	4,78
25 (26)	6,83	24 (27)	8,03	28 (15)	15,98	24 (27)	6,28	34 (3)	4,79
34 (3)	6,95	31 (9)	8,03	34 (3)	16,04	37 (35)	6,30	31 (9)	4,81
24 (27)	6,95	28 (15)	8,03	24 (27)	16,18	36 (48)	7,28	36 (48)	4,84
28 (15)	6,96	34 (3)	8,30	31 (9)	16,36	31 (9)	7,44	26 (4)	4,85

Table 4.8 cont'd.

IOB		MTR		MDT
OTU (n)	Means	OTU (n)	Means	OTU (n)
37 (35)	3,95	36 (48)	4,74	37 (35)
35 (15)	3,98	33 (7)	4,98	35 (16)
31 (9)	3,99	26 (4)	5,04	33 (7)
36 (48)	3,99	35 (16)	5,04	32 (28)
32 (28)	4,02	32 (28)	5,07	29 (7)
24 (29)	4,07	27 (24)	5,14	27 (24)
28 (15)	4,15	37 (35)	5,20	28 (15)
25 (26)	4,17	28 (15)	5,22	34 (3)
33 (8)	4,17	25 (26)	5,24	24 (27)
34 (3)	4,17	29 (7)	5,24	25 (26)
26 (4)	4,19	34 (3)	5,25	30 (10)
27 (24)	4,20	30 (10)	5,26	36 (48)
30 (10)	4,21	24 (27)	5,41	31 (9)
29 (7)	4,22	31 (9)	5,45	26 (4)

	MDH			MDL		
Means	OTU	(n)	Means	OTU	(n)	Means
4,07	32	(28)	7,34	37	(35)	16,70
4,09	37	(35)	7,63	33	(7)	17,10
4,12	33	(7)	7,67	35	(16)	17,18
4,15	35	(12)	7,78	32	(28)	17,18
4,20	26	(4)	7,79	27	(21)	17,56
4,23	27	(23)	7,81	30	(10)	18,06
4,24	25	(22)	8,03	29	(7)	18,16
4,24	30	(10)	8,07	25	(26)	18,35
4,27	28	(14)	8,24	28	(15)	18,65
4,29	24	(26)	8,42	26	(4)	18,76
4,31	29	(5)	8,44	36	(48)	18,86
4,33	36	(48)	8,46	24	(26)	19,24
4,64	31	(9)	8,70	34	(3)	19,34
4,64	34	(3)	8,74	31	(9)	19,35

Table 4.8 cont'd

PPL OTU (n)	Mean
32 (28)	9,16
37 (35)	9,22
35 (16)	9,44
33 (7)	9,54
27 (21)	9,71
30 (10)	10,03
29 (7)	10,31
25 (23)	10,34
24 (18)	10,36
26 (4)	10,67
36 (47)	10,83
28 (14)	10,84
34 (3)	11,24
31 (9)	11,40

and MDL. Coefficient of variation values were generally less than 10 % for most variables in all OTU's, indicating that variables were reliable for assessing geographic variation.

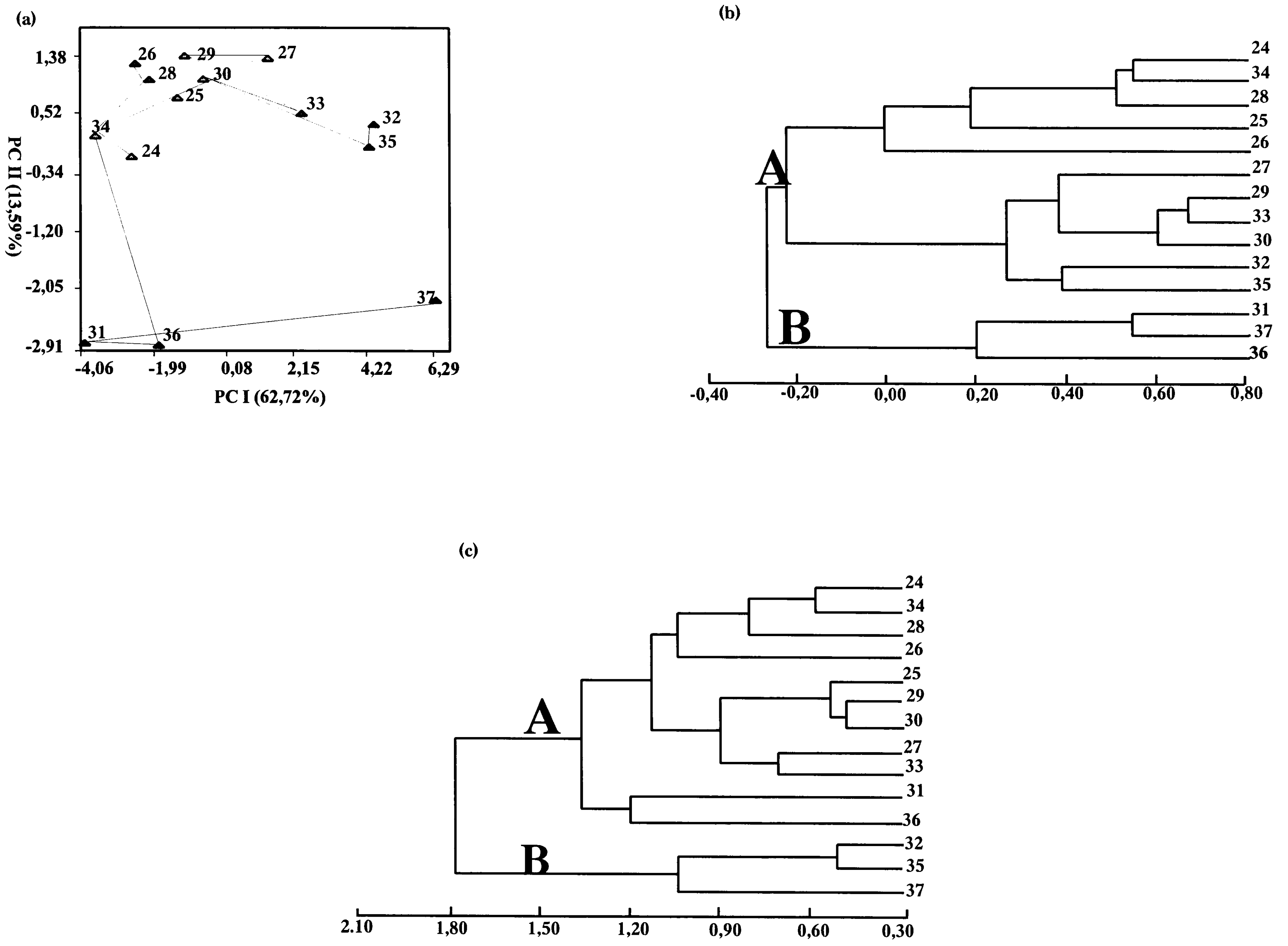
All measurements, except MAST, showed significant ($p < 0{,}01$) F - values when the 14 OTU's were subjected to Model I one - way ANOVA (Table 4. 7). SNK multiple range tests, however, resulted in overlapping nonsignificant subsets for most measurements (Table 4.8). Greatest breadth of the braincase was the only variable which indicated the Sinamotella OTU to be significantly smaller than the rest of the OTU's.

Multivariate analyses.

Pairwise comparison of PC axis I and PC axis II separated the South Africa and Namibia OTU's from those from Zimbabwe, but without any clear differentiation of any groups along PC I (Figure 4.7 a). Within the South Africa and Namibia geographic grouping the KwaZulu \ Natal Province OTU's (OTU's 28, 26, 29, 30, 25, 27) tended to cluster on the left of the OTU's from Mpumalanga and Northern Province (OTU's 35, 32, 33). Within the Zimbabwe geographic group, the Sinamotella OTU (OTU 37) clustered separately from the Norton OTU (OTU 36) along PC axis I, as in the preliminary analyses based on individual specimens (Section 4.3.2). A correlation based minimum spanning tree connected South African\ Namibian OTU's , with the exception of the Badplaas OTU (OTU 31), together. The Badplaas OTU instead connected to the Norton OTU (OTU 36) and further to the Sinamotella OTU (OTU 37). Similar to specimens from Potchefstroom, Badplaas specimens tend to group with those from Zimbabwe. Since there is no geographic basis for this , it is also possible that the identity of these specimens may have been confused during cataloguing

Loadings of most variables on PC I were relatively high and negative, indicating that this unipolar vector reflected size differences, whereas PC II was a bipolar shape vector

Figure 4.7 : Results of mean - based geographic variation analyses of 14 *M. natalensis* OTU's by (a) principal components analysis (b) correlation based cluster analysis (c) ATD based cluster analysis. OTU's are defined in Table 4.1 and Figure 4.1.


(a)
1,38
0,52
-0,34
-1,20
-2,05
-2,91
PC II (13,59%)
-4,06
-1,99
0,08
2,15
4,22
6,29
PC I (62,72%)
26
29
27
28
30
25
33
32
34
35
24
37
31
36
(b)
24
34
28
25
26
27
29
33
30
32
35
31
37
36
A
B
-0,40
-0,20
0,00
0,20
0,40
0,60
0,80
(c)
24
34
28
26
25
29
30
27
33
31
36
32
35
37
A
B
2.10
1,80
1,50
1,20
0,90
0,60
0,30

(Table 4.6). Five variables (DOB, BrBr, IOB, ROST, GBP) dominated shape variation along PC II. Three of these variables (BrBr, IOB, and GBP) were significant also in analyses based on individual specimens. Mean - value based analysis, however, obscured the importance of the greatest length of the maxillary toothrow (MTR) which loaded high in the individual based analyses, and DOB and ROST instead influenced geographic variation.

Correlation and ATD - based phenograms are presented in Figure 4.7 (b) and Figure 4.7 (c) respectively. Separation along the ATD based phenogram indicated no clear separation on a geographic basis, but two geographically - discrete clusters were evident in the correlation phenogram. Cluster A contained OTU's from South Africa and the only OTU from Namibia, whereas two OTU's from Zimbabwe (OTU 36, 37) grouped in cluster B. These analyses, therefore, supported PCA results in indicating the existence of pronounced and significant differences in cranial shape between OTU's from South Africa and Namibia and those from Zimbabwe.

The extent of geographic variation in both species, as indicated by the current results, indicated that the Zimbabwe OTU's should be treated separately from South Africa and Namibia OTU's. However, in subsequent analyses, the limited number of OTU's in the Zimbabwe geographic grouping restricted independent interspecific variation analyses by PCA or cluster analysis, as these were based on mean values. Consequently, in interspecific variation analyses, OTU's were pooled when analyses were done by PCA or cluster analyses. The two geographic grouping were only treated separately when MDA were computed, as these analyses are based on individual specimens.

4.3.4. Interspecific variation results

Principal components analyses results

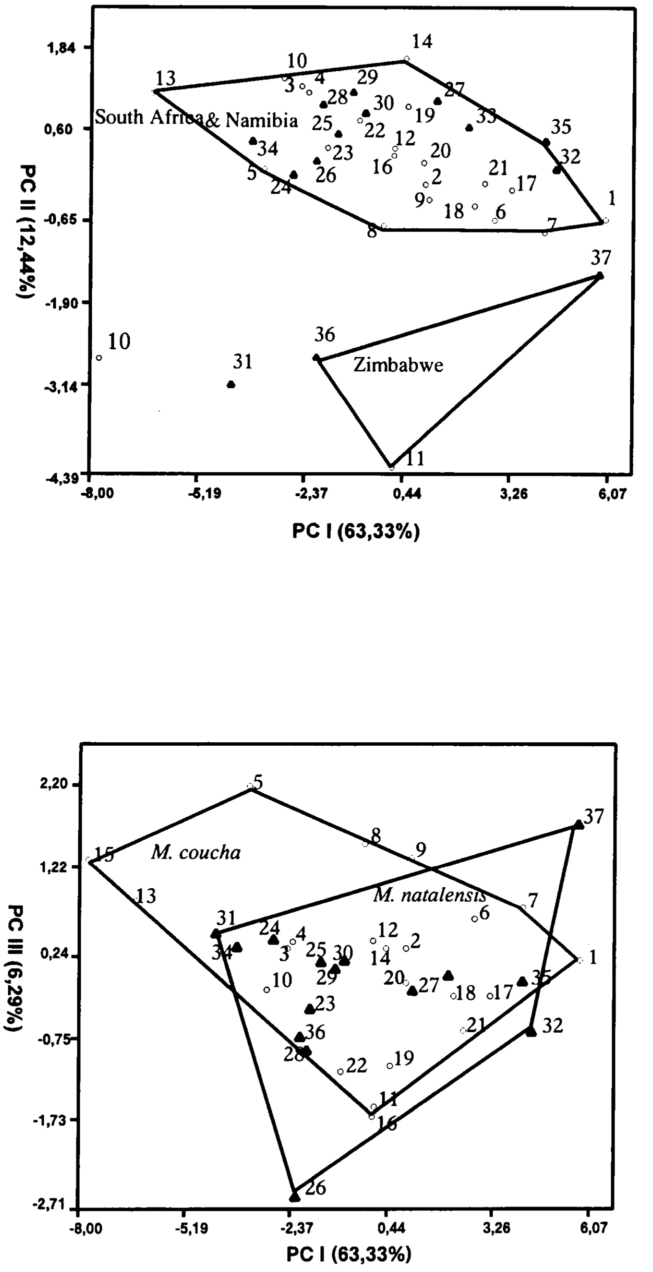
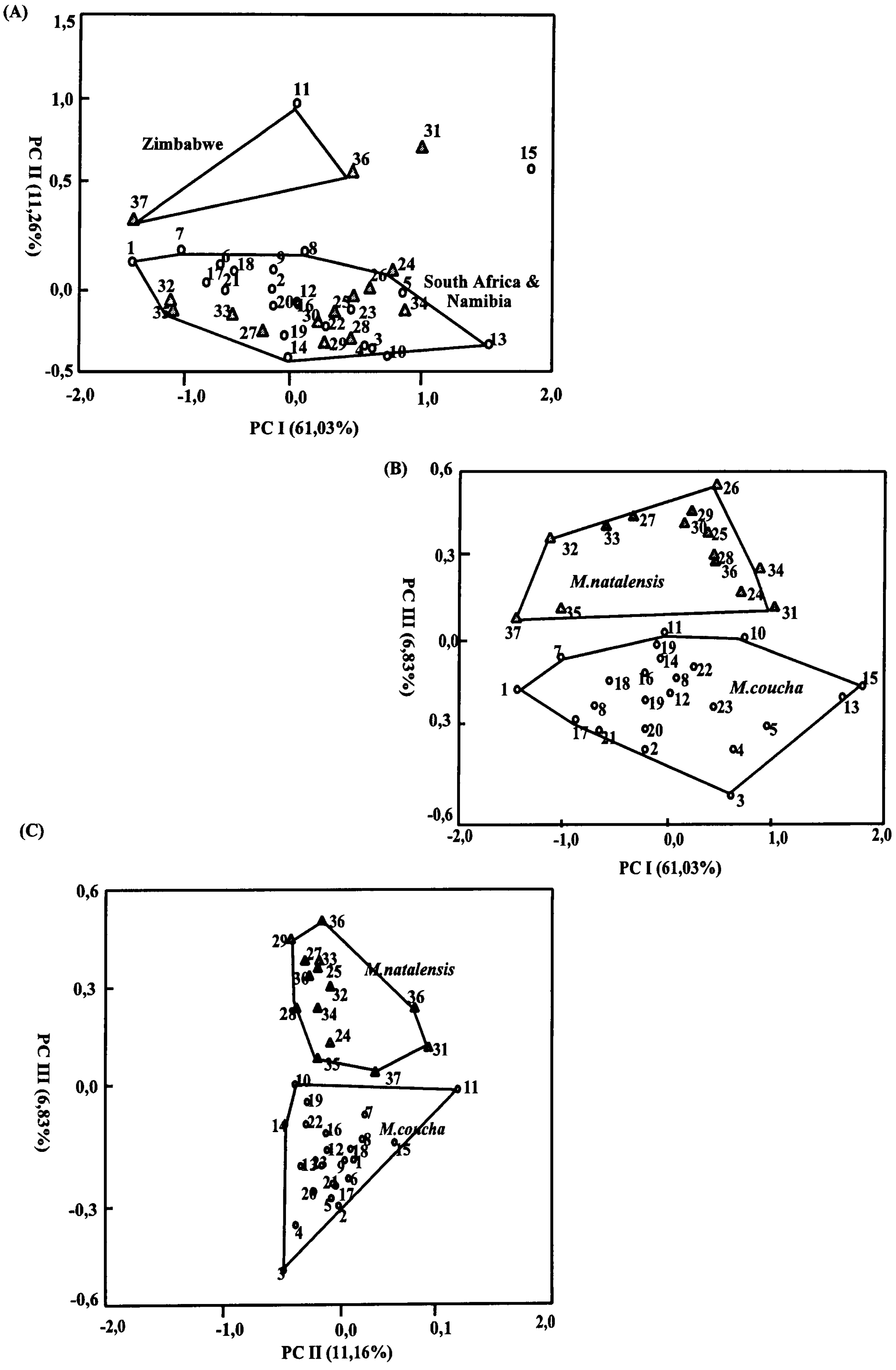


Figure 4.8 : Pairwise comparison of the first three components from a multi - group PCA of the 37 OTU's of *M. coucha* and *M. natalensis* from southern Africa. OTU's constituents are defined in Table 4.1. *M. coucha* is indicated by circles whereas *M. natalensis* is indicated by triangles.

Figure 4.9 :A composite figure showing pairwise comparisons of the first three components from a single - group principal components analysis of the 37 OTU's of *M. coucha* and *M. natalensis* from southern Africa. OTU constituents are defined in Table 4.1 and Figure 4.1. Circles denote *M. coucha* OTU's whilst triangles denote *M. natalensis* OTU's.


(A)
PC II (11,26%)
PC I (61,03%)
1,5
1,0
0,5
0,0
-0,5
-2,0
-1,0
0,0
1,0
2,0
Zimbabwe
South Africa & Namibia
(B)
PC III (6,83%)
PC I (61,03%)
0,6
0,3
0,0
0,3
-0,6
-2,0
-1,0
0,0
1,0
2,0
M.natalensis
M.coucha
(C)
PC III (6,83%)
PC II (11,16%)
0,6
0,3
0,0
-0,3
-0,6
-2,0
-1,0
0,0
0,1
2,0
M.natalensis
M.coucha

Results of MPCA and single group PCA of interspecific variation are shown in Figure 4.8 and Figure 4.9, respectively. Pairwise comparison of PC axes I and II were comparable for both techniques (Figure 4. 8 a, Figure 4.9 a), and showed no clear separation according species along PC axis I. In both analyses, OTU's representing the two species overlapped broadly along PC II, but those from Zimbabwe (OTU's 11, 36, 37) plotted apart from South African and Namibian OTU's. Principal component II which accounted for about 11 -12% of the total variance (Figures 4.8 a and 4.9 a), therefore, reflected mainly geographic variation. In a scatterplot comparing PC I and PC III from the single - group analysis, complete morphometric separation of *M.coucha* (OTU 1 -23) and *M. natalensis* (OTU's 24 - 37) was evident. Similarly, pairwise comparison of PC II and III resulted in complete segregation of OTU's representing the two species, with OTU's from Zimbabwe plotting towards the right of others along PC II as a result of pronounced geographic variation. However no such pattern of separation was evident in MPCA analyses, the two species instead overlapped broadly along PC III. Single - group PCA thus clearly differentiated the two species more effectively than MPCA.

The first three principal components from single - group PCA accounted for 79,12% of the total sample variance. The first axis was largely influenced by size, as indicated by high positive loadings for most variables (Table 4.9). Since the two species overlapped broadly along this axis, size differences between them were negligible. Eigenvector coefficients for principal component axes II and III, however, were bipolar and highlighted shape differences (Table 4.9). These two axes thus indicated that the two species and geographic groupings within each species differ mainly in cranial shape. This finding is consistent also with the results outlined in Section 4.3.2 which indicated species separation

Table 4.9 : Factor matrix of the first three axes from a 17 variable principal components analysis based on mean values for each of the 37 OTU's throughout the geographic ranges of *M. coucha* and *M.natalensis* in southern Africa. Percent contribution of each variable is indicated in parentheses.

	PRINCIPAL COMPONENTS		
Measurement	Axis I	Axis II	Axis III
GLS	**0,96** (92,79)	-0,18 (3,17)	-0,08 (0,68)
CBL	**0,96** (92,83)	-0,14 (1,89)	-0,14 (1,92)
DOB	0,64 (40,31)	0,33 (11,10)	0,10 (0,97)
BRBR	0,52 (26,70)	**-0,64** (40,67)	-0,08 (0,67)
MAST	**0,86** (73,41)	-0,15 (2,11)	0,03 (0,10)
IOB	0,33 (10,90)	**-0,56** (30,95)	**0,67** (44,98)
ROST	**0,88** (77,98)	-0,31 (9,47)	0,03 (0,11)
APF	**0,91** (82,14)	-0,04 (0,14)	0,06 (0,36)
DIAS	**0,92 (85,49)**	0,03 (0,07)	-0,02 (0,04)
PAL	**0,95** (89,62)	0,05 (0,21)	-0,17 (2,96)
GBP	0,55 (30,36)	**0,75** (56,61)	0,14 (1,87)
PPW	0,50 (25,49)	0,26 (6,74)	**0,70** (49,42)
MTR	0,54 (29,30)	-0,004(0,002)	0,02 (0,03)
MDT	0,55 (30,45)	**0,49** (24,23)	-0,03 (0,07)
MDH	**0,84** (70,33)	-0,03 (0,11)	**-0,30** (8,88)
MDL	**0,96** (92,47)	0,05 (0,20)	-0,17 (2,86)
PPL	**0,93** (87,02)	0,15 (2,13)	0,03 (0,11)
% trace	61,03%	11,16%	6,83%

regardless of size differences. The Dippenaar *et al.* (1993) study also indicated that craniometric differences between the two species were shape - related. In the current study, however, geographic variation is more pronounced than species separation, as evidenced by greater percentage of sample variance explained by PC II (Figures 4. 8 a, 4.9 a and Table 4.9).

Interorbital breadth (IOB) loaded high on both PC axes II and III, indicating that this character is variable both within and among species. This variable, which was also significant in interspecific discrimination of the Dippenaar *et al.* (1993) study is generally narrower in *M. coucha* than in *M. natalensis*. Within each species, IOB is narrower in the Zimbabwe (overall mean : *M. coucha* = 3.96, *M. natalensis* = 4.15) OTU's than in the South Africa and Namibia OTU's (overall mean : *M. coucha* = 4.13, *M. natalensis* = 4.32) . Greatest breadth of the palate (GBP), greatest length of the braincase (BRBR) , and greatest length of the mandibular toothrow at the level of the alveoli (MDT) also influenced separation of the species in the study by Dippenaar *et al.* (1993) study. However, these characters varied within geographic groupings and hardly influenced species separation during the present study. Greatest breadth of the palate (GBP) was wider, greatest length of the braincase shorter (BrBr), while the mandibular toothrow (MDT) was longer in the Zimbabwe OTU's than in South Africa and Namibia OTU's.

Measurements outlining palatal region of the skull proved to be significant not only in geographic variation, but also in interspecific variation. Post - palatal width (PPW) loaded the highest on PC III, and thus markedly influenced species separation. This character is generally wider in *M. natalensis* than in *M. coucha,* regardless of locality southern African region. As in Dippenaar *et al.* (1993), greatest height of the mandible (MDH) also loaded

Table 4.10 : Differential cranial ratios (percentage ± standard deviation) of *M.coucha* and *M. natalensis*, calculated from variables that loaded high positive and high negative on PCA (Table 4. 9).

Cranial ratio	*M. coucha*	*M. natalensis*
IOB : MDH	49,13 ± 3,60	53,04 ±4,07
Range (Min - Max)	41,55 - 60,78	44,43 - 63,13
Sample size	187	122
PPW : MDH	54,68 ± 4,36	60,08 ± 4,99
Range (Min - Max)	44,00 - 68,71	50,28 - 71,53
Sample size	187	122

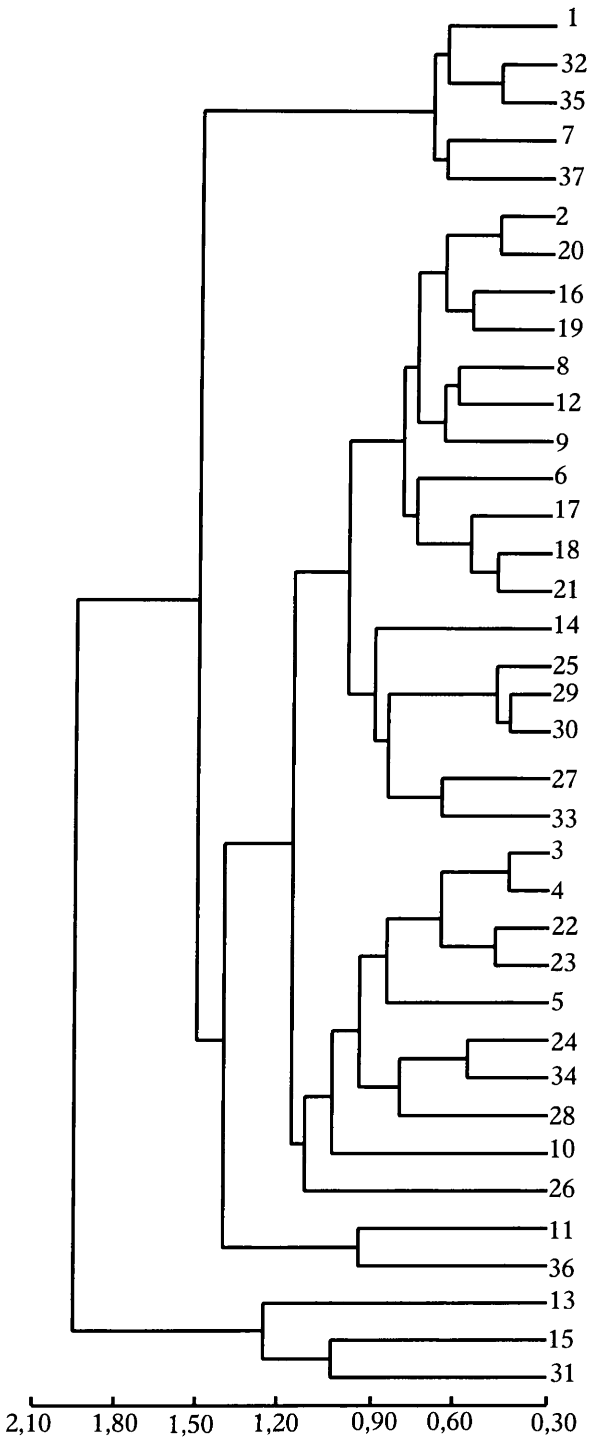


Figure 4.10: ATD based phenogram from cluster analysis (UPGMA) of mean values of *M. coucha* and *M. natalensis* from various localities in southern Africa. OTU's 1 - 23 = *M. coucha* and OTU's 24 - 37 = *M. natalensis*. Each OTU constituent is defined in Table 4.1. Cophenetic correlation coefficient = 0,73.

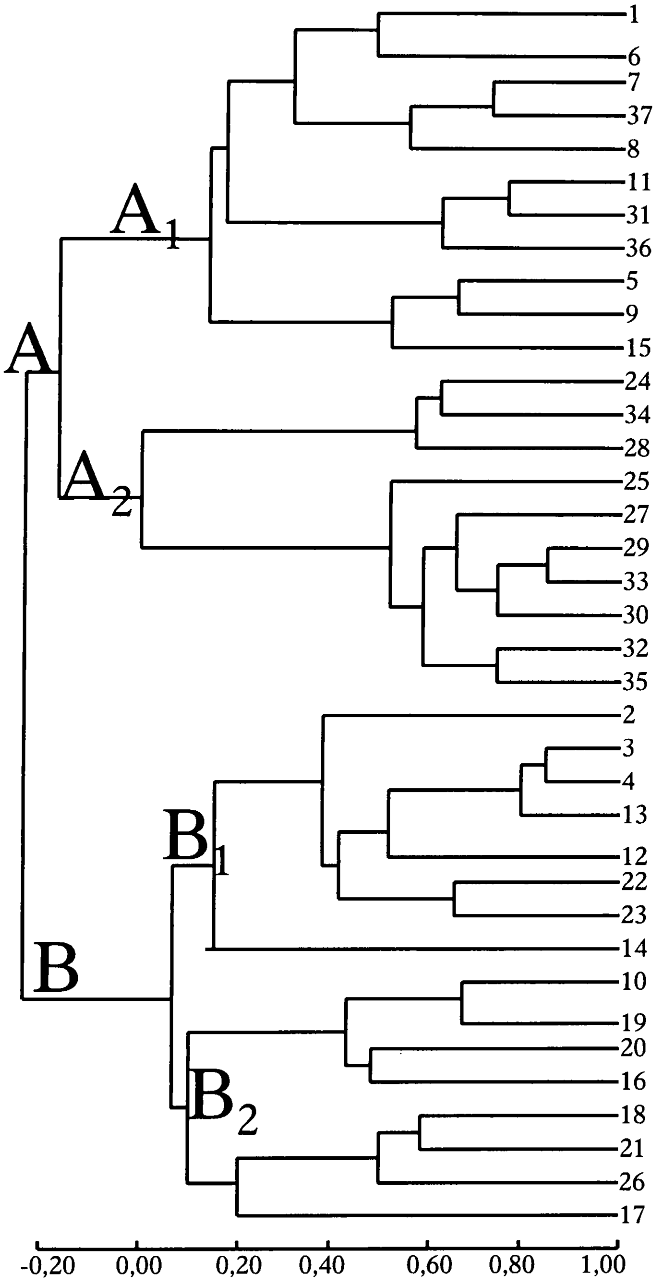


Figure 4.11: Correlation phenogram from cluster analysis (UPGMA) of mean values of *M. coucha* and *M. natalensis* from various localities in southern Africa. OTU's 1 - 23 = *M. coucha* and OTU's 24 - 37 = *M. natalensis*. Each OTU constituent is defined in Table 4.1. Cophenetic correlation coefficient = 0,67.

relatively high and influenced species separation heavily.

Differential cranial ratios were computed by contrasting variables that loaded high negatively and positively on PC III (IOB, PPW, MDH), to assist with the diagnostic procedure (Table 4.10). These ratios, however, overlapped considerably, and thus cannot be used to diagnose the two species reliably. This indicates that cranial differences between the species, although sufficiently pronounced to allow multivariate separation, are too complex and subtle for diagnosis at bivariate level.

Cluster analyses results

No clear separation of species nor of any geographic groupings was evident in an ATD phenogram (Figure 4.10).This concurred with PCA results which showed that size does vary markedly on a geographic or interspecific basis. In a correlation phenogram reflecting mainly shape differences, OTU's representing the two species tended to group apart (Figure 4.11). Two major clusters were evident : cluster A (with subclusters A_1 and A_2)contained mainly *M. natalensis* (62 % OTU's) and cluster B (with subclusters B_1 and B_2) comprised mainly of *M. coucha* (93,75% OTU's). The influence of geographic variation in this analysis was indicated by the overlap of the species in subcluster A_1 of the correlation phenogram. The three OTU's from Zimbabwe (OTU 11, 36, and 37) along with other *M. coucha* and *M. natalensis* OTU's from various localities in South Africa, grouped together in this subcluster.

4.3.5. Development of the interspecific discrimination technique.

A two-group discriminant functions analysis of all cytogenetically known *M. coucha* and *M. natalensis* from southern Africa resulted in 91% correct classification of individuals to their *a priori* groups. Misclassifications were apparently the result of

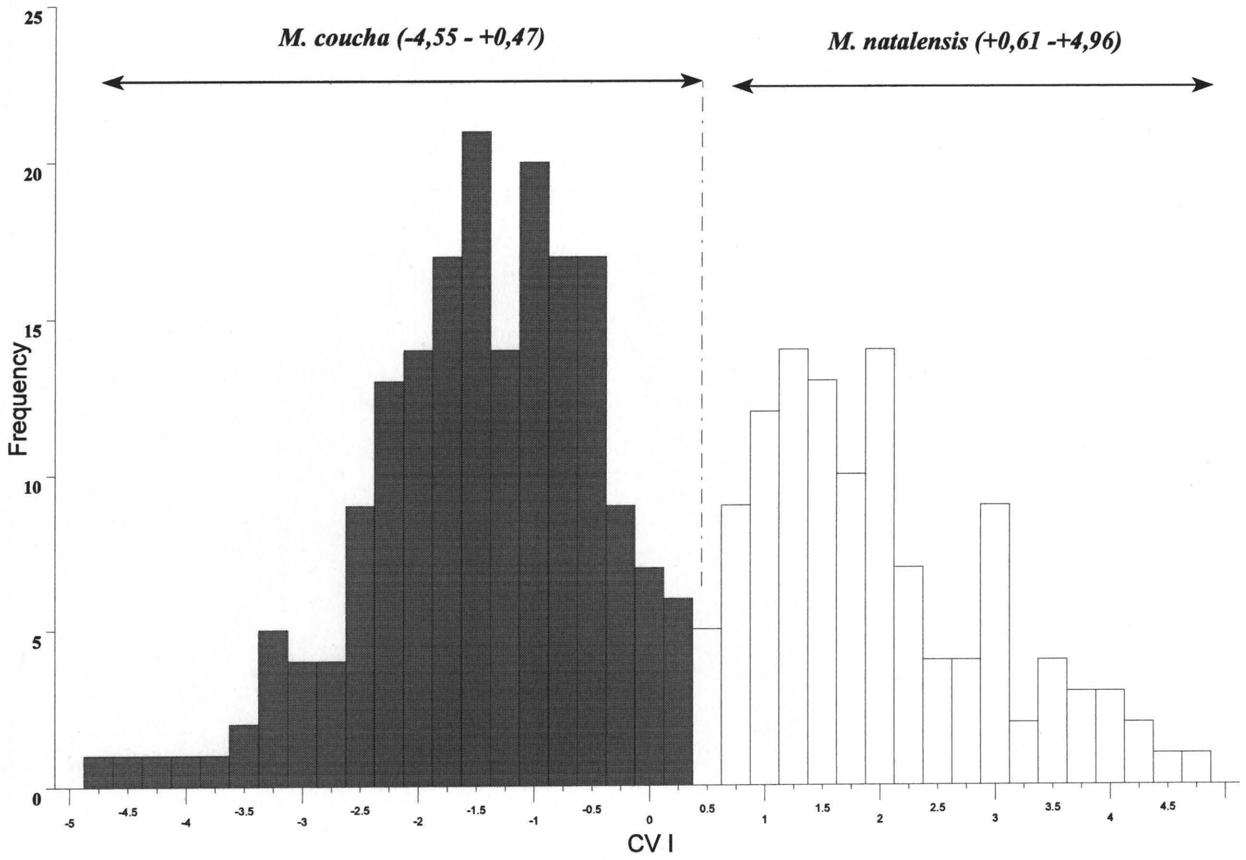


Figure 4.12 : Frequency histogram showing results of a two-group discriminant functions analysis (MDA) of known *M. coucha* and *M. natalensis* from South Africa. Discriminant score ranges are: *M. coucha* = -4,55 - +0,47 and *M. natalensis* = +0,61 - +4,96.

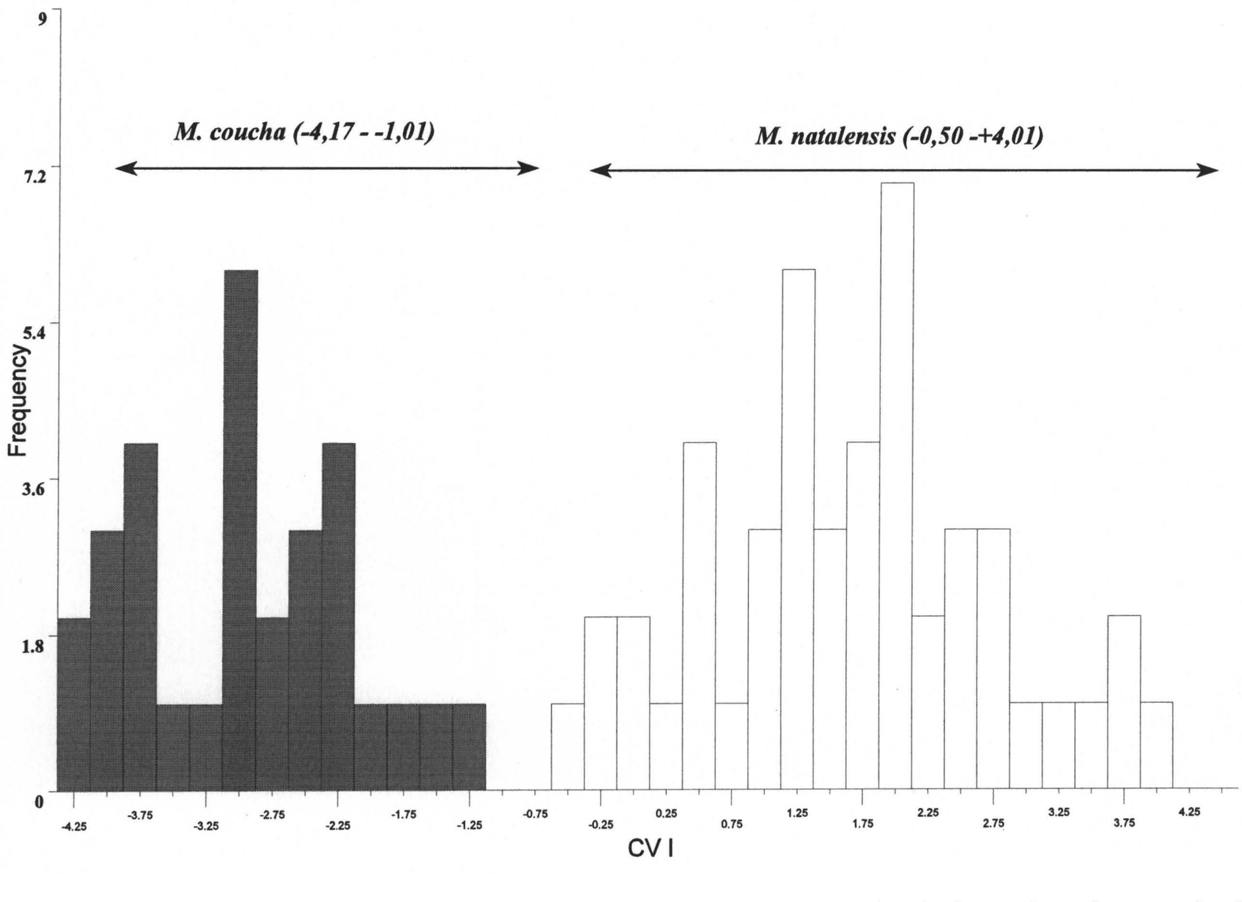


Figure 4.13 : Frequency histogram showing results of a two-group discriminant functions analysis (MDA) of known *M. coucha* and *M. natalensis* from Norton, Zimbabwe. Discriminant score ranges are: *M. coucha* = -4,17 - -1,01 and *M. natalensis* = -0,50 - +4,01.

geographic variation confounding interspecific discrimination. Of the 78 specimens from Zimbabwe, 18% of these were misclassified to the other species, compared to six percent and nine percent misclassifications of 301 South African OTU's and 17 Namibian OTU's, respectively. Although the two species overlapped when OTU's from throughout southern Africa were pooled, multivariate analysis of variance (MANOVA) results nevertheless indicated that significant differences existed between the species - group centroids ($F = 31_{(17, 378)}$, $p < 0.01$)

The existence of significant geographic variation implies that any reliable discrimination procedure to be developed, needs to focus on discrete geographic regions. Given that the Zimbabwe OTU's consistently grouped apart from Namibia and South Africa OTU's , separate discrimination procedures were computed for : (i) Norton, Zimbabwe OTU's, a single locality which had adequate sample sizes of both species (Figure 4.13) and (ii) South African localities, which included all available known specimens of both species (Figure 4.12). By treating samples geographically, the two species separated clearly, resulting in 100% correct classification of all individuals in both of the regional discrimination

Classification procedure.

The results of the regional MDA's are summarized in Tables 4.11 and 4.12, and were used to classify all measured unknown *M. natalensis s.l.* from greater South Africa (this included Lesotho and Swaziland specimens) and Zimbabwe respectively.

To classify *M. natalensis s.l.* specimens of unknown species affinity one should take the 17 cranial measurements listed in Figure 2.1, and then :

1. Subtract the overall mean of each measurement from the corresponding measurement of the unknown (X -Y) .

Table 4.11: Mean values and the canonical variate scores from a regional two-group discriminant functions analysis (MDA) of *M.coucha* and *M.natalensis* from South Africa, required to differentiate the two species. Calculating a discriminant score for an unknown *Mastomys* specimen from the greater South Africa region can be done following the technique explained in Section 4.3.5.

Variable	Measurement of unknown	Overall mean		Standardized coefficient	Score
	X	Y	(X - Y)	CV	(X - Y) x CV
GLS		27.47		1.06	
CBL		26.17		-1.67	
DOB		10.39		0.09	
BRBR		11.40		0.24	
MAST		10.95		- 0.15	
IOB		4.02		4.24	
ROST		4.46		0.65	
APF		6.48		1.07	
DIAS		7.43		0.80	
PAL		15.25		0.07	
GBP		5.98		-1.10	
PPW		4.51		3.71	
MTR		5.10		0.68	
MDT		4.28		-2.84	
MDH		7.99		-1.03	
MDL		17.98		-0.84	
PPL		9.87		1.36	
Discriminant Score =					

Table 4.12 : Mean values and canonical variate score from a two group discriminant functions analysis (MDA) of *M. coucha* and *M. natalensis* from Norton, Zimbabwe, required to differentiate the two species . Calculating a discriminant score for an unknown *Mastomys* specimen from Zimbabwe can be done following the technique explained in Section 4.3.5.

Variable	Measurement of the unknown	Overall mean		Standardized coefficient	Score
	X	Y	(X-Y)	CV	(X-Y) x CV
GLS		27.81		2.36	
CBL		26.68		-0.09	
DOB		10.71		0.69	
BRBR		11.18		-0.28	
MAST		10.99		0.34	
IOB		3.93		-0.13	
ROST		4.52		-1.56	
APF		6.63		-1.20	
DIAS		7.74		0.48	
PAL		15.78		-2.91	
GBP		7.25		0.42	
PPW		4.84		0.32	
MTR		4.80		-0.32	
MDT		4.48		-3.65	
MDH		8.45		-0.51	
MDL		18.77		0.34	
PPL		10.55		0.19	

Discriminant Score =

2. Multiply the difference by the corresponding canonical vector (X - Y x CV)

3. Summate the derived values for all the 17 measurements and determine the group to which the unknown is closely related to.

Mastomys coucha scores of specimens derived from greater South Africa range from - 4,55 to + 0,47, whereas those of *M. natalensis* range from + 0,61 to + 4,96 (Figure 4.12). To classify unknown specimens from Zimbabwe localities to *M. coucha*, scores should fall from - 4,17 to -1,01 range, whereas *M. natalensis* scores should fall within the - 0,50 to + 4,01 range (Figure 4.13). MANOVA of both regional MDA's indicated that the differences between species group centroids were highly significant (South Africa MDA: $F = 46{,}42_{(17, 283)}$, $p < 0{,}00$; Norton MDA: $F = 18{,}20_{(17, 60)}$, $p < 0{,}00$) range.

The classification procedure detailed in this section, is further explained in Taylor *et al.* (1995), Dippenaar *et al.* (1993), Chapman *et al.* (1992) and Dippenaar & Rautenbach (1986)

Application of discrimination technique to cytogenetically known specimens.

Twenty eight karyotypically identified specimens collected during this study from five localities in South Africa were classified with 100 % accuracy by this craniometric discrimination technique. Since these specimens were not included in the development of this technique, this amounted to an *a posteriori* test of the discrimination technique. Similarly, thirty five individuals of *M. natalensis* from Sinamotella (Zimbabwe) which were excluded from the development of the analyses, were identified with 91,43% accuracy. These results show that the craniometric discrimination technique developed is robust. However, application of the discrimination procedures to areas outside the geographic confines of this study may lead to misclassification of specimens owing to the pronounced nature of geographic variation in both species. This was exemplified by results of the classification of known specimens from two localities in Namibia (OTU's 2 and 24). In all the exploratory analyses, these two OTU's consistently grouped with South African OTU's, thereby suggesting that unknown specimens from Namibia could be identified using the discrimination procedure based on South African OTU's. However, when the South African discrimination procedure was applied, 100% of the Windhoek specimens (OTU 2), and only 63% of Mahango specimens (OTU 24), were correctly identified. When a two-group discriminant function based on Norton (Zimbabwe) specimens was used, none of the Windhoek specimens were correctly identified, while 88% of the Mahango specimens were correctly identified. Unknown specimens from Namibia were, therefore, excluded from the classification procedure, pending further analysis of geographic variation throughout southern Africa.

Application of the discrimination technique to the available type specimens.

The holotypes of both *M.coucha* and *M. natalensis* were not available for examination. However, the discrimination technique was applied to four type specimens housed at the Transvaal Museum, Pretoria. *Mus limpompoensis* (Roberts, 1914 - TM 1341), and *Mus socialis* (Roberts, 1913 - TM 388, TM 389) specimens, which are considered synonyms of *Mastomys coucha limpompoensis* and *Mastomys coucha coucha* (Meester *et al.* 1986) respectively, were correctly classified to *Mastomys coucha*. The *Mus komatiensis* (Roberts, 1926 - TM 1794) specimen, however, which is considered a synonym of *Mastomys coucha komatiensis,* was identified to *M. coucha* rather than to *M.natalensis* (Meester *et al.* 1986). This suggests that *M. coucha komatiensis* should be synonymised under *M. coucha.*

4.4. CONCLUSIONS.

Geographic variation analyses, even when based on individual specimens provided a useful picture in showing that in both species shape - related, and to some extent size -related geographic variation is pronounced. In both species SNK multiple range comparison tests failed to categorise localities in any interpretable manner, whereas PCA indicated the existence of two geographic groupings. Cluster analyses results in *M. natalensis* were congruent with PCA results, but in *M. coucha* no clustering according to geographic origin was evident. Thorpe (1976) stated that PCA is adequate in explaining all types of geographic variation, whereas cluster analyses is useful in elucidating only a few types. The lack of congruence between PCA and cluster analyses results in *M. coucha,* and the presence thereof in *M. natalensis*, indicates that the nature of geographic variation differs between the two species.

Geographic variation has been documented in other small mammals such as, *Myosorex longicaudatus* (Dippenaar, 1995), *Cynictis penicillata* (Taylor & Meester,1990), *Acomys subspinosus* and *A. spinosissimus (*Dippenaar & Rautenbach, 1986), and in the two sibling species of *Aethomys chrysophilus* (Chimimba[3], pers comm). In these studies the nature of geographic variation was shape - related, size - related or a compound of shape and size components. While the study of patterns of geographic variation often leads to causal analysis (Gabriel & Sokal, 1969), the limited number of OTU's in the current study hampered such an assessment of the possible influential causes of geographic trends in either species.

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Results of multivariate interspecific variation analyses, in particular PCA results (Figure 4. 3 b), clearly support the existence of two morphometrically - distinct taxa within the *M. natalensis* species complex in southern Africa. These results are comparable with the findings based on chromosomal and biochemical data (Gordon,1984; Robbins *et al.* 1983; Green *et al.* 1980; Hallett, 1979; Gordon, 1978; Green *et al.* 1978). Morphometric differences between *M. coucha* and *M. natalensis* are shape rather than size related, and in this regard my results are congruent with those of Dippenaar *et al.* (1993). In this study the two species proved to be very similar in cranial morphology, and this was illustrated by the overlap of the differential cranial ratios of the two species (Table 4.10). However, differences in interorbital breadth, post-palatal width and height of the mandible (which loaded high on PC III - Table 4.2) allowed discrimination of the two species by multivariate techniques. The qualitative dental character (t3 cusp), proved not to be a taxonomically useful character, as it varied with toothwear, and also geographically in *M. coucha*. Morphometric techniques, therefore, remain the only available taxonomic tool that can be used to delineate the two species in the absence of cytogenetic or electrophoretic information.

The extent of geographic variation in both species was substantial, and confounded species separation. Other studies have indicated presence of geographic variation in small mammals (Dippenaar, 1995 ; Dippenaar & Rautenbach, 1986; Chimimba, pers comm), but in none of these studies did the magnitude geographic variation perturb specific differences. It is not clear if Gordon (1984) examined geographic variation in *M. coucha* and *M. natalensis,* but he treated Zimbabwe OTU's separately from South Africa and Namibia OTU's when analysing interspecific differences by the multivariate techniques. While the present study, was useful in highlighting the extent of geographic variation, the limited number of OTU's used did not allow a comprehensive analysis of geographic variation. A comprehensive assessment of the nature and possible causes of geographic variation in both species is clearly needed. Such an analysis would require large sample sizes derived from a broad range of localities in southern Africa, and should form part of future investigations of these taxa.

CHAPTER 5

GEOGRAPHIC DISTRIBUTION AND THE BIOGEOGRAPHY OF *M. COUCHA* AND *M. NATALENSIS* IN SOUTHERN AFRICA .

5.1. INTRODUCTION.

Member species of the genus *Mastomys* occur widely in subSaharan Africa, and extend even into the southern part of Morocco (Figure 1.1; Davis 1962). In southern Africa, *M. natalensis s.l.* occurs widely in Namibia, Botswana, Zimbabwe, Mozambique, South Africa, Lesotho and Swaziland. (Figure 1.1, Smithers, 1983). Before the recognition of two genetic species within *M. natalensis* species complex in southern Africa, the wide geographic range of this taxon suggested that it occurred in diverse climatic, biogeographic and habitat ranges (Meester *et al.*, 1979). Since *Mastomys* is a semi - commensal rodent, and also because it thrives in disturbed habitats (Coetzee, 1975; Veenstra, 1958), Gordon (1984) suggested that its distribution range may have been influenced by human dispersal, agricultural development and establishment of transport lines.

Only upon identification of the two chromosome forms within *M. natalensis s.l.* did it become apparent that the two cytotypes show characteristic distributions with respect to biotic zones and some climatic factors, such as rainfall. Gordon (1984) showed that *M. coucha* is widely distributed primarily in Southern Savanna Woodland, Grassland and, South West Arid biotic zones. Conversely, *M. natalensis* appears to be restricted to the Southern Savanna Woodland biotic zone, with only a marginal intrusion into South West Arid and Grassland biotic zones. Based on limited cytogenetic data suite available, Gordon (1984) further illustrated a positive relationship between distribution of the two species and mean annual rainfall, with

M. natalensis generally occurring in mesic areas receiving more than 600 mm rainfall per annum, whereas *M. coucha* appeared to be restricted to drier areas with less than 700 mm rainfall per annum.

Gordon (1984) pointed out, however, that the apparent correlation between biotic zones and species distributions were tenuous at best. Furthermore, the cytogenetic data on which the distributions established by Gordon (1984) and Green *et al.* (1980), were based on a relatively limited number of localities. Green *et al.* (1980) also emphasized that their sampling was biased as large areas were not surveyed, and others were oversampled. Detailed distribution patterns and limits of the two species are thus needed to clarify the biogeography of *M. natalensis s.l.* in southern Africa.

The history of plague in southern Africa dates back to early 1900. The early phase was confined to urban areas infested by house rats (Davis, 1964 ; Innes, 1952). This murine phase lasted until 1912, but the next phase, the sylvatic phase, began in 1914 - 1915 on farms near Uitenhage in the Eastern Cape (Mitchell, 1921). These outbreaks had their origins in wild rodent species, and *Yersinia pestis (*the plague bacillus*)* was isolated from dead gerbils (*Tatera brantsi*), multimmamate mice (*Mastomys*) and other rodents (*Rattus rattus*, *Mus musculus*). Primary sylvatic foci became established at three widely separated areas: Uitenhage, North West Orange Free State and South West Transvaal (Davis, 1948). From 1919 onwards, the primary foci extended their range, with the North West Orange Free State focus expanding westwards to Kimberly and south and eastwards to Bloemfontein (Mitchell, 1927). Between 1925 - 1931, plague foci expanded westwards across the Karroo and the Kalahari Desert and into Namibia and Botswana (Fourie, 1932). The extension of plague outside South Africa went on to reach Angola and Zambia.

In recent years plague has been less of a public health problem in southern Africa, however, it is enzootic in wild rodents particularly in areas receiving less than 625 mm rainfall (Davis, 1964). Mitchell, Pirie & Ingram (1927) gave an account of epidemiological, bacteriological and entomological aspects of sylvatic plague, and provided information on the circulation of the plague bacillus, *Y. pestis*. Although other rodents and hares have been implicated in the plague cycle, *Tatera brantsi* in particular is a primary sylvatic plague reservoir (Twigg, 1978). This species is widely distributed in southern Africa (Skinner & Smithers, 1990), and Davis (1949) noted that it is an ecologically suitable rodent host for the plague bacillus. Davis (1948) further confirmed the role of *Mastomys* as an intermediary agent in carrying *Y. pestis* from the primary reservoir to man. In the wild *Mastomys* uses unoccupied burrows infested by fleas, and when the food is scarce it moves towards human habitation and its fleas come in contact with humans. *Tatera brantsi* carries three flea species, of which *Xenopsylla philoxera* is thought to be the most important in circulation of *Y. pestis*. The distribution of this flea species in southern Africa also coincides with areas receiving less than 625 mm annual rainfall. *Mastomys* also carries a mixed flea fauna, but the house - rat flea *Xenopsylla brasiliensis* is thought to be chiefly responsible for transmitting the disease from infected semi - commensal rodents to man (Twigg, 1978).

The recognition of the two species within *M. natalensis* species complex had important epidemiological implications (Gordon, 1984) with respect to the role of *M. coucha* and *M.natalensis* in plague epidemiology. The study by Isaacson *et al.* (1981) showed *M. coucha* to be more susceptible when both species were experimentally infected with the plague bacillus. This, and the positive correlation between the range of karyotyped *M.coucha* and human plague outbreaks (Taylor *et al.* 1981), suggested that only *M. coucha* may be responsible for transmission of plague bacillus to humans. But the latter results were based on limited cytogenetic data, and a subsequent study by Shepherd, Leman & Hummitzch (1986) showed that *M. natalensis* is also susceptible to plague infection, although the degree of susceptibility differed between the two populations they used.

The main aims of this chapter, therefore, were to determine the ranges of *M. coucha* and *M.natalensis* in southern Africa, and to relate these to available climatic and environmental data, as well as to data of historical plague outbreaks.

5.2. MATERIALS AND METHODS

Distributions of the two species were established by plotting localities for morphometrically identified *M. coucha* (302 sites) and *M. natalensis* (210 sites) on a 1: 250 000 scale map. Owing to limited availability of data for other southern African countries, only the South African distributions of the following five data sets were subjected to spatial analysis with the Geographic Information System (GIS): karyotyped *M. coucha* (56 localities); karyotyped *M. natalensis* (23 localities); morphometrically identified *M. coucha* (300 localities); morphometrically identified *M. natalensis* (168 localities); and plague outbreak distributions (274 localities). Spatial analyses were performed using ArcView GIS version 2.0c for Windows (Environmental Systems Research Institute Inc, Redlands, CA, U.S.A). Species distribution data were derived largely from museum collections, and in the case of karyotyped specimens, some locality data points were extrapolated from Keogh & Price (1981). The plague distribution database was derived from published papers by Taylor *et al.* (1981) and Davis (1964). Although point locality data were available for some localities, most museum and literature records were given as only quarter degree squares, necessitating that analyses be restricted to this scale of resolution. Correlations of the distributions with mean annual rainfall zones, altitude zones, and vegetation types were determined using data extracted from Environmental Potential Atlases (ENPAT), Version 1 for ArcView (Department of Environmental Affairs and Tourism , Pretoria, South Africa).

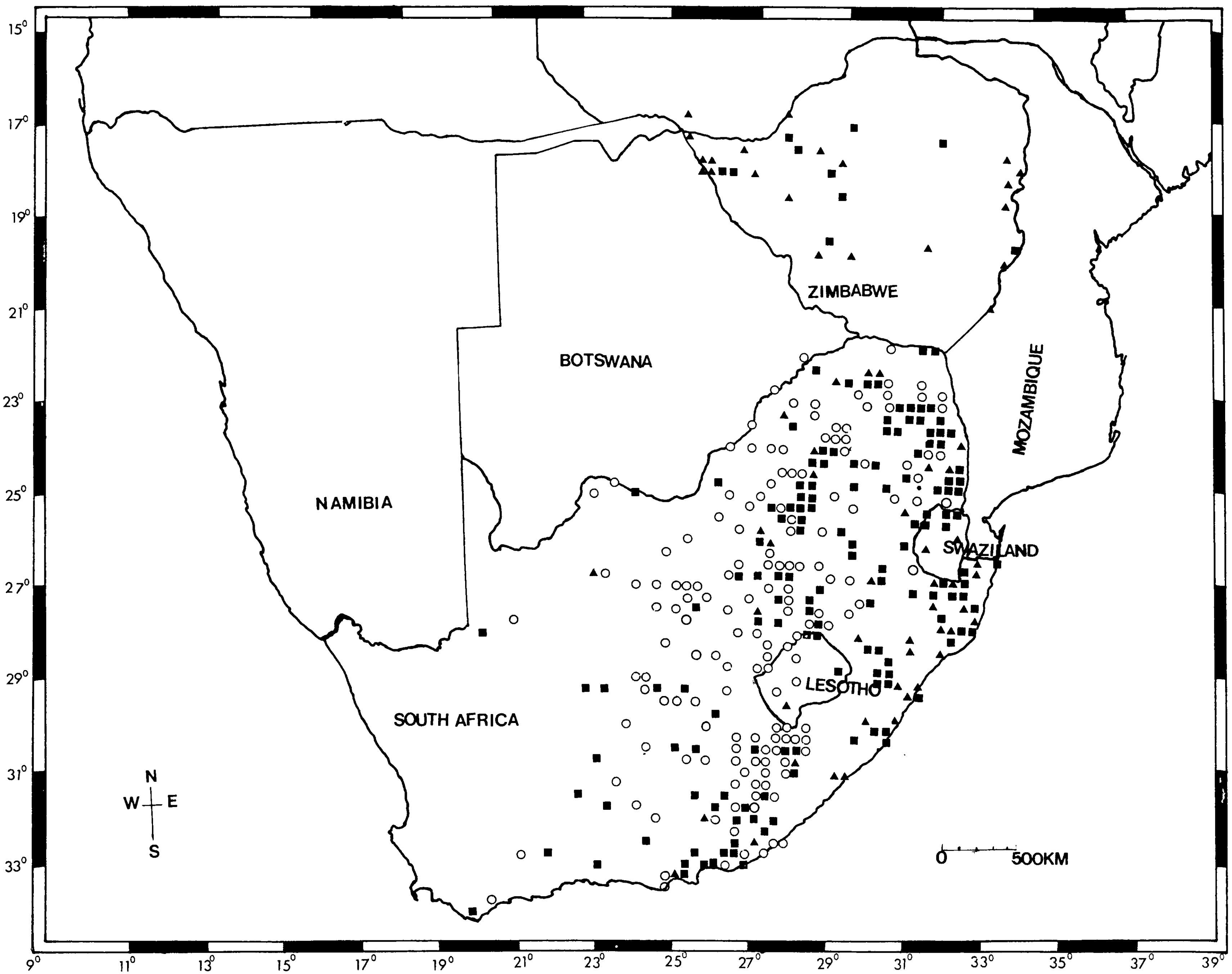
5.3. RESULTS AND DISCUSSION.

Geographic distributions of *M. coucha* and *M. natalensis*.

A map of the distributions of *M. coucha* and *M. natalensis* in southern Africa based on morphometrically identified specimens, is shown in Figure 5. 1. This indicates that *M. coucha* and *M. natalensis* occur together at 149 (41%) of the QDS sampled. *Mastomys coucha* occurs independently of *M. natalensis* in 153 QDS, and *M. natalensis* occurs in absence of *M. coucha* in 59 QDS. The distributions of the two species, as implied by the current results, are broadly sympatric. This contradicts the results based on limited cytogenetic data, which instead suggested a largely parapatric distribution pattern of the two species (Gordon,1984 ; Green *et al.* 1980). Such incongruence may be attributed to either discrimination error, or differences in data scope and quality.

While it is possible that the influence of geographic variation on interspecific discrimination may have resulted in discrimination errors, this seems most unlikely as discrimination techniques were computed according to regions to maximise the probability of correct assignment of specimens to their respective species. Furthermore, *a posteriori* tests of the identification procedure using samples of cytogenetically identified specimens not utilised during development of the morphometric discrimination technique, achieved high levels of accuracy (100% and 91,43% for South Africa and Zimbabwe, respectively). Clearly, therefore, the discrimination technique is robust, and identification error is too negligible to account for the contrasting distribution patterns of cytogenetic and

Figure 5.1 : A map showing the distribution of morphometrically identified specimens of *M. coucha* and *M. natalensis* from southern Africa. OTU's are listed in appendix 1.


ZIMBABWE
BOTSWANA
NAMIBIA
MOZAMBIQUE
SWAZILAND
LESOTHO
SOUTH AFRICA
N
W
E
S
0
500KM
15°
17°
19°
21°
23°
25°
27°
29°
31°
33°
9°
11°
13°
15°
17°
19°
21°
23°
25°
27°
29°
31°
33°
35°
37°
39°

morphometrically identified data suites.

It is likely that the actual distributions of the two species are indeed wider than limited cytogenetic data suggested. Taylor *et al.* (1995), who also found the two sibling species of *Thallomys paedulcus sensu lato* to occur widely in sympatry, attributed the disparities between cytogenetic data and morphometric data to lack of thorough sampling at localities. They pointed out that two sibling species might be broadly sympatric but utilize different microhabitats at any specific locality. Gordon (1984) similarly emphasised that possible differences at microhabitat level warranted further investigation in *Mastomys*. Similar distribution patterns of sibling species occurring in broad sympatry has also been found in *Aethomys chrysophilus* species complex (Chimimba, pers comm). It seems reasonable, therefore, to assume *M. coucha* and *M. natalensis* have broad overlapping distributions in southern Africa. Extensive sampling and karyotyping is, however, clearly needed to verify this, and also to confirm the nomenclatural validity of the names *M. coucha* and *M. natalensis.* These names were assigned (Green *et al,* 1980) on the basis of lack of sympatry in Durban (type locality for *M. natalensis*) and Kuruman (type locality for *M. coucha*), but my data indicate sympatry at these localities.

Comparison of the species distributions and environmental parameters.

Rainfall.

Results showing the frequency of locality distributions of *M. coucha* (karyotyped and morphometrically identified) and *M. natalensis* (karyotyped and morphometrically identified) in specified rainfall zones are given in Table 5 .1, and rainfall zones are further illustrated in Figures 5.2 - 5.5. Distributions based on cytogenetic data show a positive correlation with mean annual rainfall isohyets (Figures 5.2, 5.3; Table 5.1). Seventy seven percent of QDS in which *M. coucha* was recorded fall in drier areas receiving less than 600 mm mean annual rainfall, whilst 95,65% of QDS in which *M. natalensis* has been recorded fall areas in with more than 700 mm mean annual rainfall (Table 5.1, Figure 5. 2). A similar pattern was evident when morphometrically identified data sets were analysed (Table 5.1 ; Figures 5.4, Figure 5). The majority of *M. coucha* localities (68%) occur in drier areas receiving less than 700 mm, whereas most *M. natalensis* localities (69,64%) fall in areas receiving more than 700 mm annual rainfall. This pattern is, therefore, similar to that shown by Gordon (1984), and suggests that even though both species occur in either rainfall zone, *M. coucha* is more xeric - adapted than *M. natalensis*.

Altitude

The frequency of QDS of *M. coucha* (karyotyped and morphometrically identified) and *M. natalensis* (karyotyped and morphometrically identified), within specified altitudinal zones is given in Table 5.1. The relationship between the distribution of these data sets and given altitude zones are further illustrated in Figures 5.6 - 5.9. All the data sets showed a similar distribution pattern across altitude zones, with the majority (more than 78% in all data suites) of distribution points occurring below 1800 m altitude zone. There were no overt differences between karyotyped and morphometrically identified data sets of both species. There does not seem to be major differences between the two species in terms of altitudinal tolerance or preference. The apparent absence of any differences between the two species in altitudinal preference may, therefore, indicate that they have similar broad habitat requirement. Since the altitude classes were very broad, this does not necessarily negate the possible existence of subtle microhabitat differences between them.

Table 5.1: Frequency of localities of karyotyped *M. coucha*, karyotyped *M. natalensis*, morphometrically identified *M. coucha*, morphometrically identified *M. natalensis* and plague distribution data sets in each of the specified rainfall, altitude and vegetation zones. K = karyotyped, M = morphometrically identified.

RAINFALL (mm\yr)

Data set	0-200	201-300	301-400	401-500	501-600	601-700	701-800	801 - 900	900-1000	1001 - 1100
M.coucha (K)	0	3 (5,4%)	13 (23,2%)	6 (10,7%)	3 (5,4%)	18 (32,1%)	8 (14,3%)	5 (8,9%)	0	0
M. coucha (M)	3 (1%)	11 (3,7%)	12 (4%)	50 (16,.7%)	40 (13,3%)	88 (29,3%)	41 (13,7%)	35 (11,7%)	13 (4,3%)	7 (2,3%)
M.natalensis (K)	0	0	0	0	1 (4,4%)	0	6 (26,1%)	7 (30,4%)	2 (8,7%)	7 (30,4%)
M.natalensis (M)	1 (0,6%)	7 (4,2%)	10 (6%)	16 (9,5%)	17 (10,1%)	43 (25,6%)	17 (10,1%)	34 (20,2%)	17 (10,1%)	6 (3,5%)
Plague data	32 (11,6%)	30 (1,1%)	62 (22,6%)	46 (16,8%)	37 (13,6%)	54 (19,7%)	12 (4,4%)	1 (0,4%)	0	0

ALTITUDE (m)

Data set	0-400	401-800	801-1200	1201-1400	1401-1800	1801-2200	2201-2601	2601-3000
M. coucha (K)	0	4 (7,1%)	16 (28,6%)	8 (14,3%)	21 (37,5%)	1 (1,8%)	4 (7,1%)	2 (3,6%)
M. coucha (M)	7 (2,3%)	34 (11,3%)	52 (17,3%)	40 (13,3%)	134 (44,7%)	9 (3%)	13 (4,3%)	11 (3,7%)
M. natalensis (K)	0	8 (34,8%)	8 (34,8%	0	2 (8,7%)	4 (17,4%)	1 (4,4%)	0
M.natalensis (M)	6 (3,6%)	30 (17,9%)	34 (20,2%)	15 (8,9%)	68 (40,5%)	5 (3%)	9 (5,4%)	1 (0,6%)
Plague data	2 (0,7%)	3 (1,1%)	61 (22,3%)	63 (23%)	129 (47,1%)	2 (0,7%)	13 (4,7%)	1 (0,4%)

Table 5.1 cont'd.

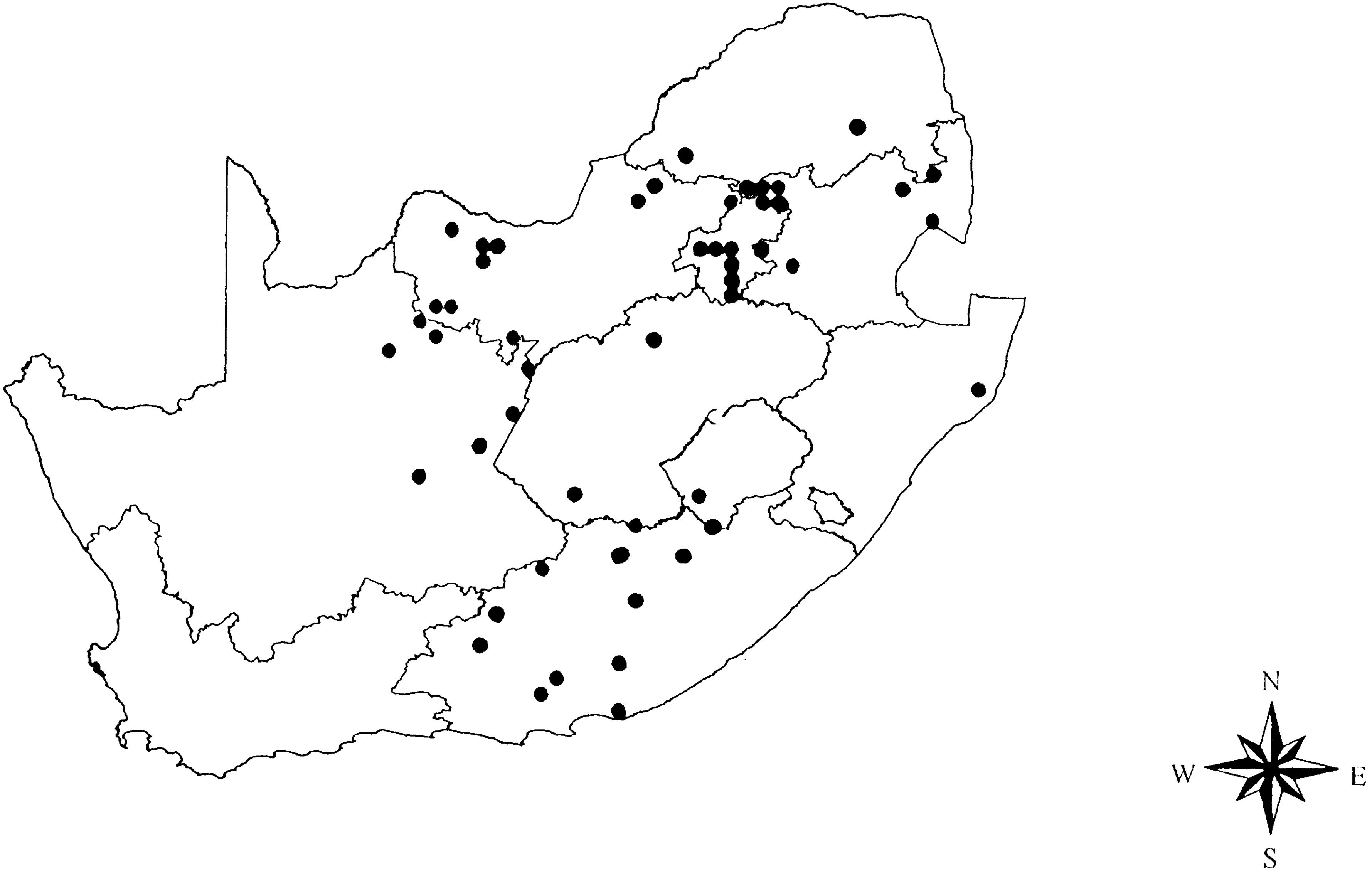
VEGETATION

TT = temperate and transitional forest, FK = false karoo, TBS = tropical bush and savanna, FG = false grassveld, KK = karoo and karroid, CTF = coastal tropical forest, PG = pure grassveld, ITF - inland tropical forest, FSCL = false sclerophyllous bush, SCL = sclerophyllous bush.

Data set	TT	FK	TBS	FG	KK	CTF	PG	ITF	FSCL	SCL
M.coucha (K)	0	4 (7,1%)	25 (44,6%)	18 (32,1%)	5 (8,9%)	0	0	3 (5,4%)	0	0
M.coucha (M)	30 (10%)	27 (9%)	87 (29%)	25 (8,33%)	21 (7%)	13 (4,33%)	74 (24,67%)	10 (3,33%)	2 (0,67%)	0
M.natalensis (K)	2 (8,7%)	0	10 (43, 0%	0	1 (4,4%)	6 (26,09%)	2 (8,7%)	2 (8,7%)	0	0
M.natalensis (M)	22 (13,09%)	14 (8,33%)	47 (28%)	20 (11,90%)	18 (10,71%)	11 (6,55%)	26 (15,5%)	4 (2,4%)	0	0
Plague data	4 (1,4%)	59 (21,53%)	64 (23,36%)	9 (3,3%)	56 (20,4%)	0	81 (29,56%)	0	0	1 (0,4%)

Figure 5.2 : Map showing the relationship between mean annual rainfall and cytogenetically identified *M. coucha* from various localities in South Africa. Rainfall ranges are indicated as follows : 1 = 99 - 236 mm\yr, 2 =236 - 372 mm\yr,
3 = 372 - 509 mm\yr, 4 = 509 - 646 mm\ yr, 5 = 646 - 782 mm\yr,
6 = 782 - 919 mm\yr, 7 = 919 - 1055 mm\yr,
8 = 1055 - 1192 mm\yr

Cytogenetically Identified M.coucha



Rainfall Map

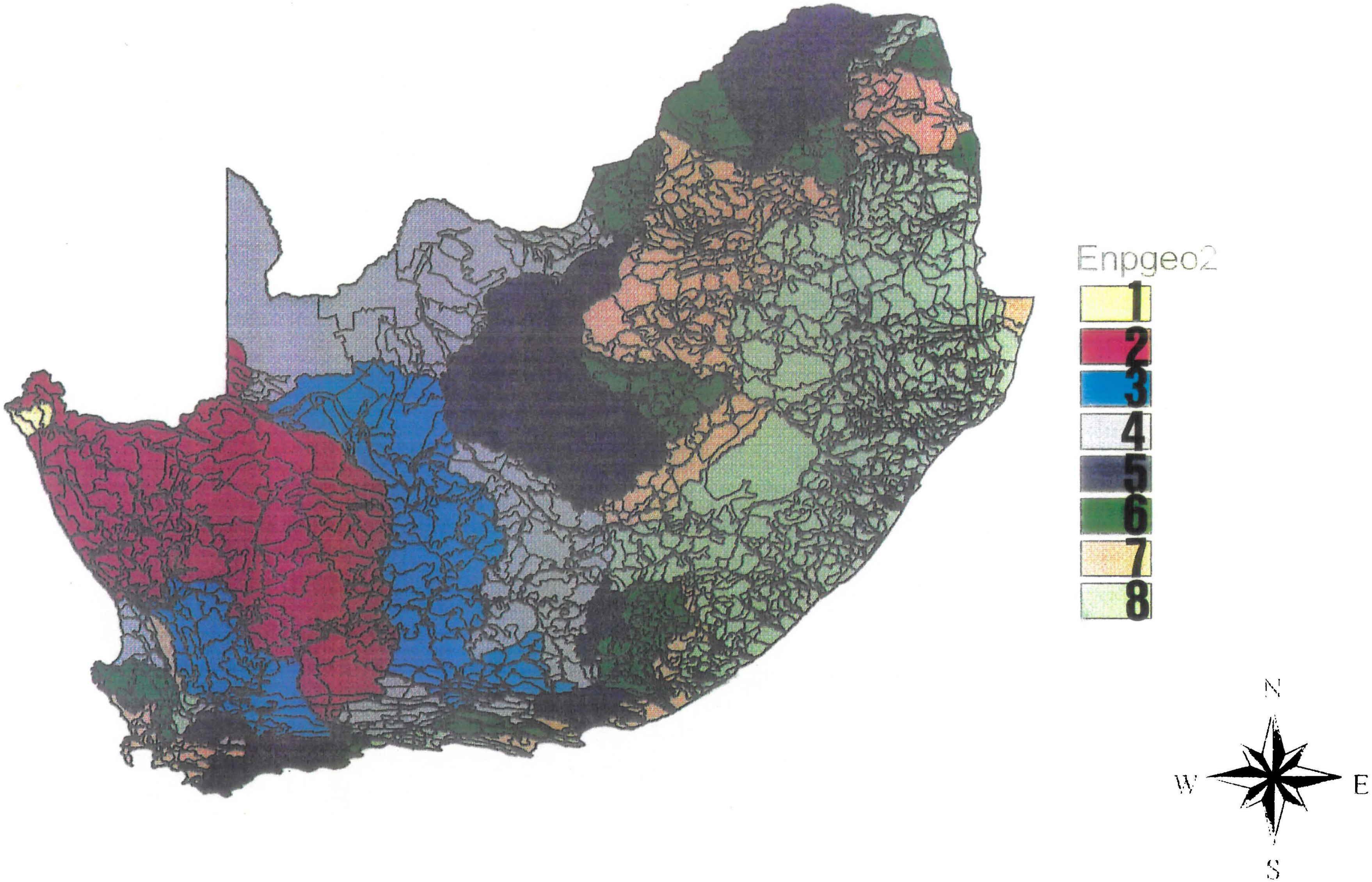


Figure 5.3 : Map showing the relationship between the mean annual rainfall and cytogenetically identified *M. natalensis* from various localities in South Africa. Rainfall ranges are indicated as follows : 1 = 99 - 236 mm\yr,
2 =236 - 372 mm\yr, 3 = 372 - 509 mm\yr, 4 = 509 - 646 mm\ yr,
5 = 646 - 782 mm\yr, 6 = 782 - 919 mm\yr,
7 = 919 - 1055 mm\yr, 8 = 1055 - 1192 mm\yr

Cytogenetically Identified M.natalensis

Rainfall Map

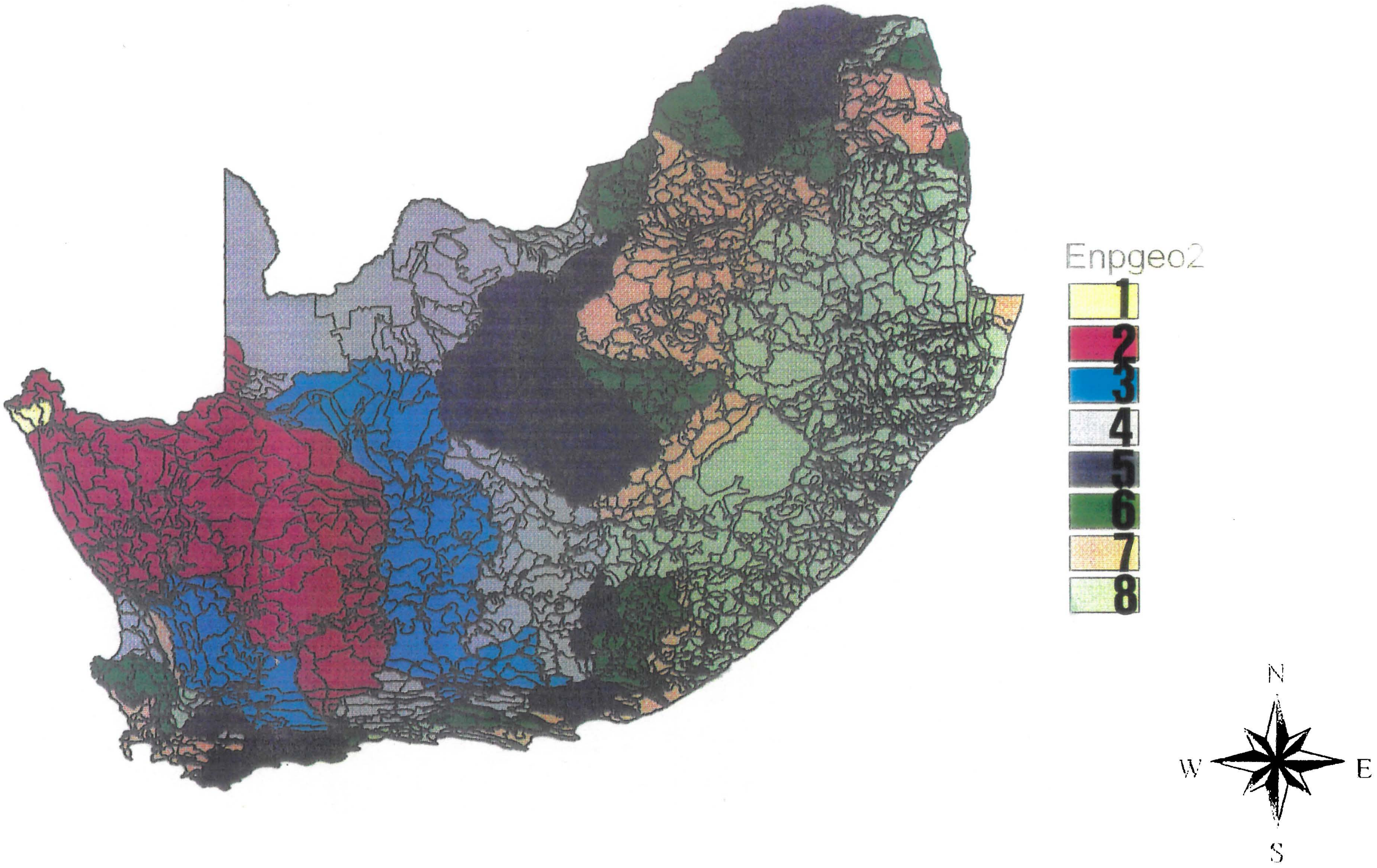
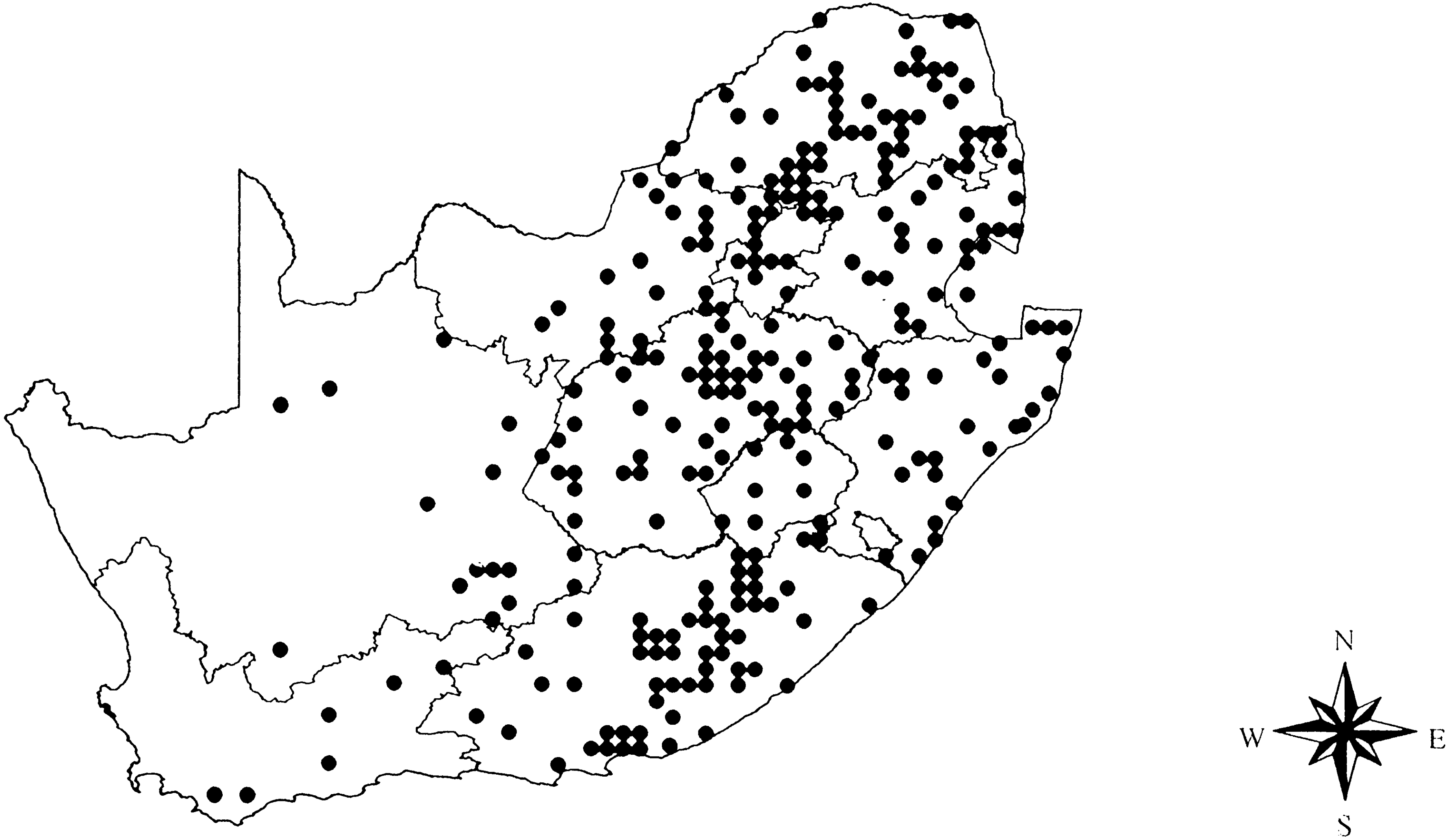


Figure 5.4 : Map showing the relationship between mean annual rainfall and morphometrically identified *M. coucha* from various localities in South Africa. Rainfall ranges are indicated as follows : 1 = 99 - 236 mm\yr, 2 =236 - 372 mm\yr, 3 = 372 - 509 mm\yr, 4 = 509 - 646 mm\ yr, 5 = 646 - 782 mm\yr, 6 = 782 - 919 mm\yr, 7 = 919 - 1055 mm\yr, 8 = 1055 - 1192 mm\yr

Morphometrically Identified M. coucha



Rainfall Map

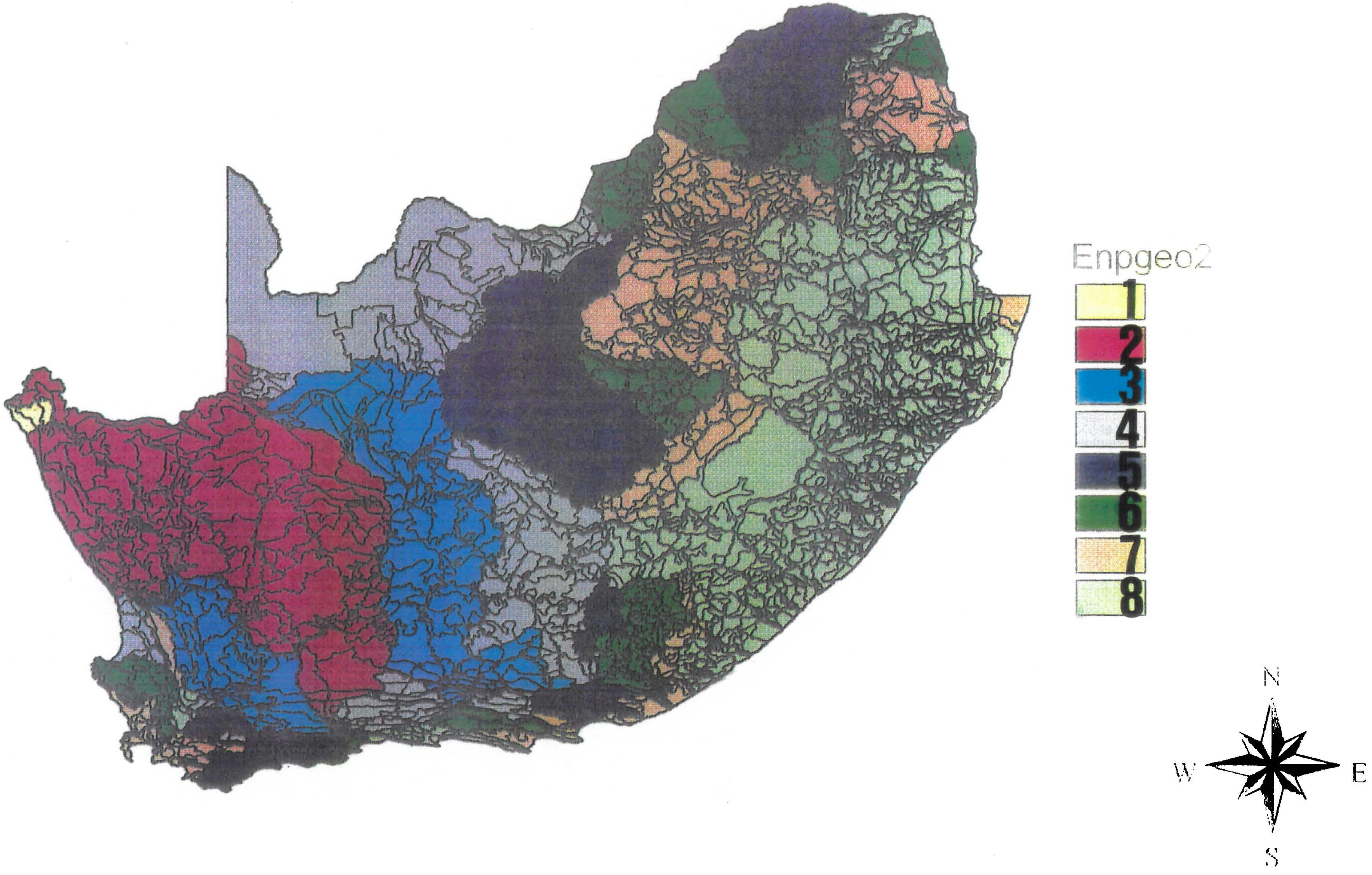
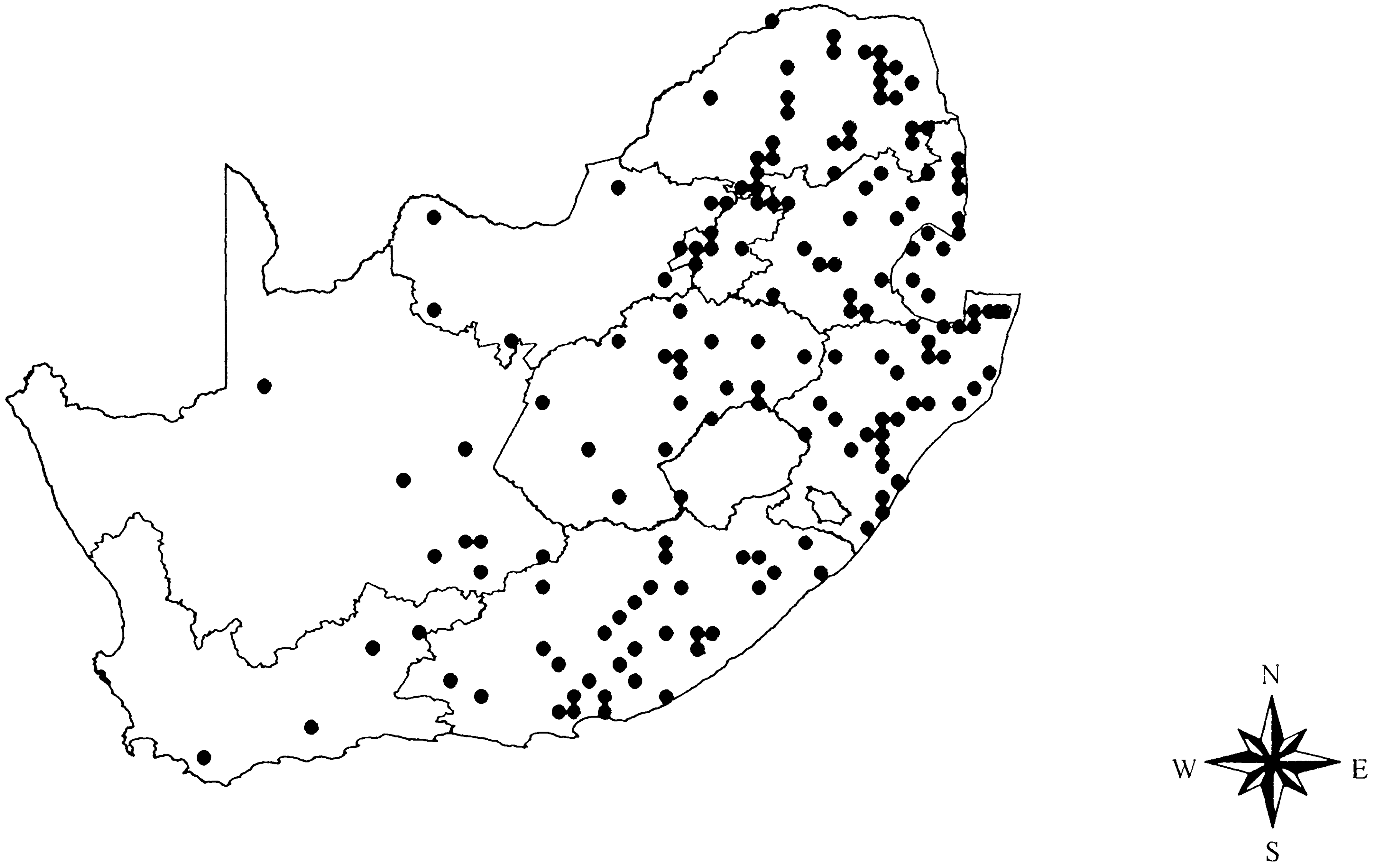
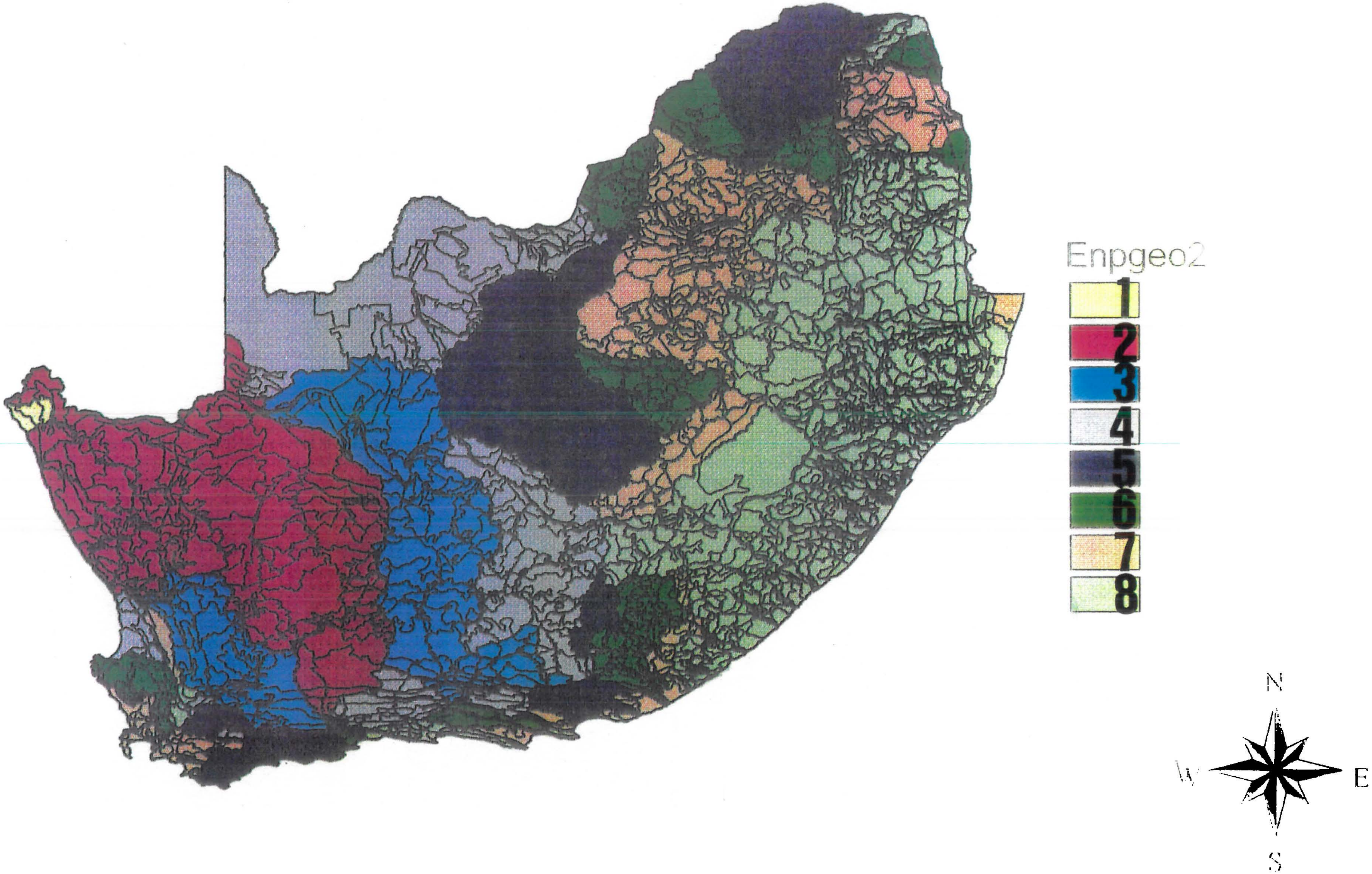


Figure 5.5 : Map showing the relationship between mean annual rainfall and morphometrically identified *M. natalensis* from various localities in South Africa. Rainfall ranges are indicated as follows : 1 = 99 - 236 mm\yr,
2 =236 - 372 mm\yr, 3 = 372 - 509 mm\yr, 4 = 509 - 646 mm\ yr,
5 = 646 - 782 mm\yr, 6 = 782 - 919 mm\yr,
7 = 919 - 1055 mm\yr, 8 = 1055 - 1192 mm\yr

Morphometrically Identified M. natalensis



Rainfall Map



Vegetation

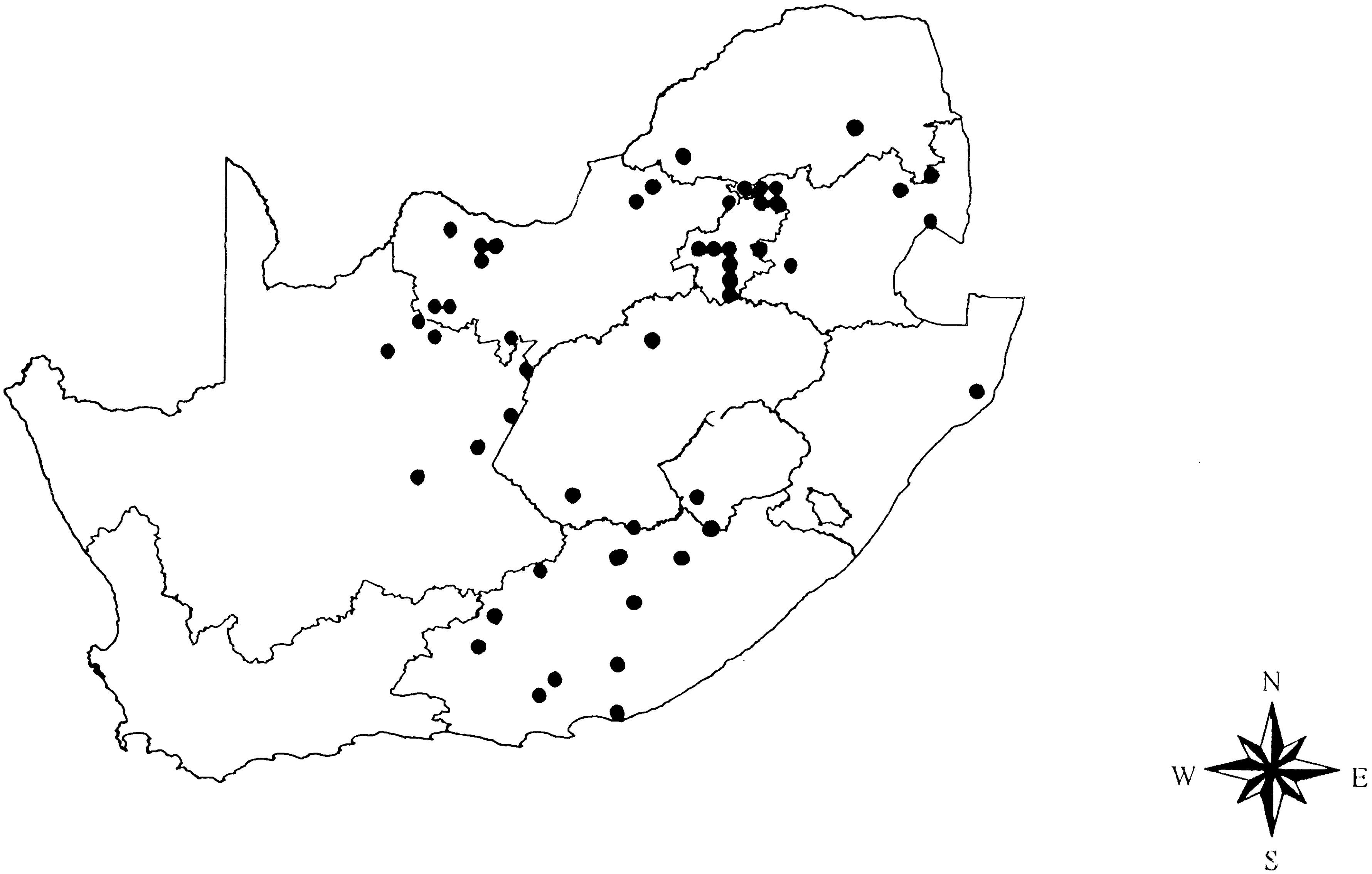
Results showing the frequency of QDS containing *M. coucha* (karyotyped and morphometrically identified) and *M. natalensis* (karyotyped and morphometrically) within 10 vegetation types (Acocks, 1988) are shown in Table 5.1. Maps showing the relationship between the species distributions and the 10 vegetation types, are shown in Figures 5.10 - 5.13. Karyotyped *M. coucha* QDS fall mainly into the False Karoo, Tropical Bush and Savanna, False Grassland, Karoo and Karroid and Inland Tropical Forest vegetation types. Karyotyped *M. natalensis* QDS, on the other hand, fall mainly into the Temperate and Transitional Forest, Tropical Bush and Savanna, Karoo and Karroid, Coastal Tropical Forest, Pure Grassland and in the Inland Tropical Forest. The two species overlap in the Tropical Bush and Savanna vegetation zone, with 45% of *M. coucha* and 43% of *M. natalensis* locality data points falling in this vegetation zone. Other vegetation types containing both species are the Karoo and Karroid and Inland Tropical Forest vegetation zones. *Mastomys coucha* was also abundant in the False Grassland vegetation zone, whereas *M. natalensis* was prevalent in the Coastal Tropical Forest vegetation area.

The distribution of karyotypically identified specimens did not extend into the Sclerophyllous Bush vegetation area. *Mastomys coucha* appears to prevail in False Karoo and False Grassland vegetation, whereas *M. natalensis* is restricted to the Temperate and Transitional Forest, Coastal Tropical Forest and Pure Grassland zones.

When morphometrically identified data were examined, the distribution of the two species across vegetation zones predictably proved to be wider than suggested by karyotyped material. *Mastomys coucha* occurred in all except the Sclerophyllous Bush vegetation type. *Mastomys natalensis* also occurred in all vegetation types except for .

Figure 5.6 : Map showing the relationship between the altitude range and cytogenetically identified *M. coucha* from various localities in South Africa. Altitude ranges are indicated as follows, 1 = 0 - 400m, 2 = 401 - 800m, 3 = 801 - 1200, 4 = 1201-1400, 5 = 1401 - 1800m, 6 = 1801 - 2200m, 7 = 2201 - 2601m, 8 = 2601 -3000m

Cytogenetically Identified M.coucha



Altitude Map

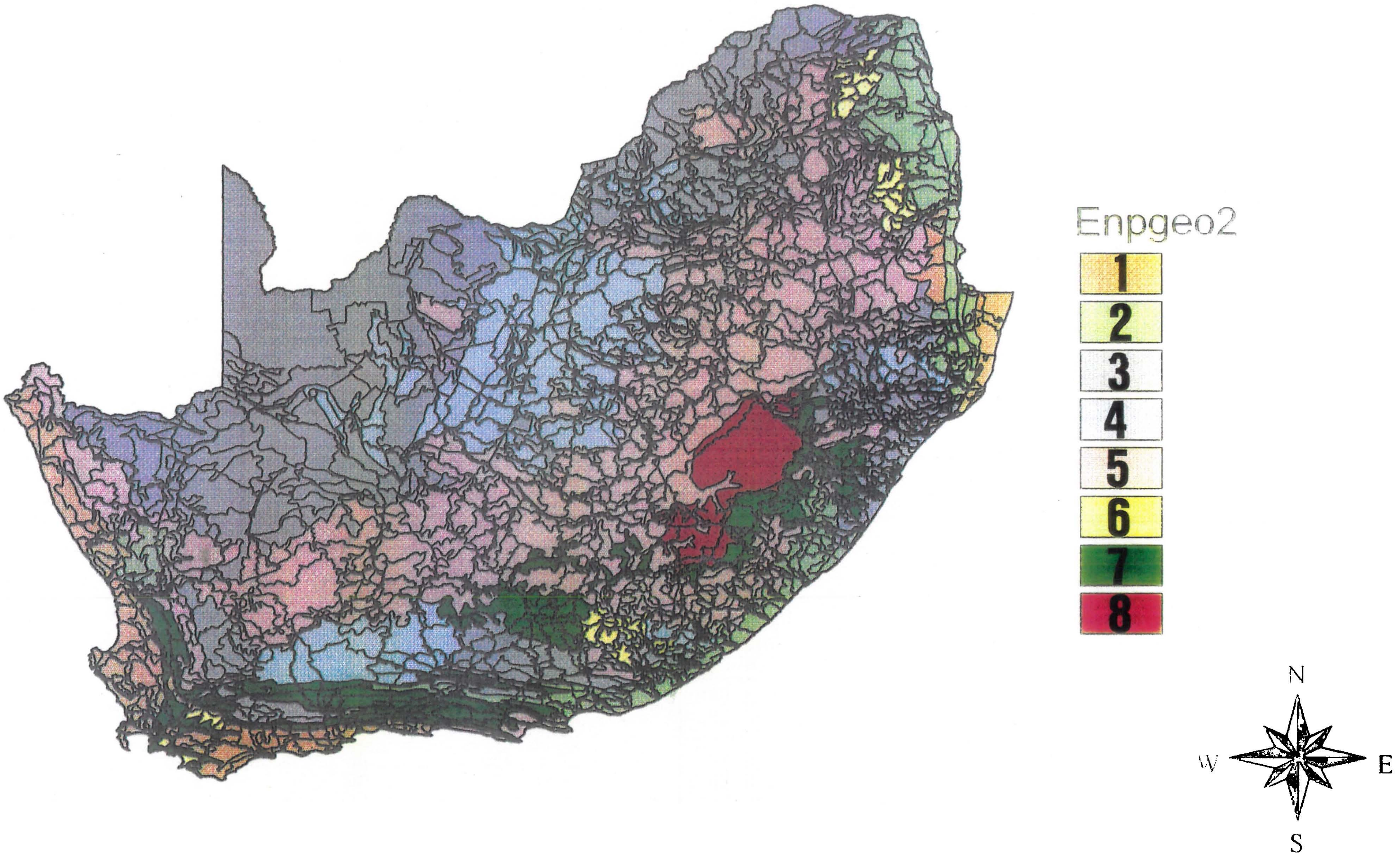


Figure 5.7 : Map showing the relationship between the altitude ranges and cytogenetically identified *M. natalensis* from various localities in South Africa.Altitude ranges are indicated as follows, 1 = 0 - 400m, 2 = 401 - 800m, 3 = 801 - 1200, 4 = 1201-1400, 5 = 1401 - 1800m, 6 = 1801 - 2200m, 7 = 2201 - 2601m, 8 = 2601 -3000m.

Cytogenetically Identified M.natalensis

Altitude Map

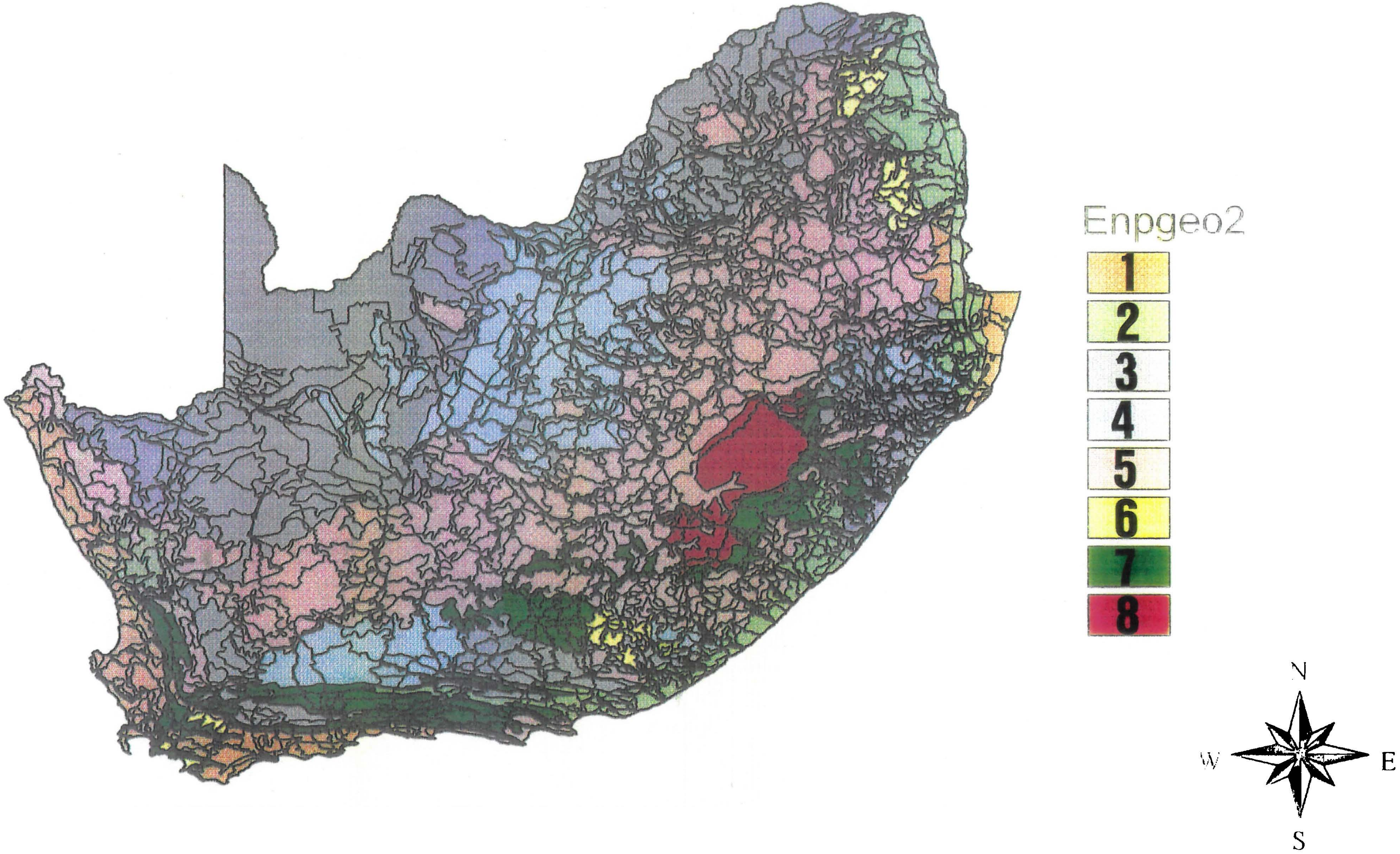
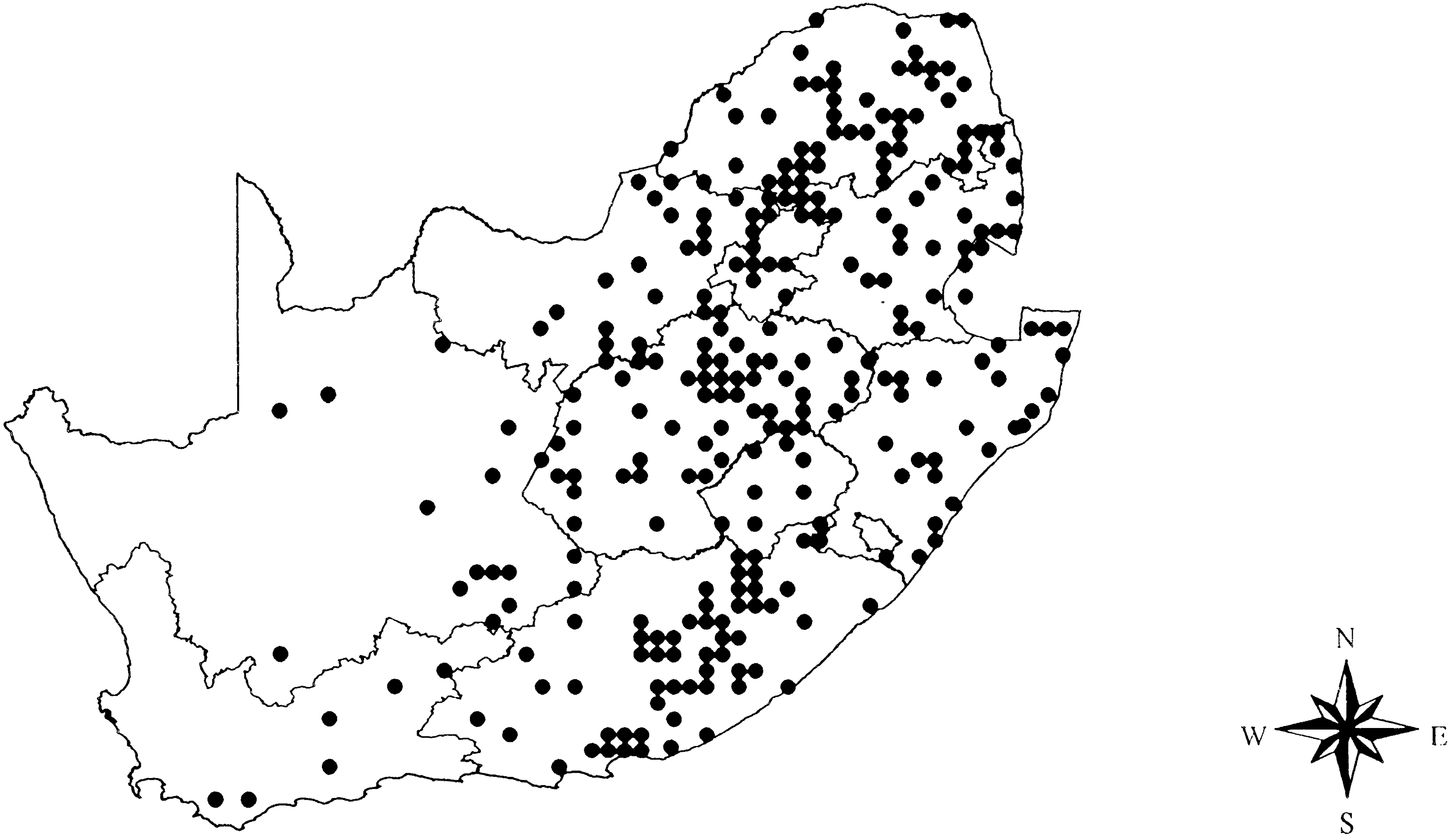


Figure 5.8 : Map showing the relationship between the altitude ranges and morphometrically identified *M. coucha* from various localities in South Africa.Africa.Altitude ranges are indicated as follows, 1 = 0 - 400m, 2 = 401 - 800m, 3 = 801 - 1200, 4 = 1201-1400, 5 = 1401 - 1800m, 6 = 1801 - 2200m, 7 = 2201 - 2601m, 8 = 2601 -3000m.

Morphometrically Identified M. coucha



Altitude Map

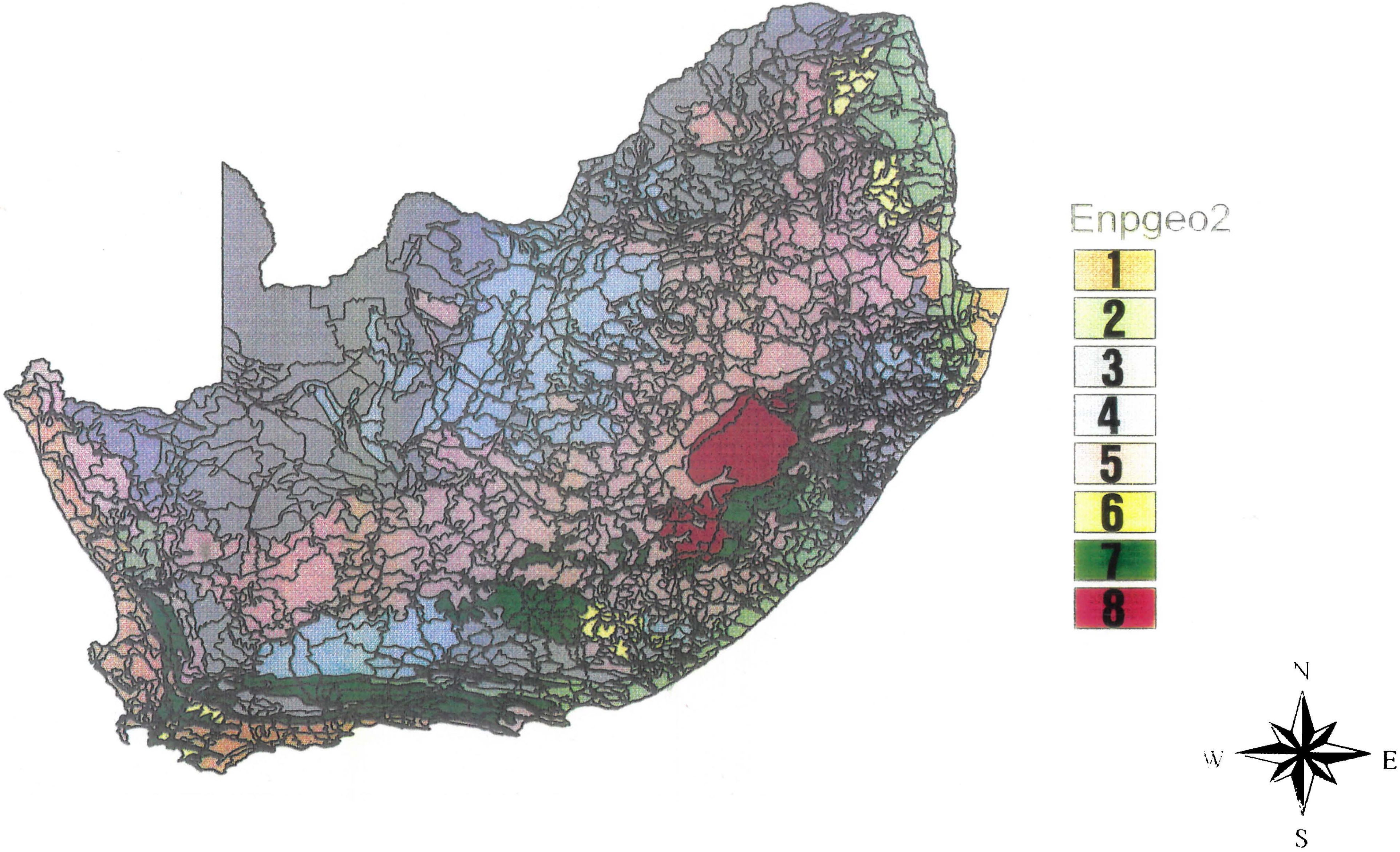
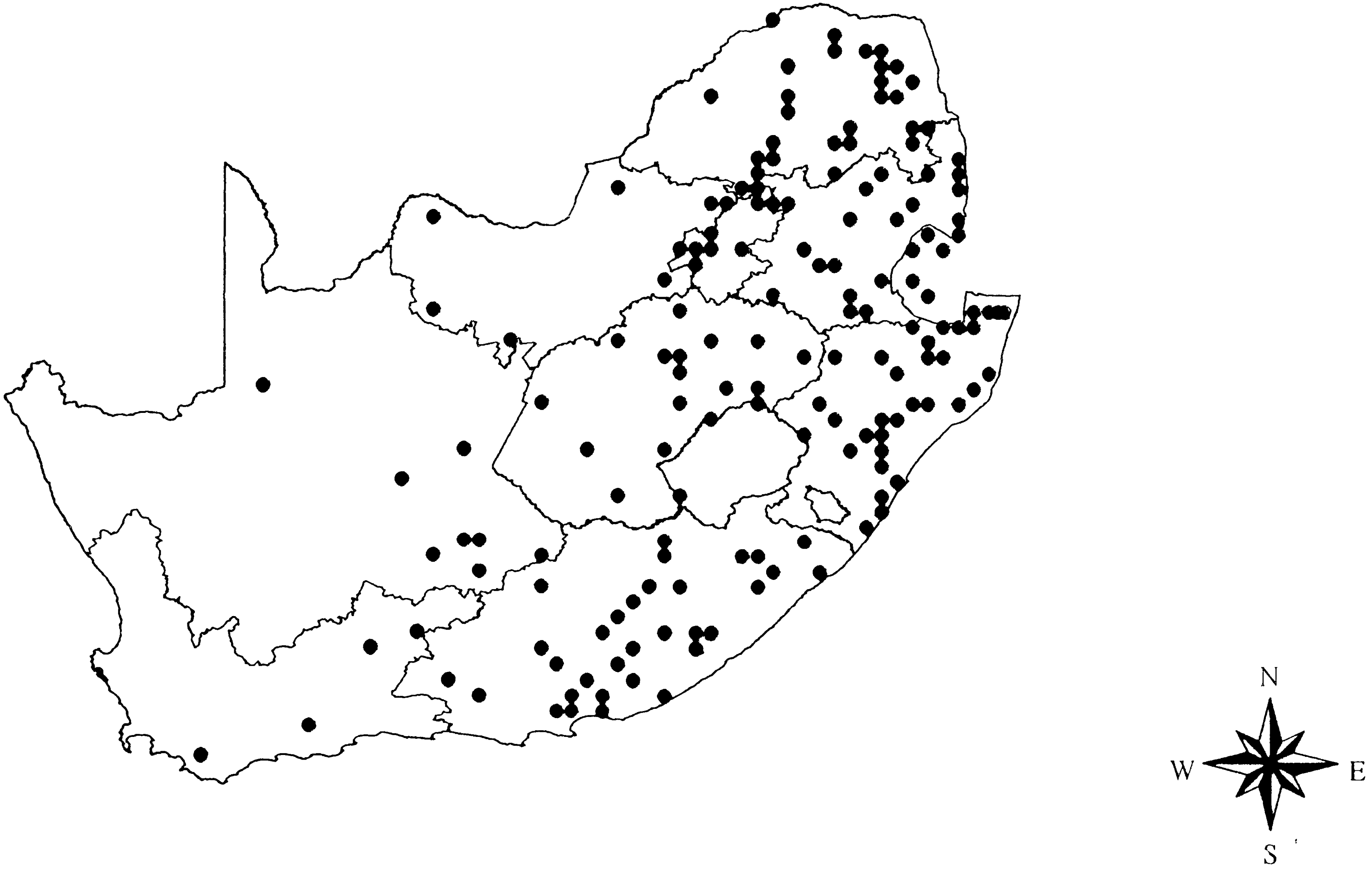
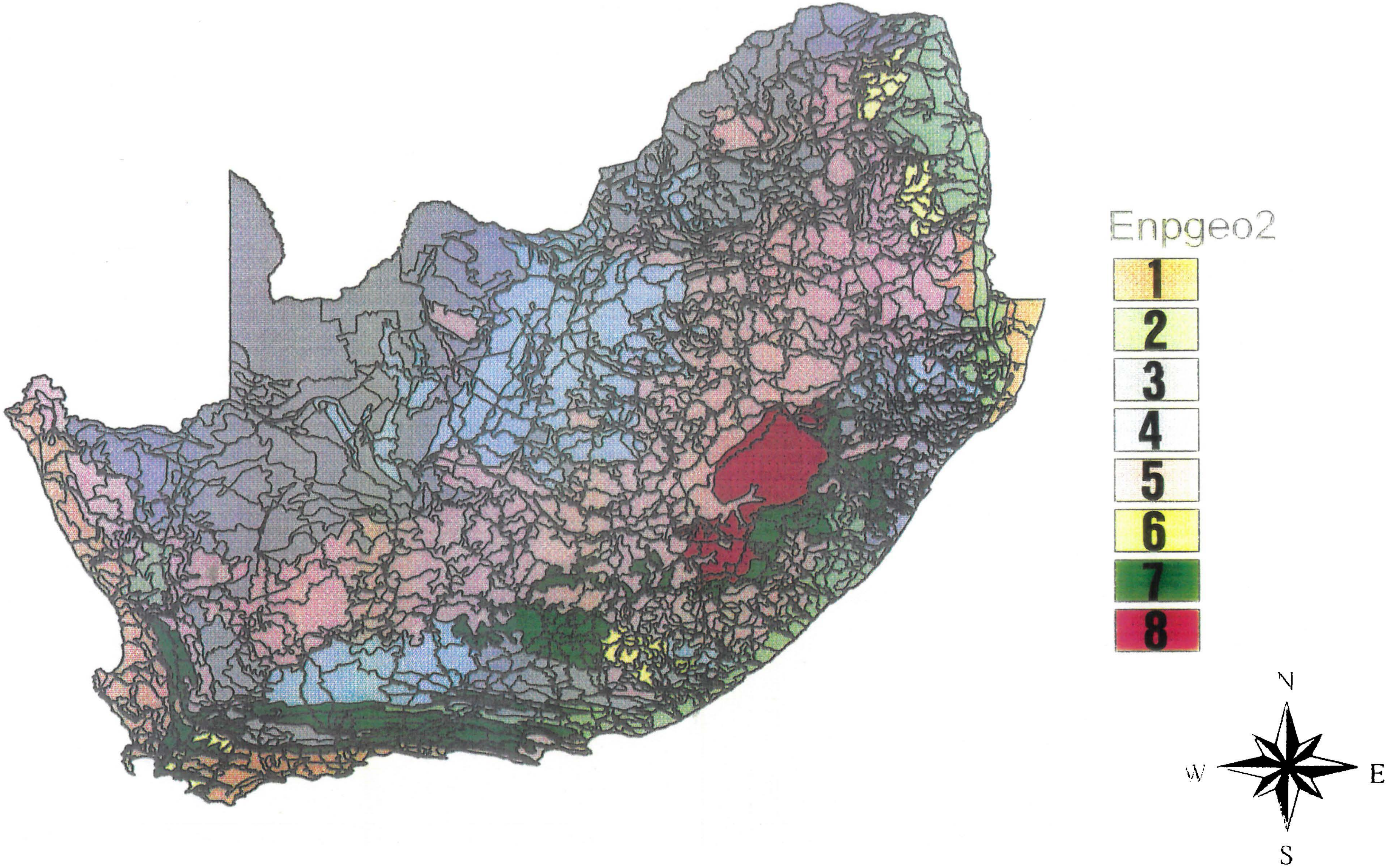


Figure 5.9 : Map showing the relationship between altitude ranges and morphometrically identified *M.* natalensis from various localities in South.Africa.Altitude ranges are indicated as follows, 1 = 0 - 400m, 2 = 401 - 800m, 3 = 801 - 1200, 4 = 1201-1400, 5 = 1401 - 1800m, 6 = 1801 - 2200m, 7 = 2201 - 2601m, 8 = 2601 -3000m.

Morphometrically Identified M. natalensis



Altitude Map



Sclerophyllous Bush and False Sclerophyllous Bush

Although Gordon (1984)'s study was based on wider vegetation zones, there seems to be a correlation between his findings and the current study. He found that the distributions of the two species largely separated according to biotic zones. Similarly, in this study karyotyped data sets showed that the two species distributions largely follow characteristic vegetation zones, but the distributions based on morphometrically identified specimens indicate overlap of the two species in most vegetation zones. Given these results it is, therefore, expected that a similar picture would have emerged if the distributions of morphometrically identified data were analysed according to biotic zones.

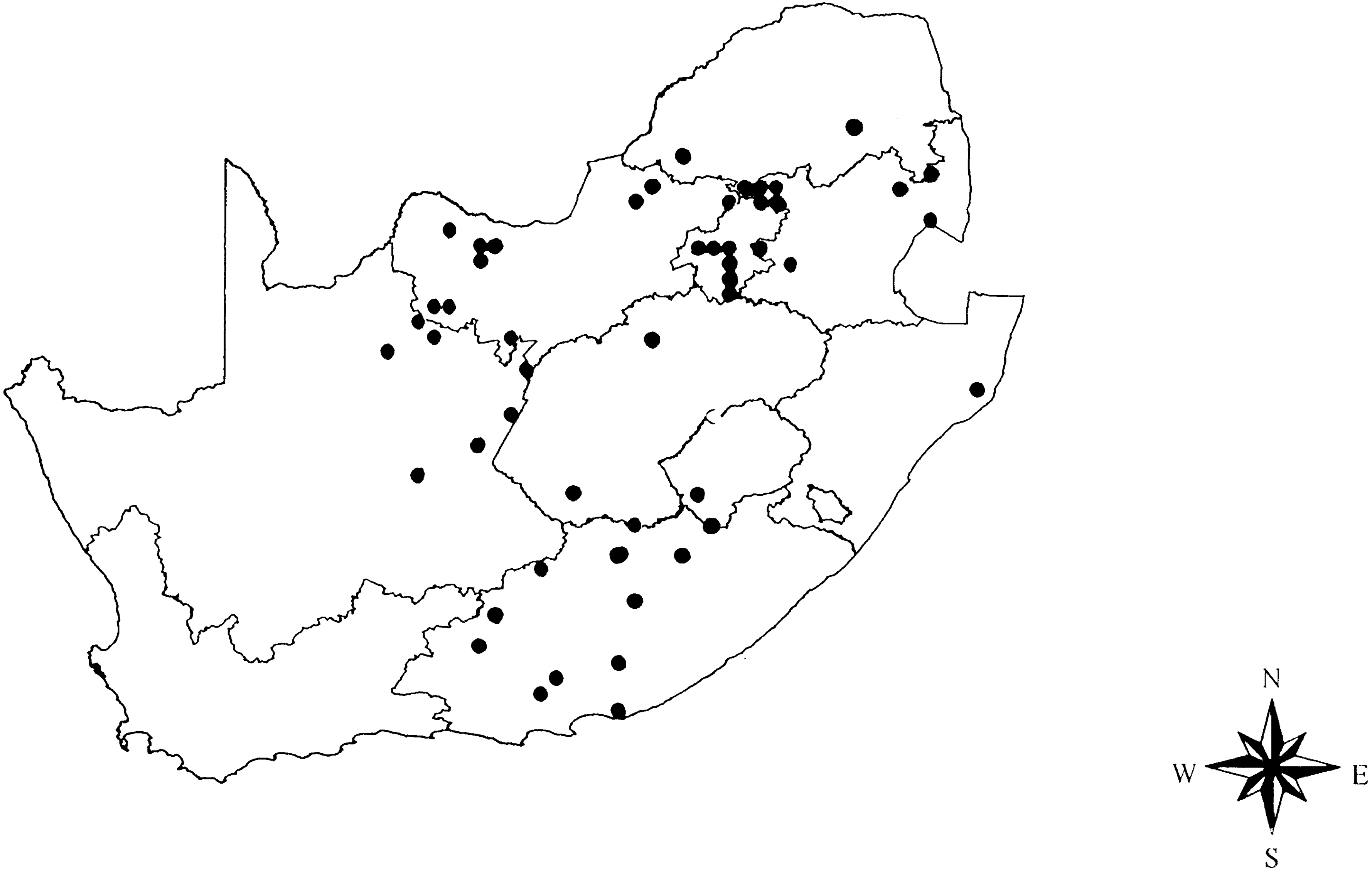
Plague outbreaks distributions

Plague outbreaks distributions and the two species distributions.

The relationship between bubonic plague outbreaks, and the two species distribution data are illustrated in Figures 5.14 - 5.17. Of the 56 QDS in which karyotyped *M. coucha* occurs, 14 (25%) coincided with QDS in which plague outbreaks have been recorded. None of the 23 QDS in which karyotyped *M. natalensis* occurs coincide with bubonic plague outbreak areas. These findings are in agreement with previous studies by Gordon (1984) and Taylor *et al.* (1981), and show positive correlation between *M. coucha* distribution and plague

Figure 5.10 : Map showing the relationship between the 10 vegetation types in South Africa and cytogenetically identified *M. coucha* from various localities in South Africa. Keys indicate vegetation types.

Cytogenetically Identified M.coucha



Vegetation types Map

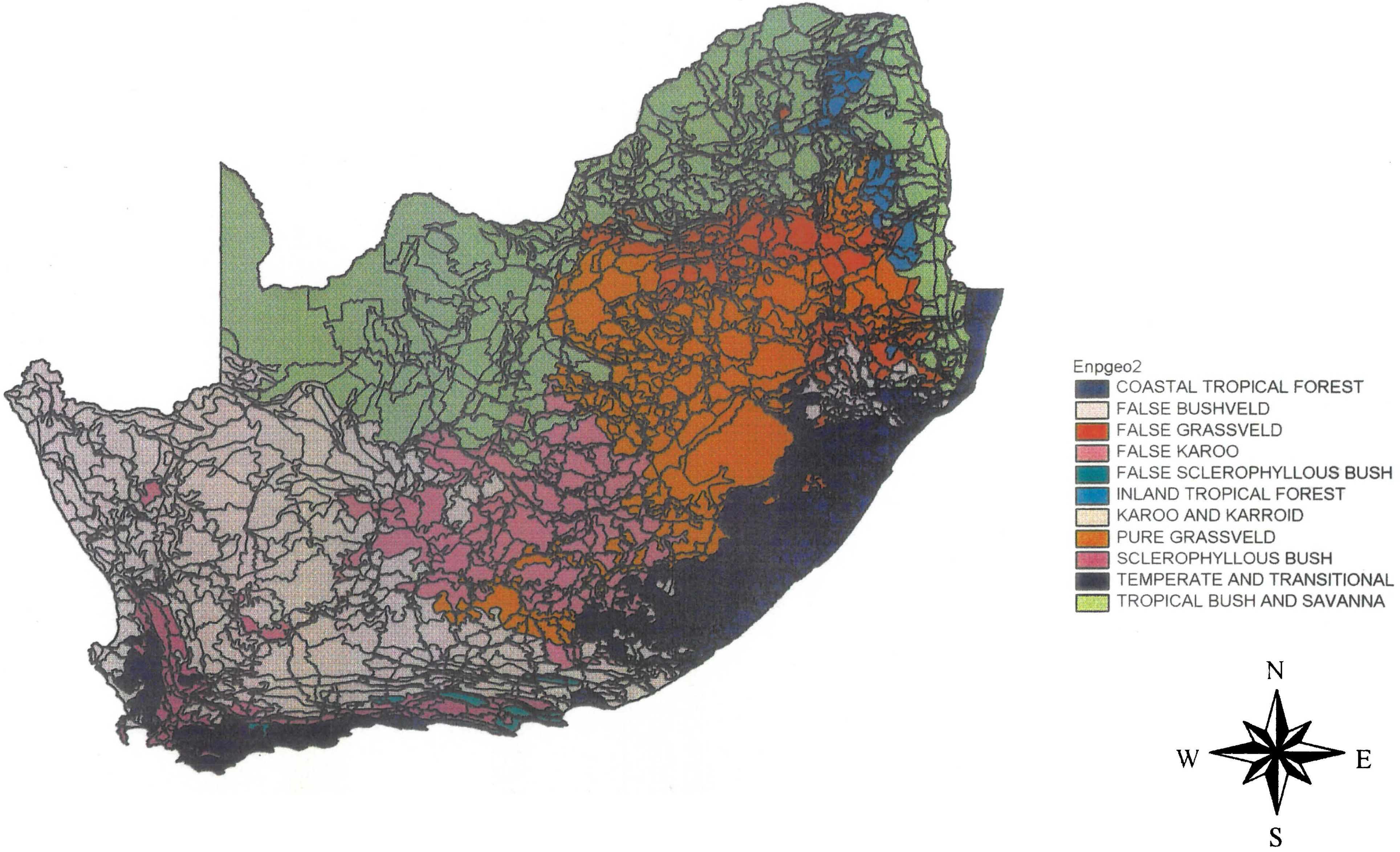


Figure 5.11 : Map showing the relationship between the 10 vegetation types in South Africa and cytogenetically identified *M. natalensis* from various localities in South Africa. Keys indicate vegetation types.

Cytogenetically Identified M.natalensis

Vegetation types Map

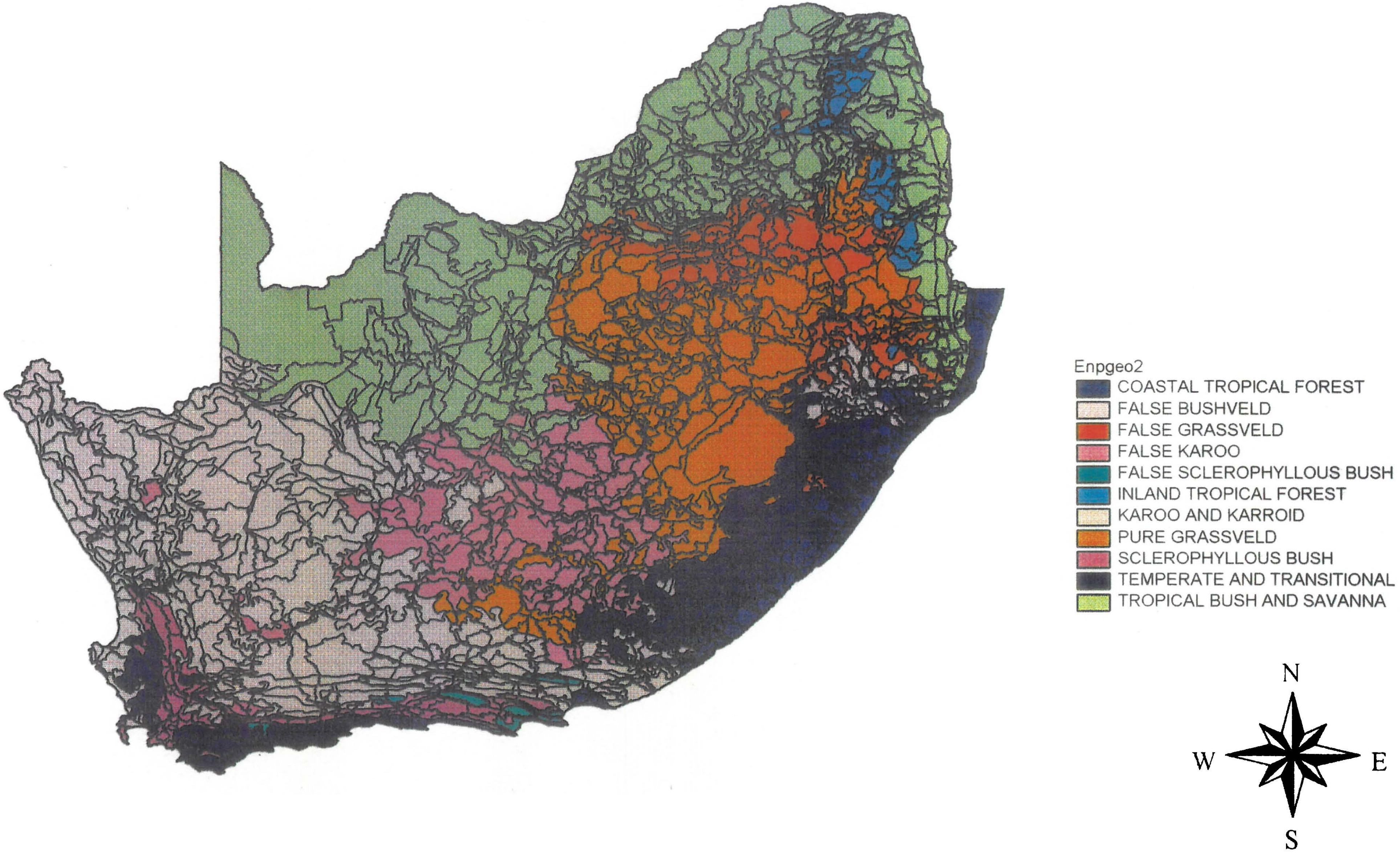
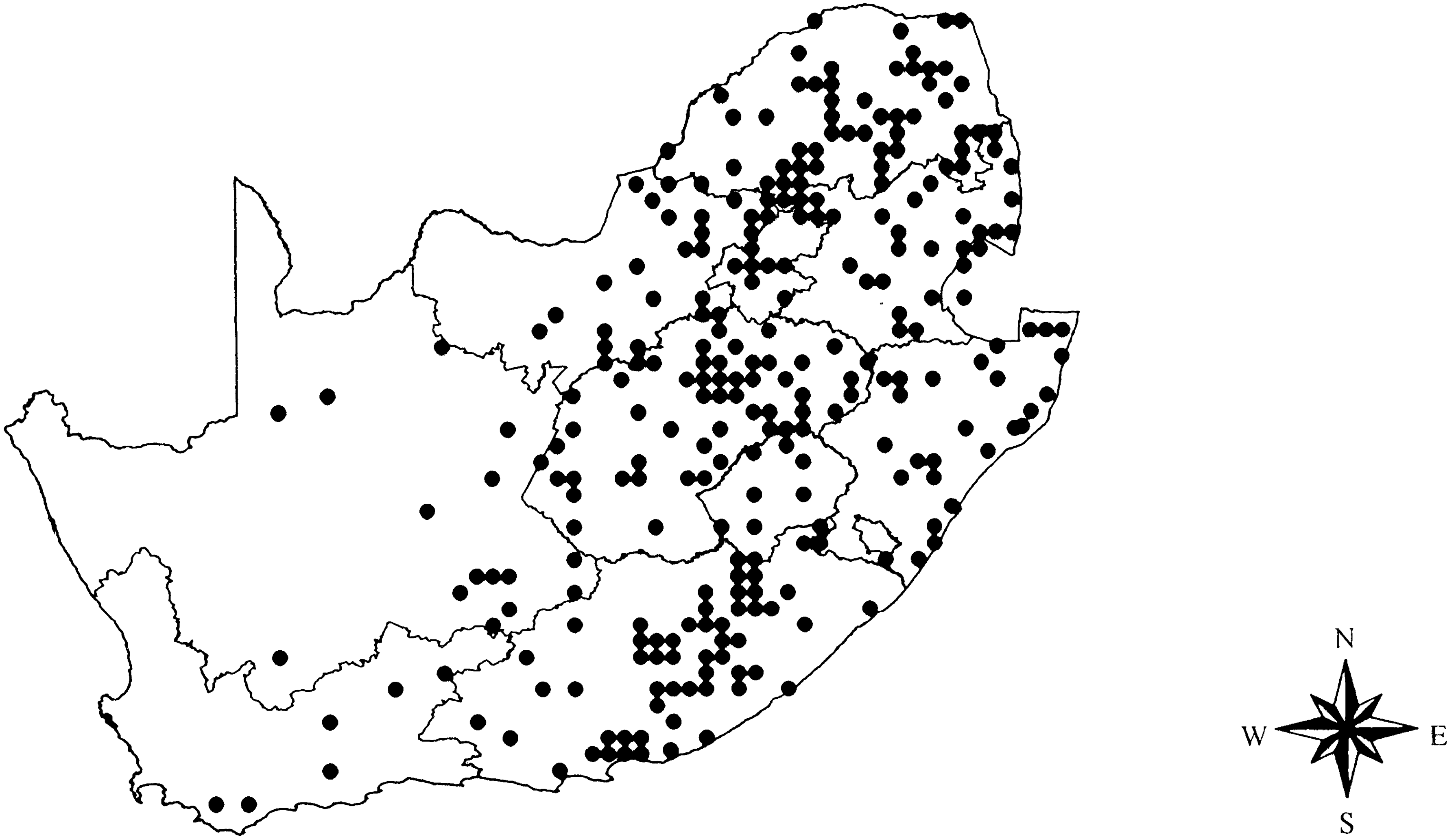


Figure 5.12 : Map showing the relationship between the 10 vegetation types in South Africa and morphometrically identified *M. coucha* from various localities in South Africa. Keys indicate vegetation types.

Morphometrically Identified M. coucha



Vegetation types Map

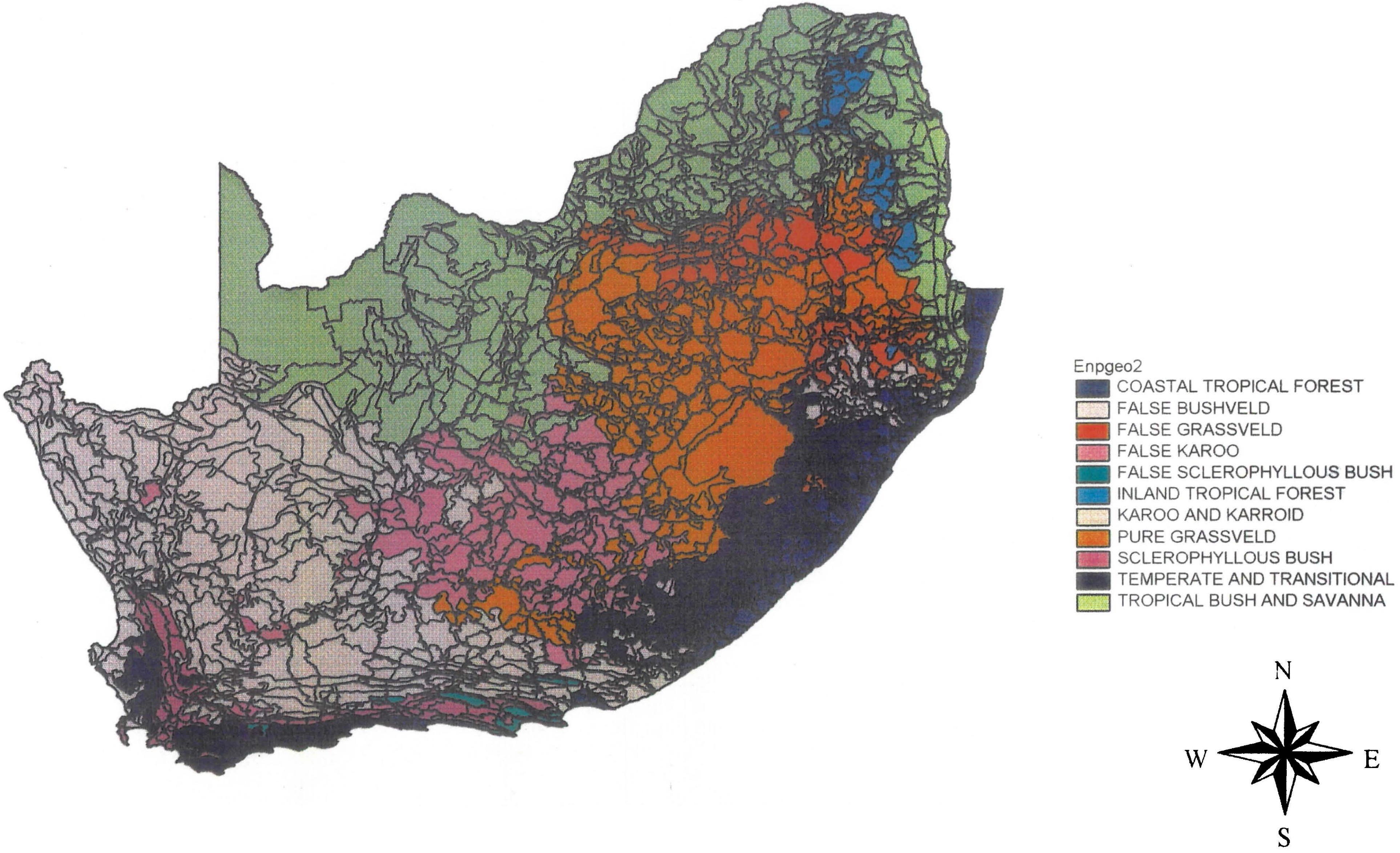
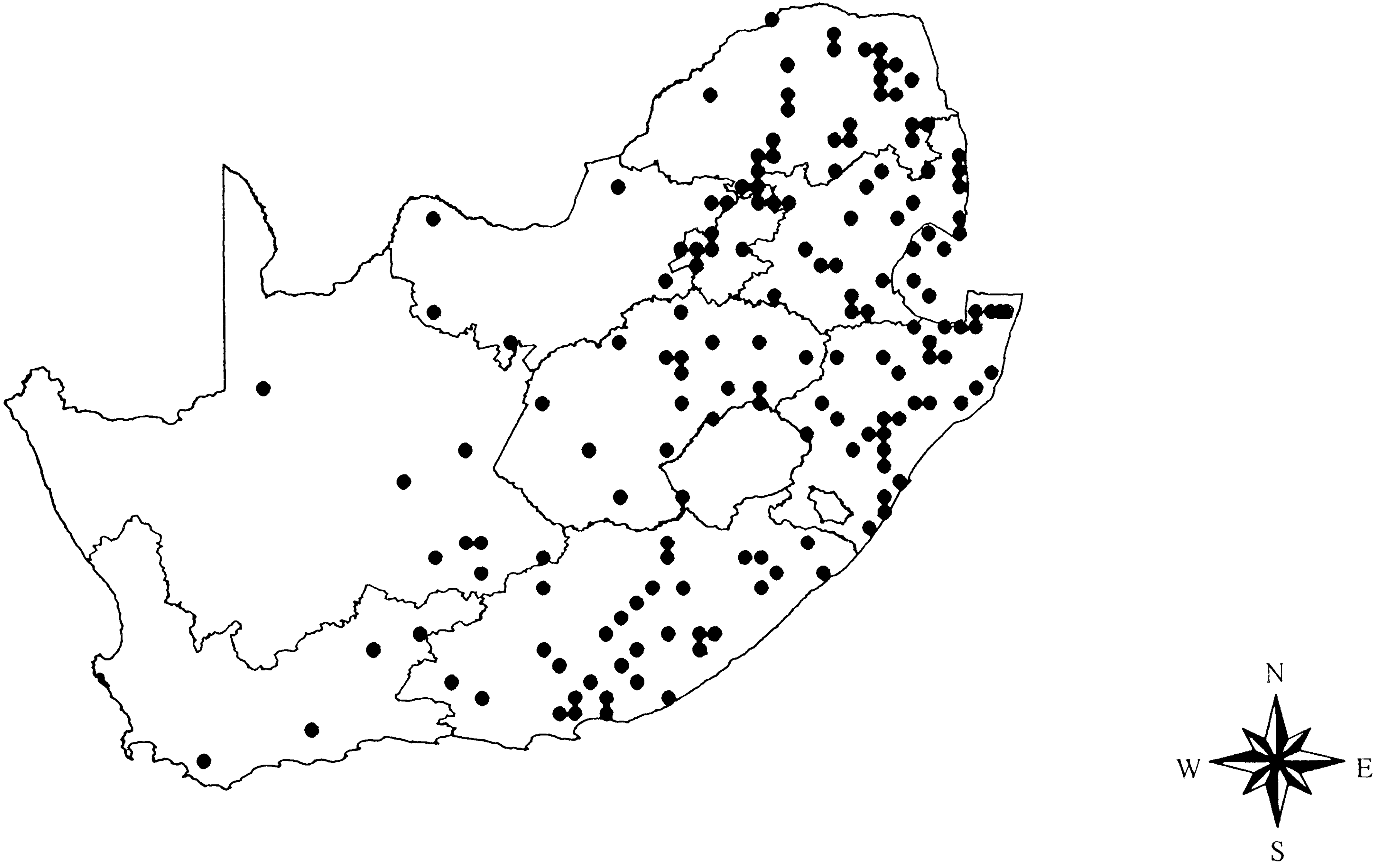
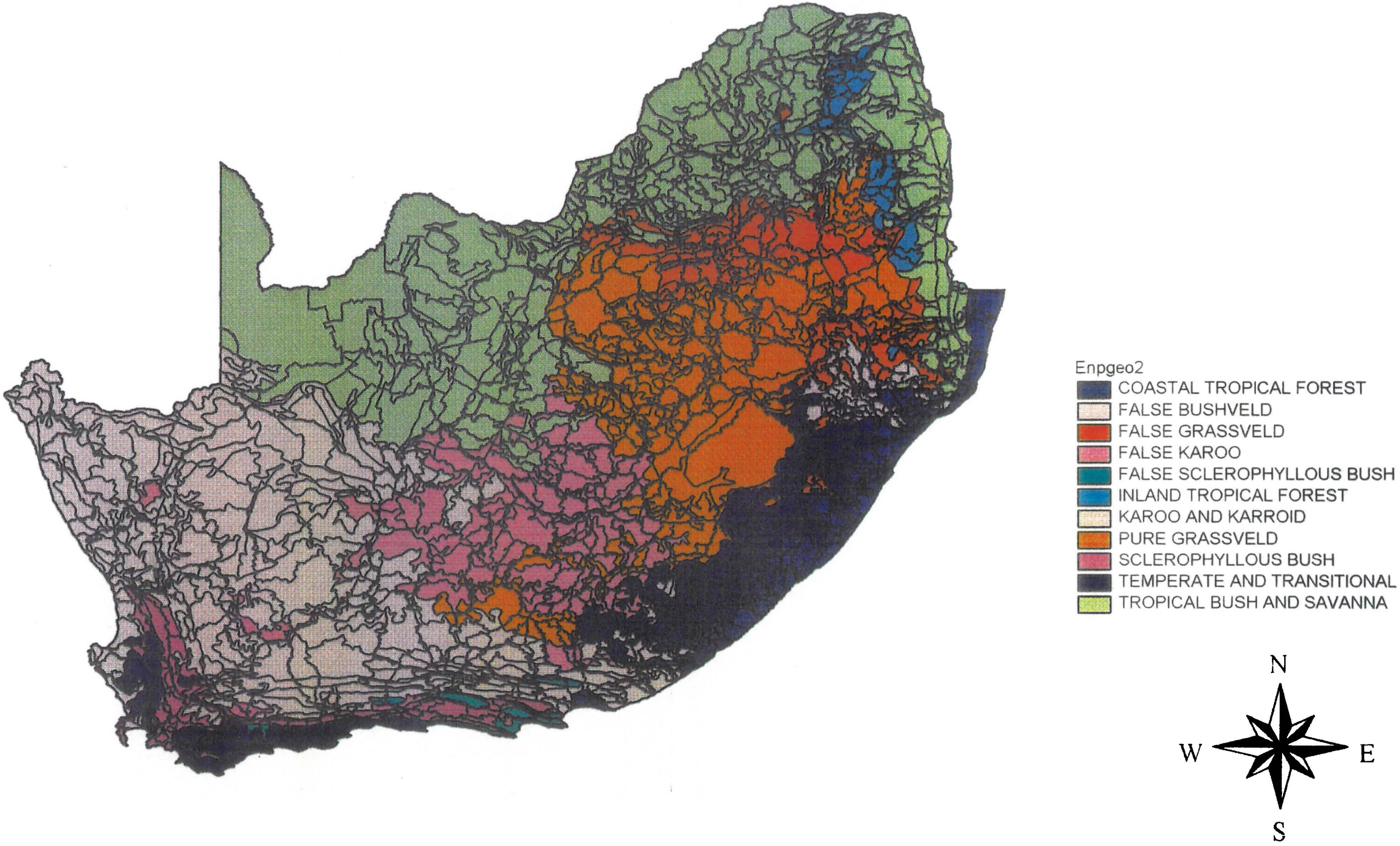


Figure 5.13 : Map showing the relationship between the 10 vegetation types in South Africa and morphometrically identified *M. natalensis* from various localities in South Africa. Keys indicate vegetation types.

Morphometrically Identified M. natalensis



Vegetation types Map



outbreak areas.

Of the 168 QDS in which morphometrically identified *M. natalensis* occurs, 1.8% (3) coincided with QDS in which plague outbreaks have been recorded. Of the 300 QDS in which morphometrically - identified *M. coucha* occurs, 15% (44) coincided with QDS in which plague outbreaks have been recorded. The correlation between historical outbreaks of bubonic plague and the morphometrically identified species distributions is not as clear as indicated by karyotyped data, but there is a stronger association between plague outbreak areas and distribution of *M. coucha*.

The studies by Gordon (1984) and Taylor *et al.*, 1981 were based on a very limited cytogenetic surveys, which indicated greater geographic separation between the two species than the morphometrically identified data shows. It is likely that both species are responsible for transmission of the disease, as several rodent species (such as *Rattus rattus*, *Otomys, Rhabdomys*, and *Tatera brantsi)* have been implicated in plague transmission (Davis, 1964). This indicates that susceptibility to the plague bacillus may not be species - specific. It is more probable that climatic factors such as rainfall (Davis, 1964), or other factors like soil types (Arntzen[4], pers comm) and the distribution of the flea species are more important in providing suitable environment for the maintenance of the plague bacillus in enzootic areas. It remains to be established if both *M. coucha* and *M. natalensis* are semi - commensal, as this aspect may help to clarify which of the two species is responsible for transmission of the plague bacillus from wild reservoirs (such as gerbils) to humans.

Plague outbreaks and environmental parameters.

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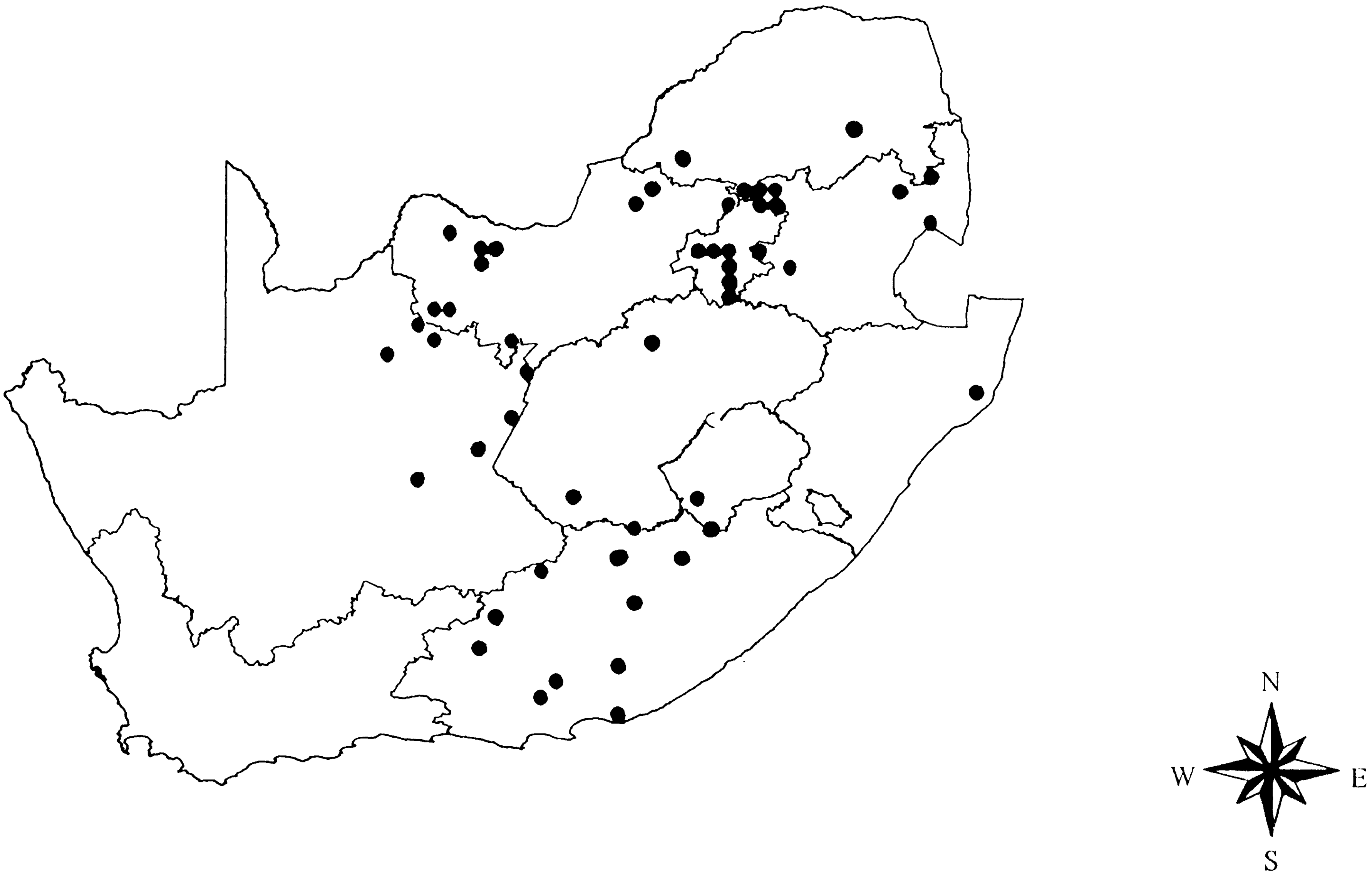
Figure 5.14 : Map showing the relationship between bubonic plague outbreak areas in South Africa and cytogenetically identified *M. coucha* from various localities in South Africa.

Cytogenetically Identified M.coucha



Plague Distribution in South Africa

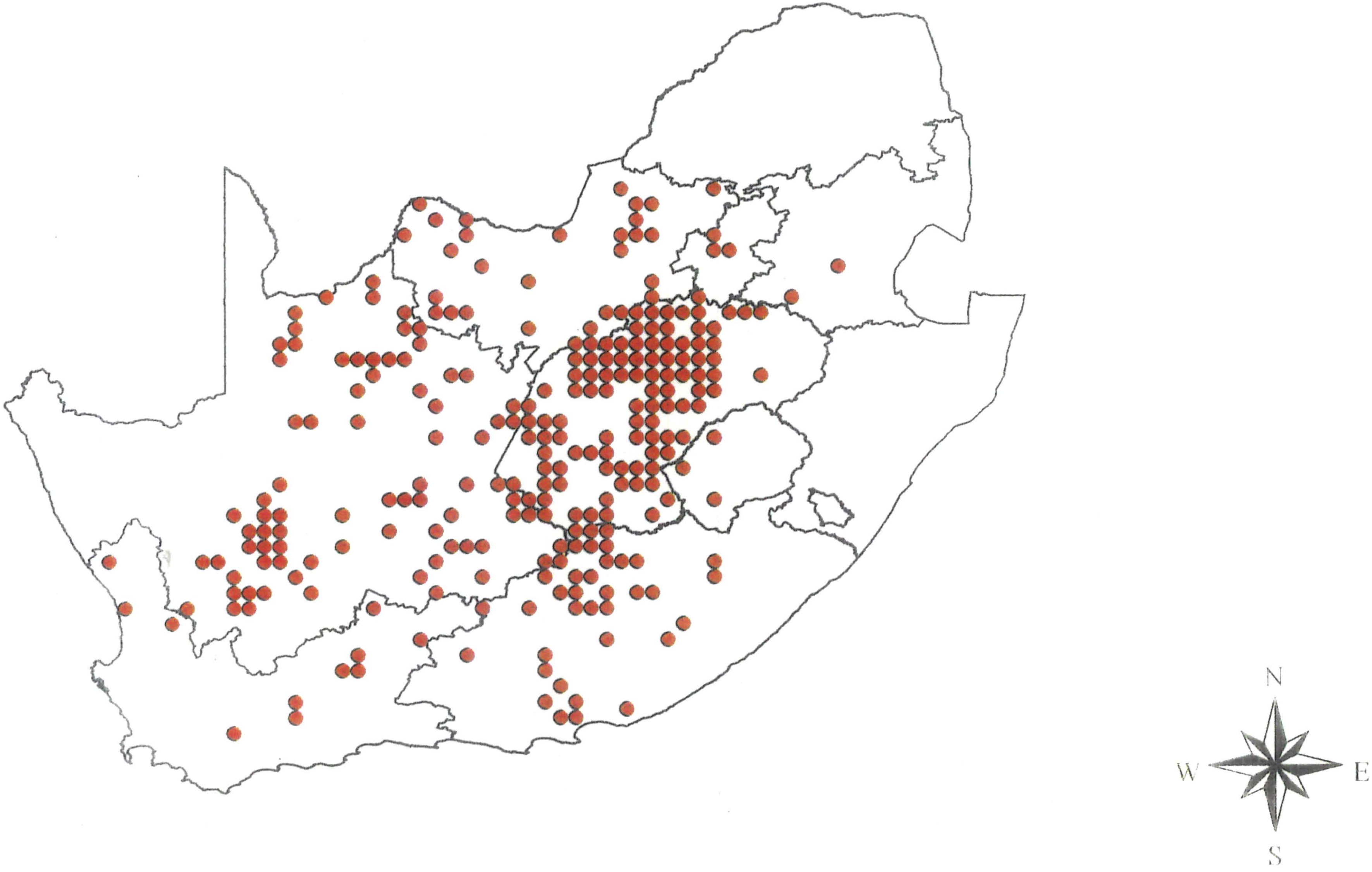
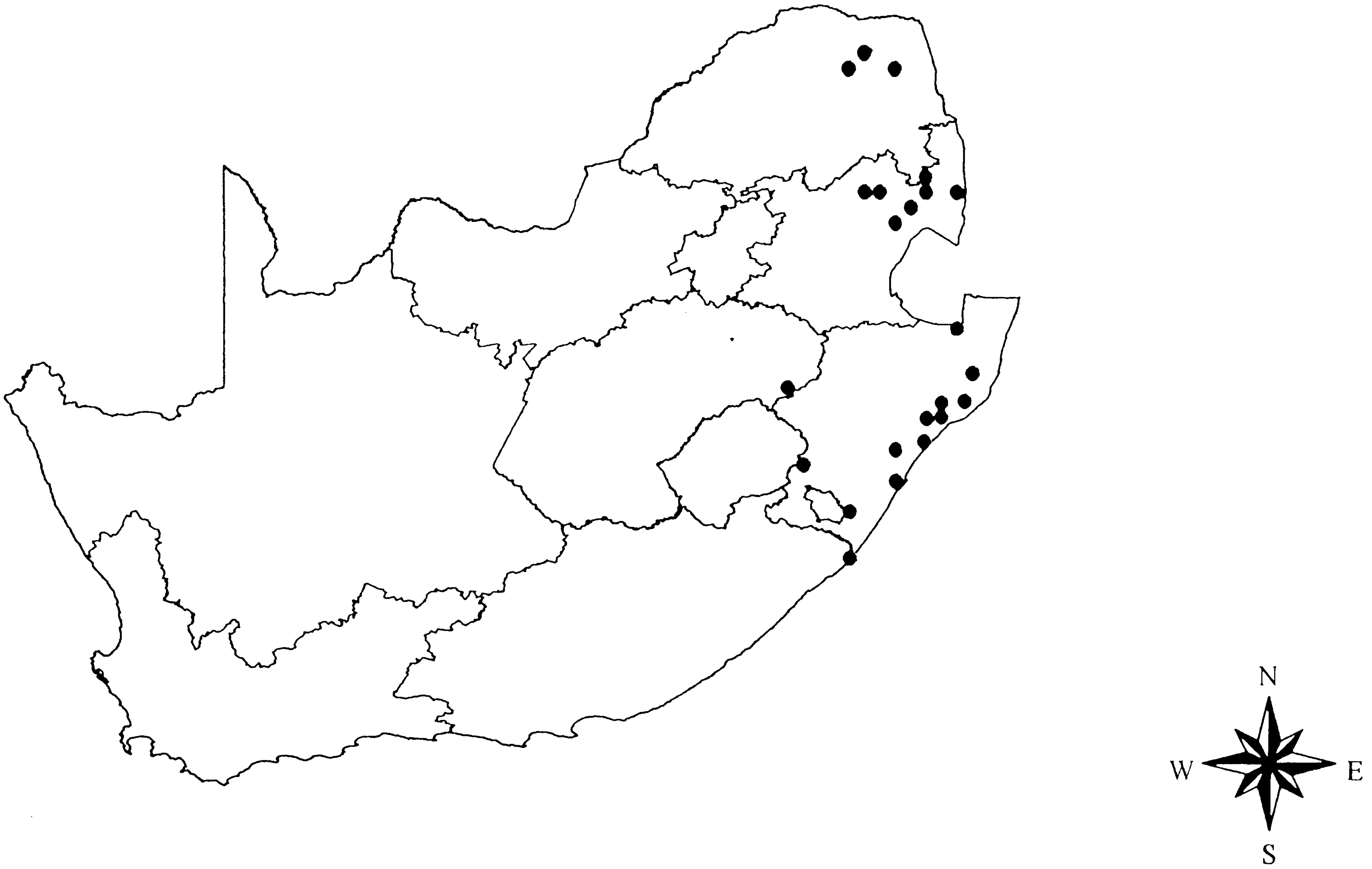


Figure 5.15 : Map showing the relationship between bubonic plague outbreak areas in South Africa and cytogenetically identified *M. natalensis* from various localities in South Africa.

Cytogenetically Identified M.natalensis



Plague Distribution in South Africa

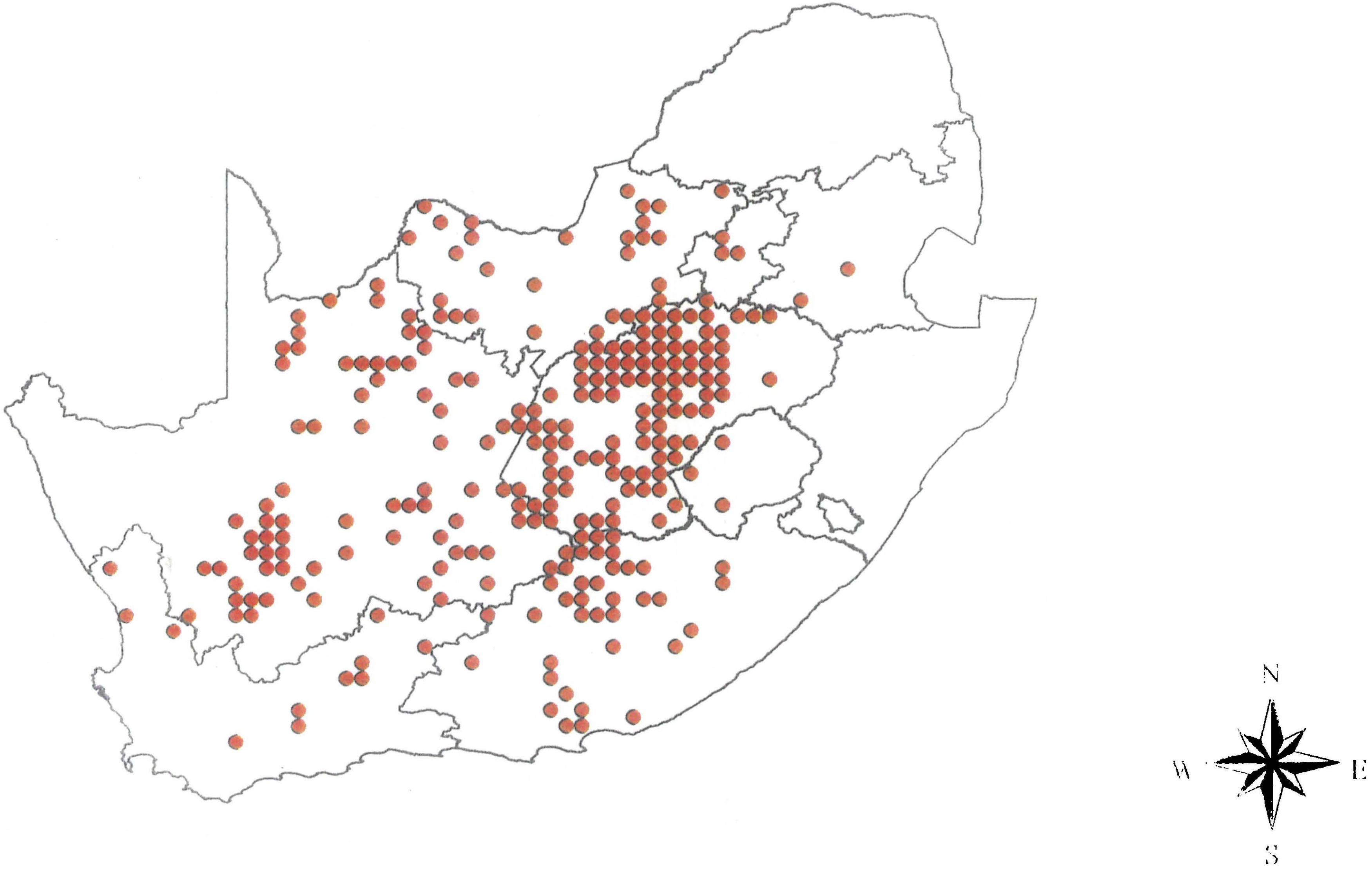
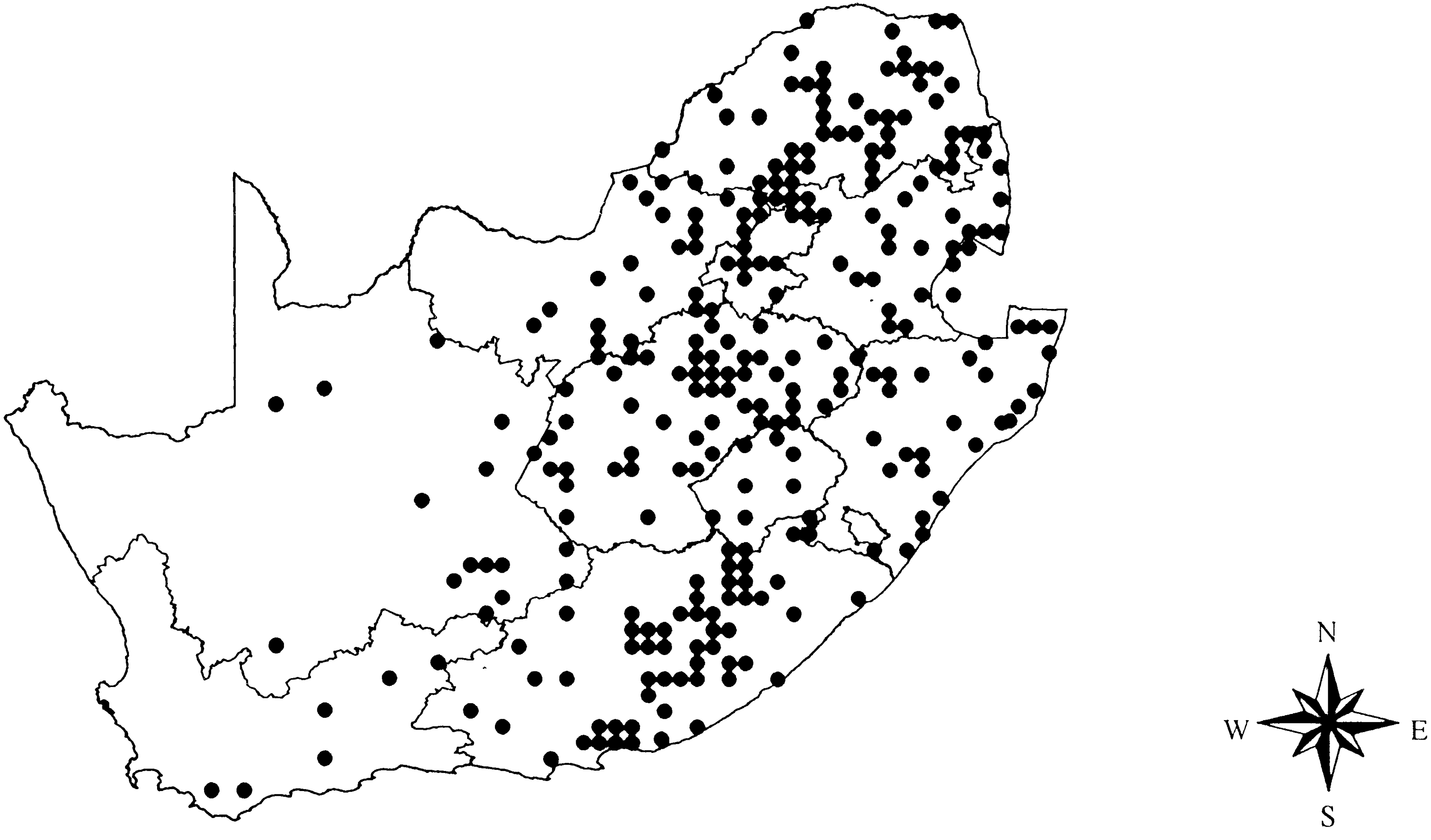


Figure 5.16 : Map showing the relationship between bubonic plague outbreak areas in South Africa and morphometricaly identified *M. coucha* from various localities in South Africa.

Morphometrically Identified M. coucha



Plague Distribution in South Africa

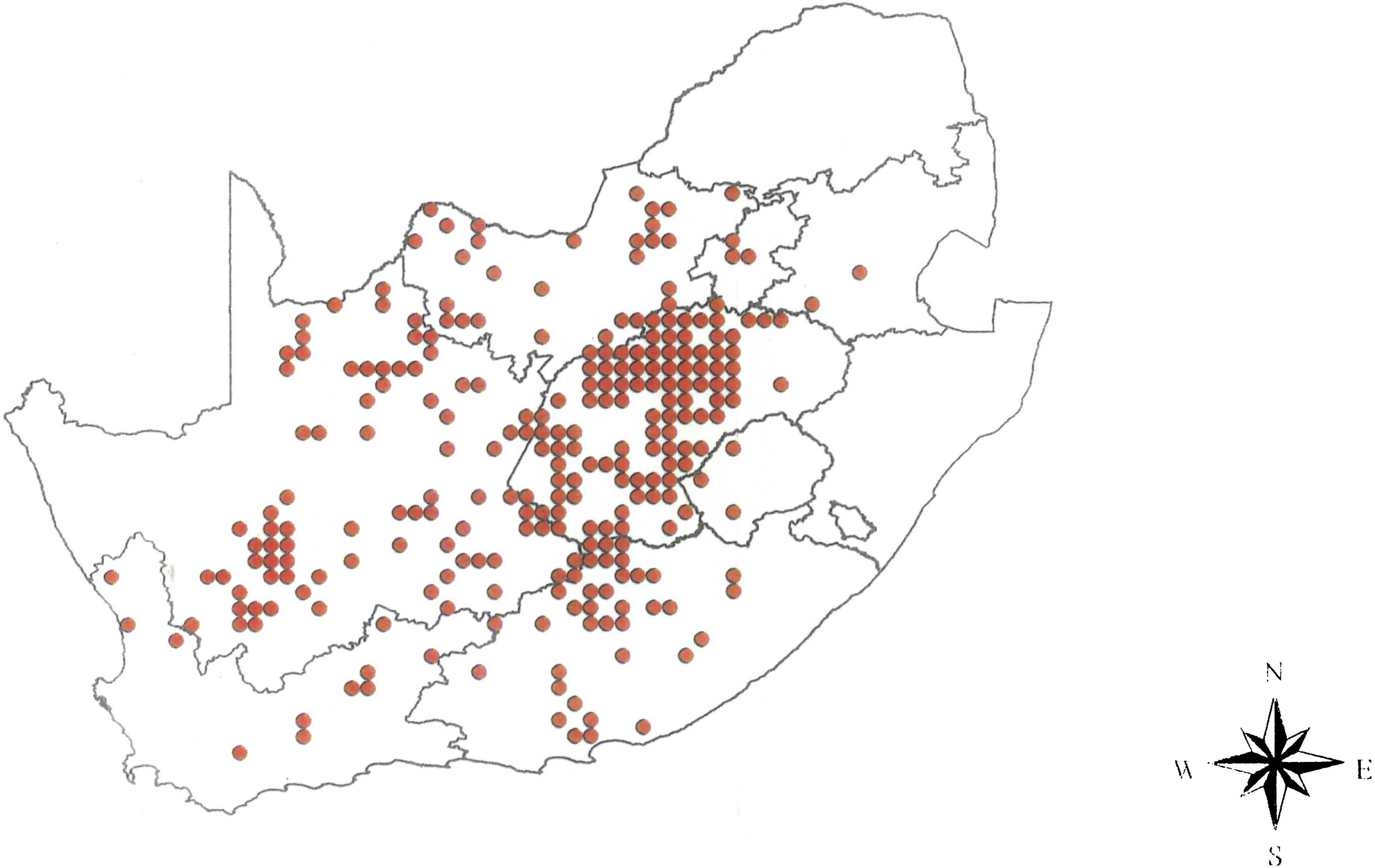
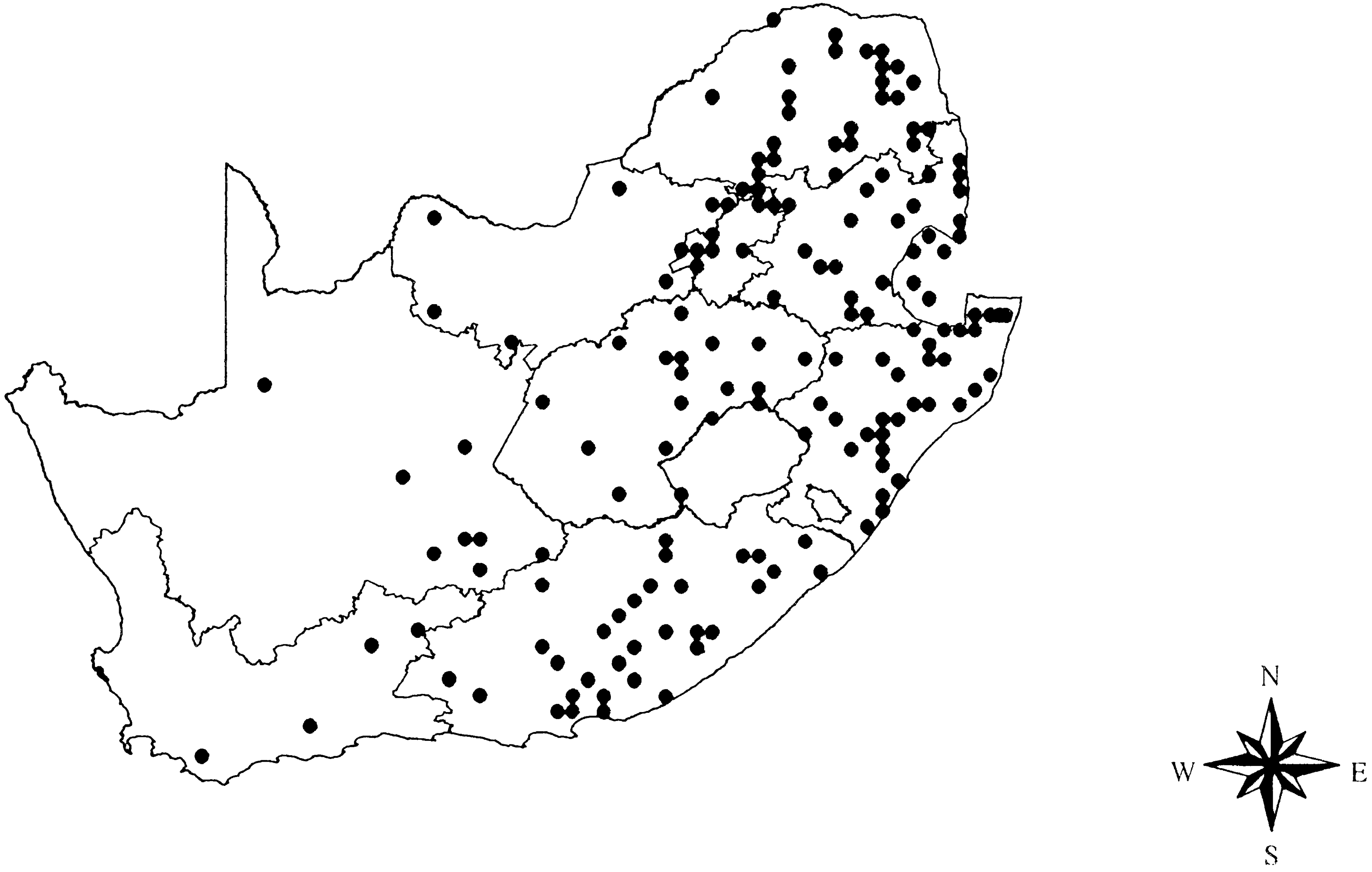
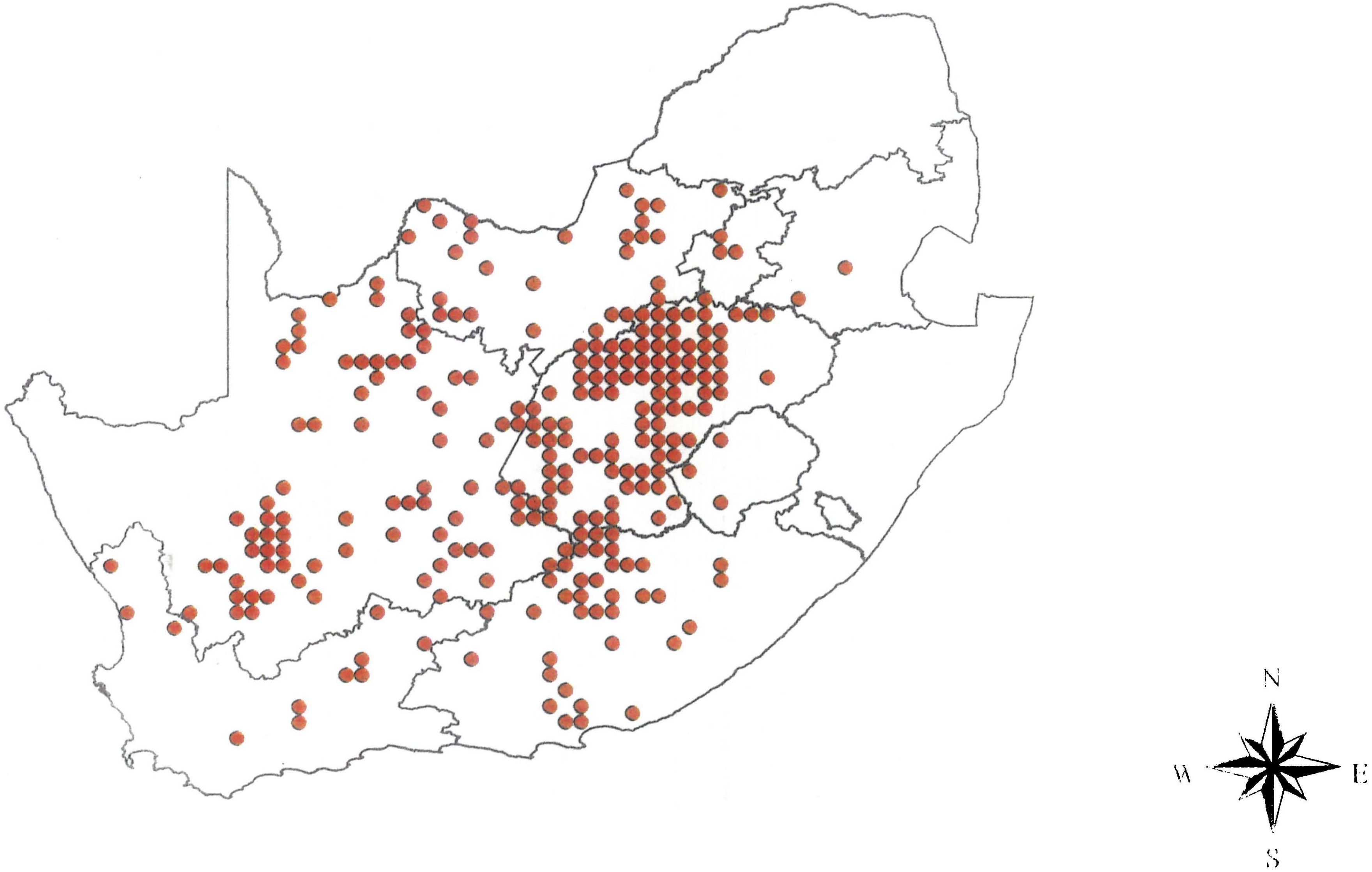


Figure 5.17 : Map showing the relationship between bubonic plague outbreak areas in South Africa and morphometricaly identified *M. natalensis* from various localities in South Africa.

Morphometrically Identified M. natalensis



Plague Distribution in South Africa



Results showing the frequency of QDS in which plague outbreaks have been recorded in relation to mean annual rainfall, altitude zones and vegetation zones are shown in Table 5.1, and are illustrated in Figures 5.18 - 5.20. A broad association between the distribution of plague outbreaks and rainfall zones was evident (Figure 5.18). Approximately 95 % of the localities fell in areas receiving less than 700 mm rainfall a year. This association between mean annual rainfall and plague distribution data was also noted by Davis (1964), who observed that human plague outbreak areas in southern Africa occur in areas receiving 123 - 613 mm rainfall a year, thus correspondence between karyotyped distribution of *M. coucha* and plague is probably coincidental.

The relationship between plague outbreak distribution and altitude was not so clear, except 94,16% of the locality data points fell below the 1800 m altitude zone (Figure 5.19).It does not seem that altitude on its own is a major factor in plague outbreak distribution. It is more likely that the altitude gradients are correlated with rodent species distribution, or other environmental factors such as rainfall and soil types.

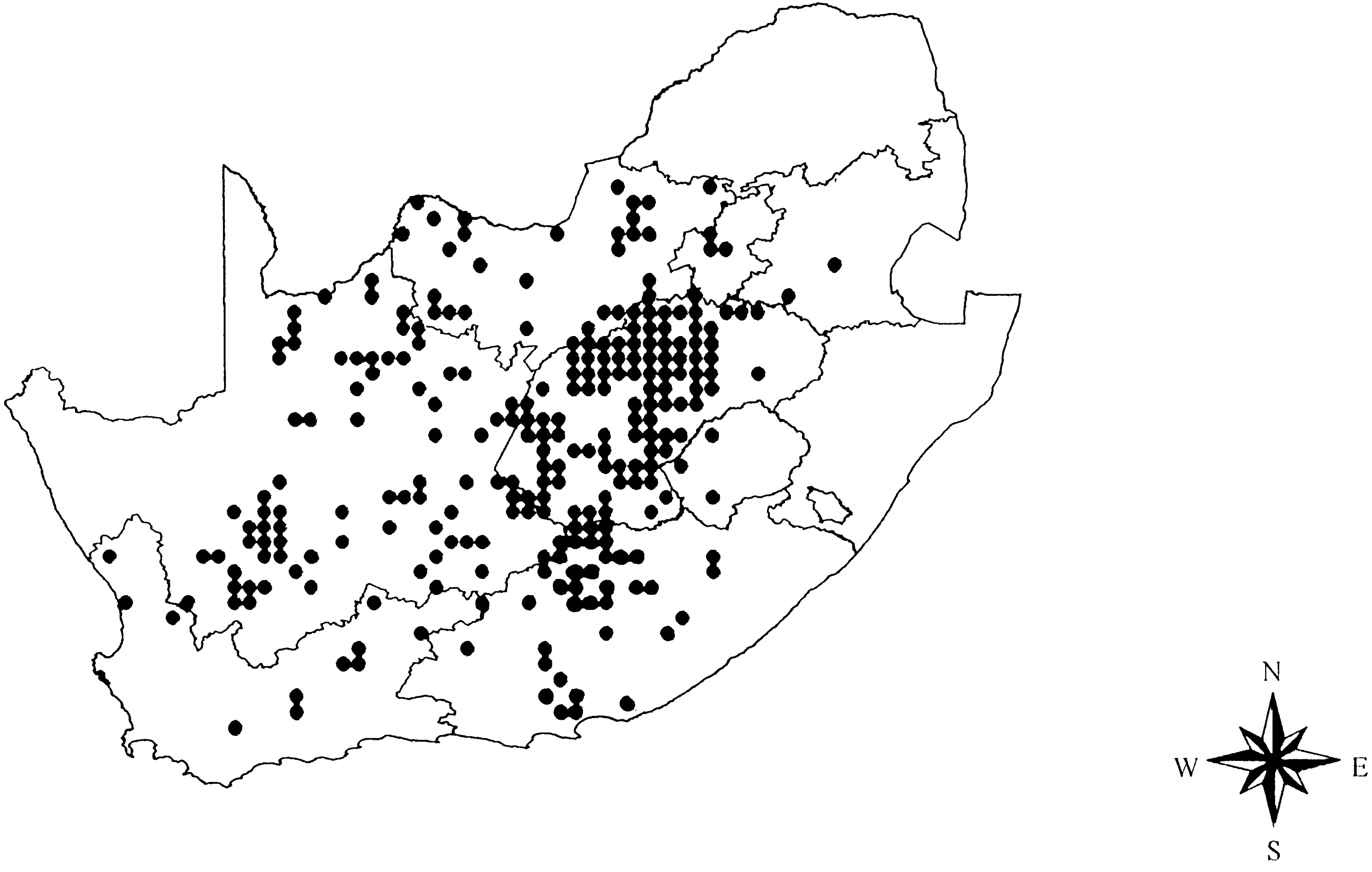
In the case of the vegetation zones, historical plague outbreaks fell in all vegetation types, except Coastal Tropical Forest and Inland Tropical Forest vegetation types. There does not seem to be a strong link between vegetation types and plague outbreak areas. In some vegetation areas, for example in the case of the Tropical Bush and Savanna vegetation area, plague outbreaks are prominent in some parts of it, whereas in other parts of this vegetation type plague outbreaks have not been recorded (Figure 5. 20).

5.4. CONCLUSION.

Although molecular or biochemical techniques are probably the only taxonomic tools that can identify species of *Mastomys* with certainty, the congruence of the morphometric

Figure 5.18 : Map showing the relationship between mean annual rainfall and bubonic plague outbreak areas in South Africa. Rainfall ranges are indicated as follows :
1 = 99 - 236 mm\yr, 2 =236 - 372 mm\yr, 3 = 372 - 509 mm\yr, 4 = 509 - 646 mm\ yr,5 = 646 - 782 mm\yr, 6 = 782 - 919 mm\yr,
7 = 919 - 1055 mm\yr, 8 = 1055 - 1192 mm\yr.

Plague Distribution in South Africa



Rainfall Map

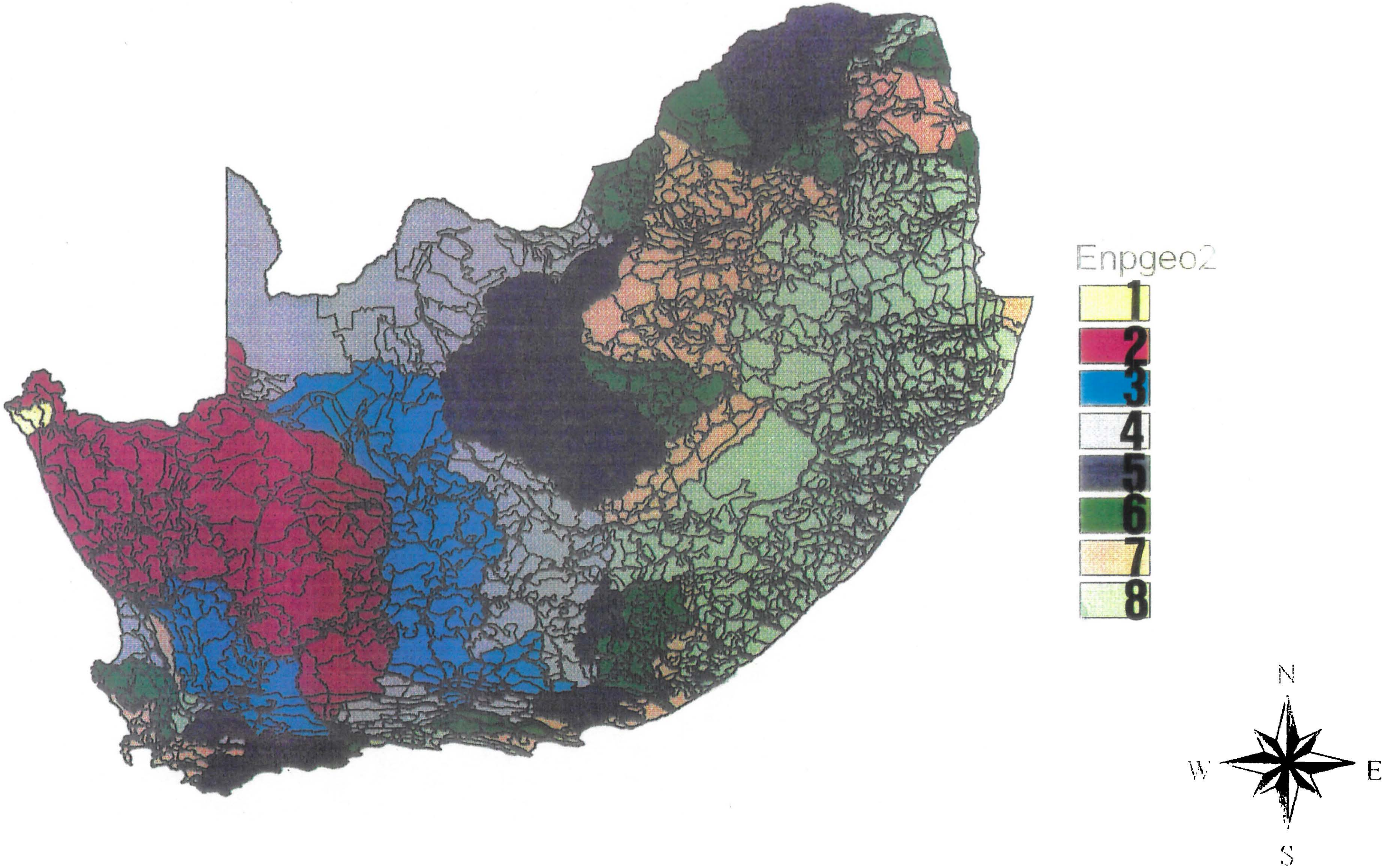
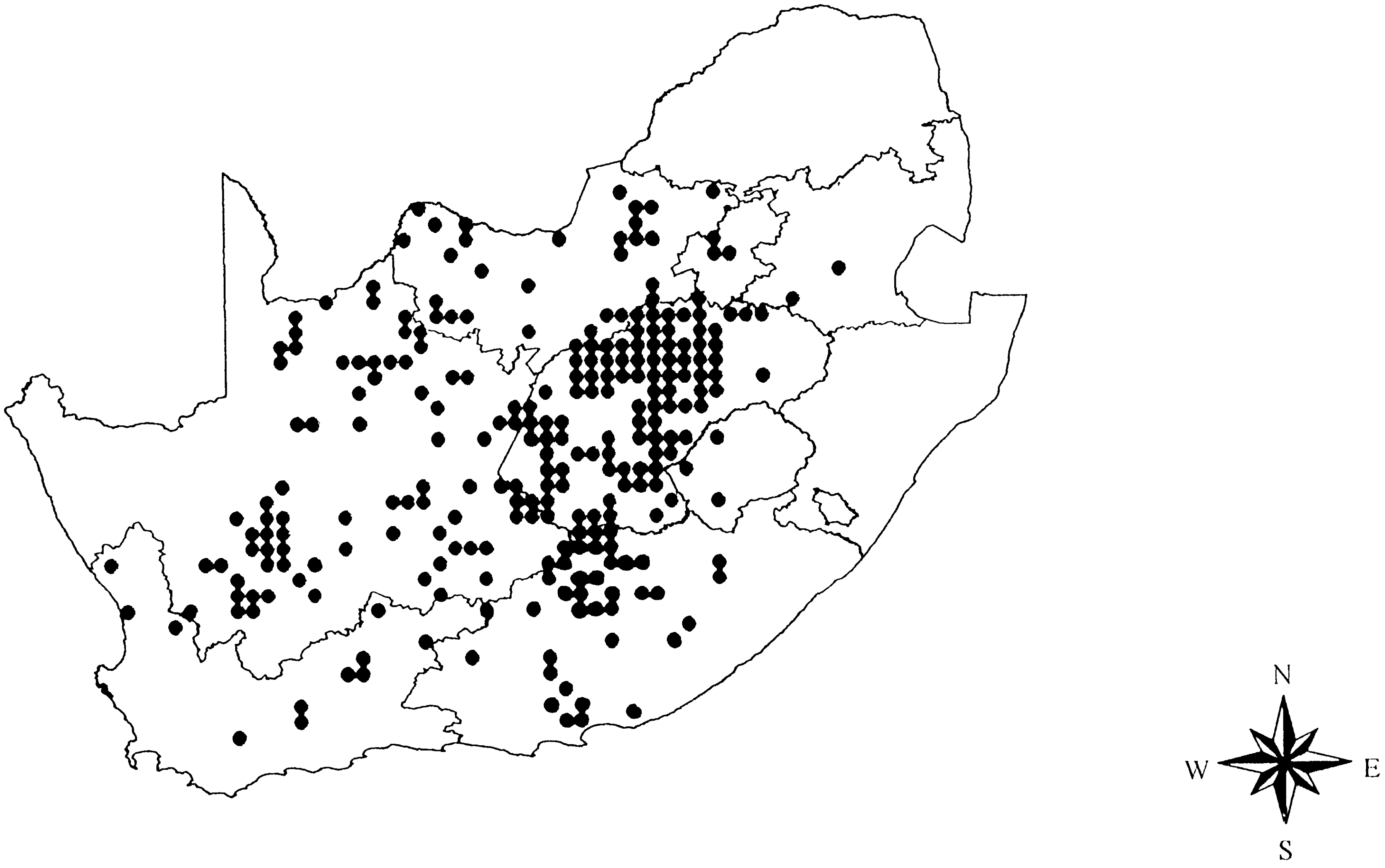


Figure 5.19 : Map showing the relationship between altitude ranges and bubonic plague outbreak areas in South Africa.Altitude ranges are indicated as follows, 1 = 0 - 400m, 2 = 401 - 800m, 3 = 801 - 1200, 4 = 1201-1400, 5 = 1401 - 1800m, 6 = 1801 - 2200m, 7 = 2201 - 2601m, 8 = 2601 -3000m.

Plague Distribution in South Africa



Altitude Map

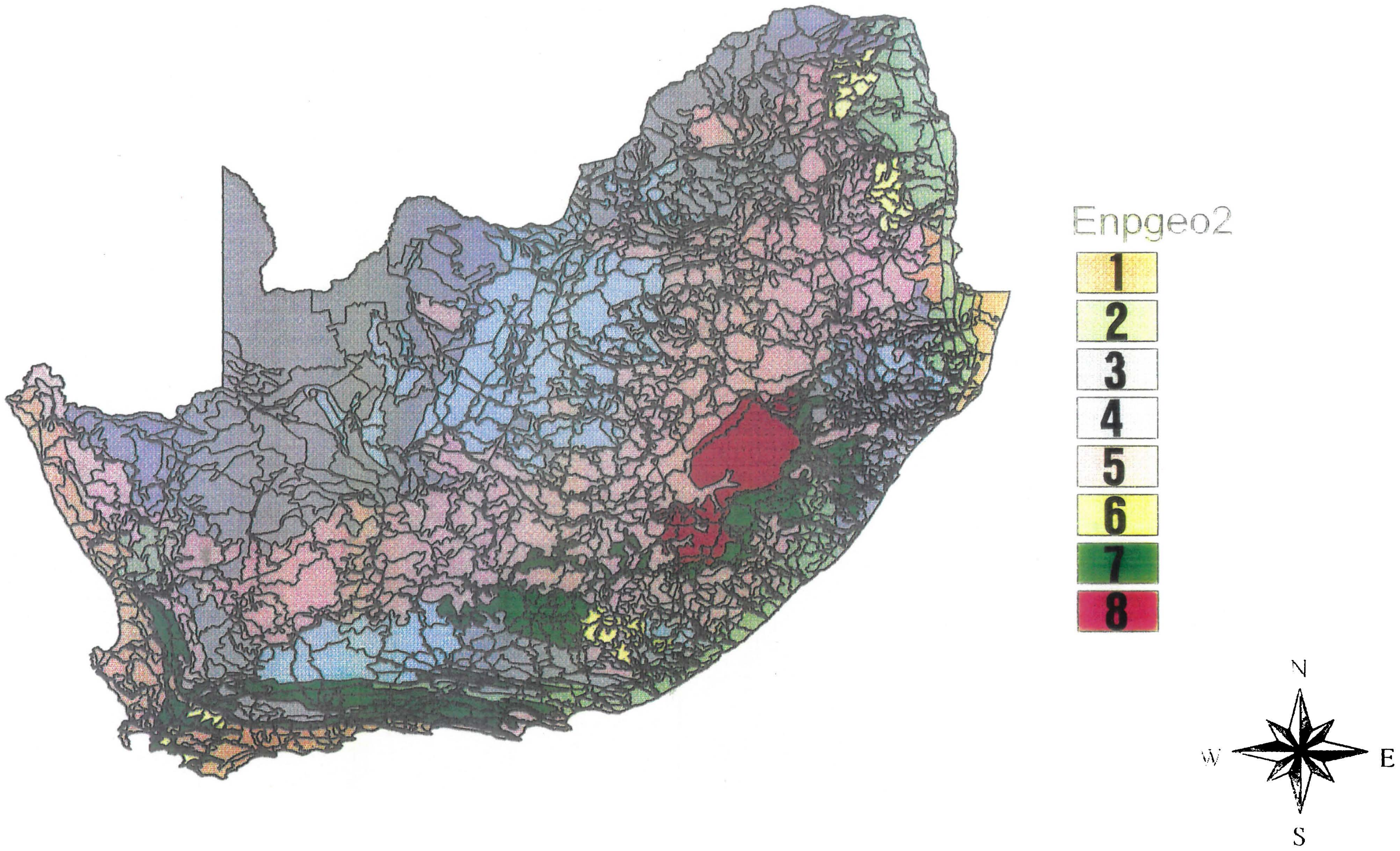
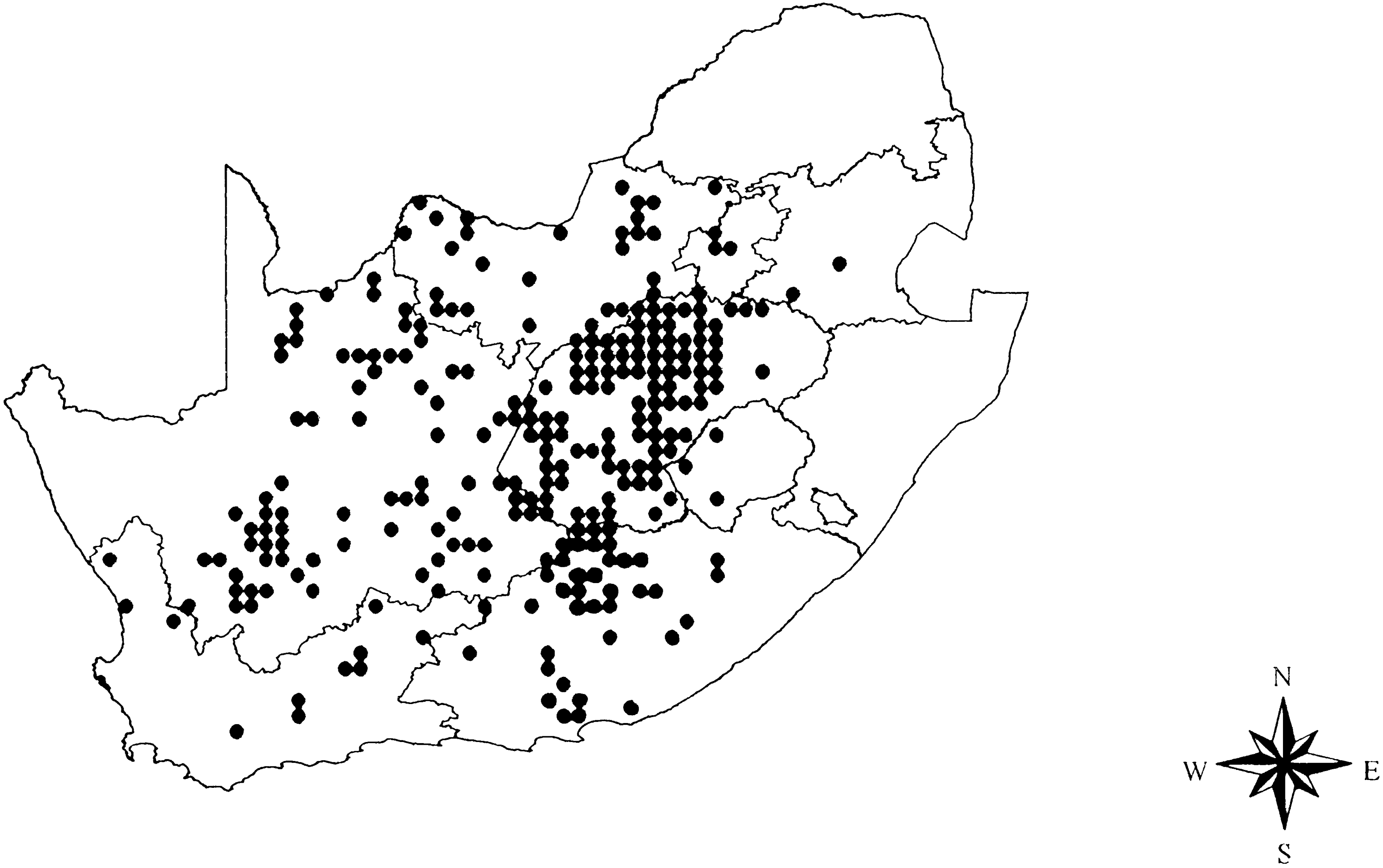
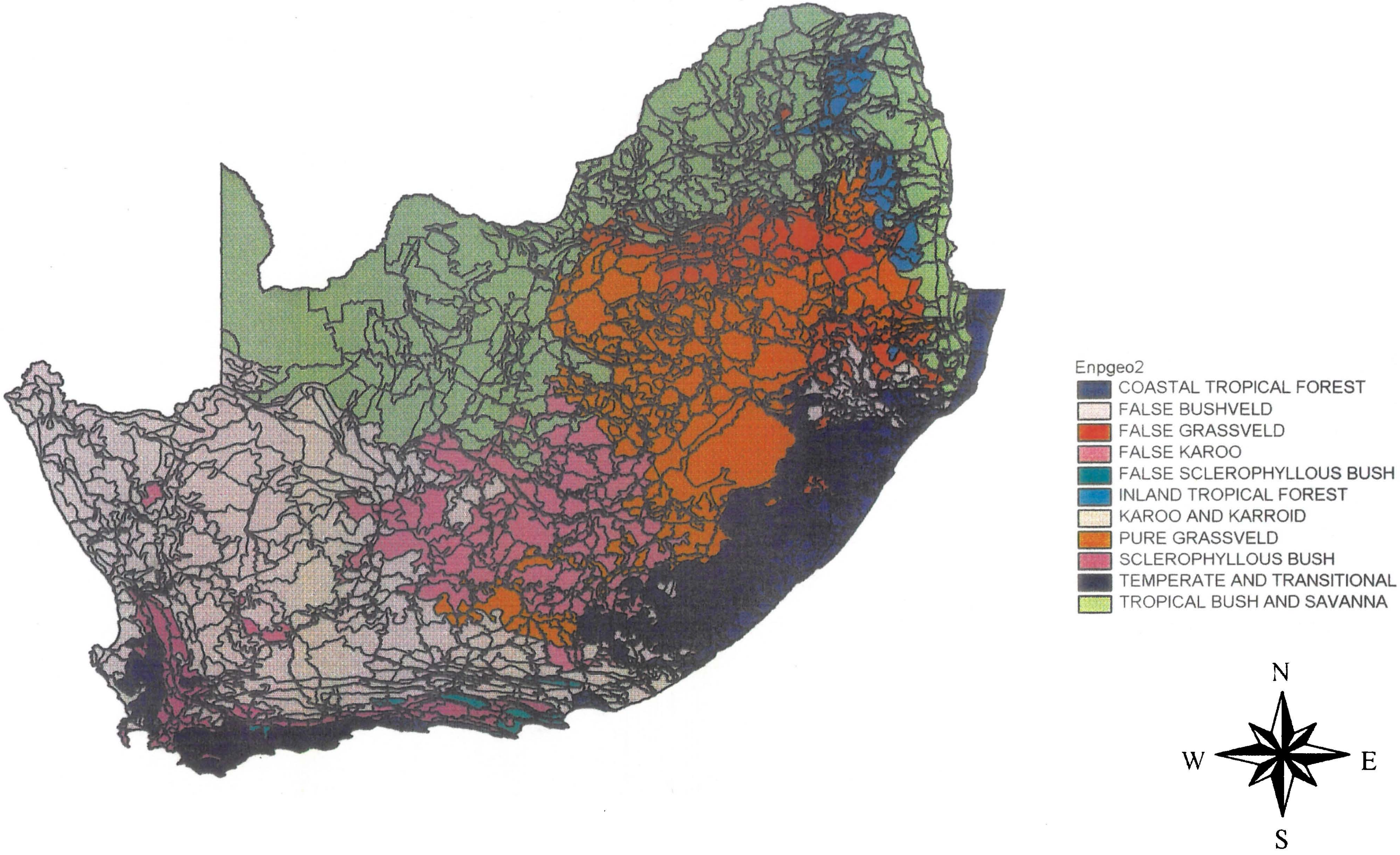


Figure 5.20 : Map showing the relationship between the 10 vegetation types and bubonic plague outbreak areas in South Africa. Keys indicate vegetation types.

Plague Distribution in South Africa



Vegetation types Map



distribution data in the current study with that of Taylor (1995) and Chimimba (pers comm) suggest that the current results are largely reliable. Furthermore, *a posteriori* applications of the discrimination techniques resulted in largely accurate identification of the species.

Current results, however, have taxonomic implications since the names *M. coucha* and *M. natalensis* were assigned on basis of lack of sympatry at type localities. My results show that both species occur in these localities. Since the two species overlap broadly in their respective distribution ranges, reliable information on the ecological and epidemiological roles of the two species cannot be extrapolated from current distribution data. The current study, however, does provide discrimination techniques which can be used to clarify the identity of the species studied in previous investigations.

SUMMARY

Multivariate morphometric techniques were used to discriminate the two - chromosomally defined sibling species of the multimammate mice, *Mastomys coucha* (A . Smith, 1836) and *M. natalensis* (A. Smith, 1834). Seventeen cranial and dental measurements were taken from cytogenetically identified specimens of both species, and from cytogenetically unknown specimens of *M. natalensis s.l.* from various localities in southern Africa. Since age variation is often important in morphometric variation, all specimens were aged and assigned to one six toothwear classes recognised.

Before examining species relationships, data of cytogenetically identified *M. coucha* and *M. natalensis* were analysed for nongeographic variation. Owing to limited sample sizes, nongeographic variation was examined only at the levels of age variation and sexual dimorphism. Results of these analyses showed that age variation was pronounced in both species, as smaller toothwear groups (TW 1 - 3) differed significantly from older toothwear groups (TW 4- 6) in both univariate and multivariate analyses. In both species sexual dimorphism was lacking, and justified pooling of sex groups.

Preliminary analyses based on individual specimens were performed and these were aimed at assessing the homogeneity of samples, and showed that : (1) in each species geographic variation was significant; and (2) age variation was not sufficiently pronounced to obscure interspecific relationships. Subsequent analyses of geographic variation based on mean values of individual or pooled localities from southern Africa showed that specimens from Zimbabwe differed in cranial shape from those from South Africa and Namibia in both species. This suggested that these two geographic groupings should be treated separately when examining interspecific variation. However, the limited number of OTU's from

Zimbabwe impeded separate treatment of Zimbabwe geographic grouping. Consequently, geographic groupings were pooled when examining interspecific variation by PCA and cluster analyses based on mean values. Geographic groupings were treated separately when interspecific relationships were examined by MDA, which was instead based on individual specimens.

Interspecific variation analyses showed that *M. coucha* and *M. natalensis* are morphometrically distinct, in agreement with molecular and biochemical data. The characters influenced discrimination of the two species most strongly, were interorbital breadth (IOB),

post - palatal width (PPW) and mandibular height (MDH). These differences were, however, subtle as evidenced by the overlap of the differential cranial ratios computed by contrasting these variables. The effect of geographic variation on interspecific discrimination was substantial. Principal components analysis, cluster analysis, and MDA all indicated that geographic variation is greater in extent than interspecific variation and thus that it may confound interpretation of patterns of interspecific variation.

To maximise the probability of correct classification of cytogenetically unknown specimens, two regional MDA were computed. When morphometrically identified specimens were plotted on a map, these showed that the distributions of the two species overlapped considerably. Overlay of the two species distributions with rainfall data, indicated that the majority of *M. coucha* localities coincided with drier areas, whereas the majority of *M. natalensis* tended to occur in wetter areas. A positive correlation was, however, lacking when the two species distribution data was overlaid with altitude, vegetation and plague data.

OPSOMMING

Meervoudige morfometriese tegnieke is gebruik om onderskeid te tref tussen twee chromosomaal-bepaalde naverwante vaalveldmuise, *Mastomys coucha* (A. Smith, 1836) en *M. natalensis* (A. Smith, 1834). Sewentien kraniale- en dentale mates is van sitogeneties-bepaalde eksemplare van beide spesies, en van sitogeneties onbekende eksemplare van *M. natalensis* s.l. van verskillende lokaliteite in suidelike Afrika geneem. Weens oudersomsverwante morfometriese variasie is alle eksemplare in een van ses bekende tandslytasieklasse geplaas.

Alvorens die ontleding van spesies-verwantskappe bestudeer is, is data van sitogeneties-geidentifiseerde *M. coucha* en *M. natalensis* geanaliseer vir nie-geografiese variasie. Weens beperkte monstergrootte was die ontleding van nie-geografiese variasie tot vlakke van ouderdomsvariasie en geslagsdimorfisme beperk. Ouderdomsvariasie was belangrik in beide spesies en jonk tandslytasiegroepe (TW 1 - 3) het betekenisvol verskil van ouer tandslytasiegroepe (TW 4 - 6) in beide enkelvoudige en meervoudige ontledings. Weens die ontbreking van geslagsdimorfisme in beide spesies was dit geregverdig om die inligting vir die geslagte saam te voeg.

Voorlopige ontledings van individuele eksemplare om die homogeniteit van die monsters te bepaal toon dat: (1) geografiese variasie betekenisvol was vir elke spesies; en (2) dat ouderdomsvariasie nie genoegsaam was om interspesifiese verwantskappe te verberg nie. Daaropvolgende ontledings van geografiese variasie, gebaseer op gemiddelde waardes van individuele of gesamentlike lokalitiete van suidelike Afrika, het in beide spesies getoon dat eksemplare van Zimbabwe in kraniale vorm verskil van die van Suid Afrika en Namibië. Dit blyk dus dat die twee geografiese groepe afsonderlik ondersoek moet word vir interspesifiese

variasie. Die beperkte operasionele taksonomiese eenhede vir Zimbabwe het egter die afsonderlike behandeling van dié geografiese groep belemmer. Gevolglik is geografiese groepe saamgevoeg in die ontleding van interspesifiese variasie in die hoofkomponentanalise en groeperingsanalises van gemiddelde waardes. Geografiese groeperings was egter afsonderlik behandel in die ondersoek van interspesifiese verwantskappe deur die gebruik van multigroepdiskriminantanalises.

Ontledings van interspesifiese variasie toon dat *M. coucha* en *M. natalensis* morfometries onderskeibaar is. Hierdie bevinding stem ooreen met die van molekulêre en biochemiese data. Interorbitale breedte (IOB), post-palatale wydte (PPW) en mandibulêre hoogte (MDH) was die mees belangrikste diskriminerende kenmerke. In hierdie geval was die ondersoek gebaseer op individuele eksemplare. Weens oorvleueling van differensiële kraniale verhoudings was hierdie verskille egter gering. Die invloed van geografiese variasie op interspesifiese variasie was aansienlik. Hoofkomponentanalise, groeperingsanalises en multigroepdiskriminantanalises het getoon dat geografiese variasie groter is in omvang as interspesifiese variasie. Die bevinding impliseer dat bogenoemde die interpretasie van interspesifiese variasie beperk.

Om die waarskynlikheid van korrekte klassifisering van sitogeneties onbekende eksemplare te optimiseer is twee streeksverwante multigroepdiskriminantanalises bereken. Die verspreiding van die twee spesies het heelwat oorvleuel wanneer morfometries identifiseerde eksemplare gekarteer is. 'n Ondersoek van die verspreiding van die twee spesies in terme van reenval inligting toon dat *M. coucha* hoofsaaklik in droër gebiede voorkom, terwyl *M. natalensis* neig om in natter gebiede voor te kom. Daar was egter geen positiewe korrelasie tuyssen spesies verspreiding en hoogte-, plantegroei- en plaaginligting nie.

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APPENDIX 1

SPECIMENS EXAMINED AND GEOGRAPHIC LOCALITIES.

Map coordinates and \ or quarter degree grid references were obtained from catalogue cards, specimen label , Southern African Place Names (Leistener & Morris , 1976) and the Zoo Historical Gazetteer (Skead, 1973). The following abbreviations indicate the museum from which specimens were obtained:

South Africa: Durban Museum of Natural Science, (DM); Kaffrarian Museum, King Williams Town (KM); National Museum, Bloemfontein, (NMB); South Africa Museum, Cape Town, (ZM); Transvaal Museum, Pretoria (TM).

Zimbabwe: National History Museum, Bulawayo (NHMZ)

United States of America: National Museum of Natural History, Smithsonian Institution, Washington D.C (USNM) .

1. **Cytogenetically known *M. coucha* and *M. natalensis* :** identified karyotypically or on the basis of Hb electromorphs.

M. natalensis :

NAMIBIA.

MAHANGO NATURE RESERVE, MUKWE, OKAVANGO, 18°10'S 21° 43'E, 1821BA
TM7790,TM37752,TM37773,TM37753,TM37765,TM37782,TM37795,TM37757,
TM37766,TM37768,TM37769,TM37770,TM37771,TM37772,TM37780,TM37783,
TM37784,TM37789,TM37781,TM37785,TM37774,TM37791,TM37794
MAHANGO VILLAGE, POPA CAMP, CAPRIVI, 18°06'S 21°57'E, 1821BB : TM33235,
TM33242
POPA CAMP, CAPRIVI, 18°06'S 21°37'E : TM33217,TM33219,TM32396
RUNTU, 17°55'S 19°46'E, 1719DD : TM32338,TM32341

SOUTH AFRICA.

KWAZULU\ NATAL PROVINCE

BLUFF NATURE RESERVE, TARA RD, DURBAN, 29°55'S 30°59'E : TM38706,

TM38657,TM38661
PIETERMARITZBURG, 29°33'S 30°18'E, 2930CB : TM32387,TM32389,TM32391, TM32392
DURBAN, 29°52'S 31°00'E : TM33428,TM33318,TM33316,TM33311,TM33314, TM33315,TM33317,TM33312,TM33308,TM33310,TM33313,TM33426, TM33424,TM33322,TM33425,TM33427,TM33321,TM33319,TM33320 TM33323,TM38666
FARM HILLTOP, RICHMOND, 30°03'S 30°18'E, 3030AB : TM41032,TM41031, TM41026,TM41033,TM41030
HAZELMERE DAM, VERULAM, 29°36'S 31°01'E, 2931CA : TM39207
HLUHLUWE GAME RESERVE, 28°05'S 32°04'E, 2832AA : TM35542,TM35544, TM35543,TM35540,TM35541,TM44391,TM44405,TM44406,TM44407,TM44408
HLUHLUWE GAME RESERVE, RESEARCH CAMP, 28°04'S 32°02'E, 2832AA : TM44388,TM44376,TM44377,TM44378,TM44379,TM44384,TM44387,TM44390, TM44402,TM44403,TM44404,TM44385,TM44386
LOTENI, DRAKENSBERG, 2929BC : TM32379
NGOME FOREST RESERVE, 27°50'S 31°24'E, 2731CD : TM39142,TM39146
PORT EDWARD, 31°03'S 30°13'E, 3130AA : TM33420,TM33405,TM33419, TM33403, TM33404,TM33406,TM33407,TM33408,TM33411,TM33417,TM33418,TM33416, TM33409,TM33412,TM33423,TM33422
UMFOLOZI GAME RESERVE, 28°20'S 31°52'E, 2831BD : TM35424
UMLALAZI NATURE RESERVE, 28°58'S 31°43'E : TM32388,TM32384,TM32382, TM32386,TM32390,TM32385,TM32383
WYFORD FARM, VAN REENEN, 28°24'S 29°25'E, 2829AD : TM44438,TM44437, TM44439,TM44440,TM43340,TM43341,TM43342,TM43345,TM43112,TM43346, TM44441

FORMER TRANSVAAL PROVINCE (includes the new Northern and Mpumalanga Provinces):

GROENVALY, 24K FROM BADPLAAS, 25°52'S 30°45'E, 2530DC : TM44798, TM44795,TM44796,TM44797,TM44670,TM44671,TM44672,TM44669,TM44668
CHAMBERIE NURSERY, BARBETON,25°47'S 31°03'E, 2531CC : TM44334, TM44347,TM44333,TM44336,TM44338,TM44340,TM44342,TM44343,TM44348, TM44349,TM44350,TM44351,TM44359,TM44360,TM44361,TM44362,TM44365 TM44366,TM44367,TM44335,TM44339,TM44341,TM44345,TM44363, TM44364,TM44346,TM44337
MILITARY BASE, BARBETON, 25°23'S 31°03'E, 2531AC : TM44331, TM44358,TM44329,TM44332,TM44357,TM44327,TM44328
DE HOEK FOREST RESERVE, MAGOEBASKLOOF, 23°50'S 30°02'E : TM40774,TM40775
ENTABENI STATE FOREST, 35 K EAST LOUIS TRICHARDT, 23°03'S 30°13'E : TM41651
PUNDA MARIA, KRUGER NATIONAL PARK, 22°43'S 31°02'E, 2231CA : TM36671
SATARA CAMP, KRUGER NATIONAL PARK, 24°23'S 31°47'E, 2431BD : TM36655, TM36656,TM36669

PRETORIUS KOP, KRUGER NATIONAL PARK, 25°10'S 31°16'E, 2531AB : TM36668
GROENVLEI, NO 353, BELFAST, 25°38'S 30°07'E, 2530CA : TM40911
MABILINGWE AREA, MADIMBO NEAR LIMPOPO, 22°26'S 31°04'E, 2231AC : TM44231,TM44227,TM44228,TM44232,TM44226
MADIMBO MILITARY AREA, 98 KM EAST OF MADIMBO, 22°18'S 30°52'E, 2230BD:TM44294,TM44291,TM44292,TM44293,TM44300,TM44301,TM44302,TM44303 TM44304,TM44305,TM44306
VERLORENVALLEI NATURE RESERVE, 2530AC : TM43755

ZIMBABWE

NORTON, NEAR HARARE, 17°53'S 30°42'E, 1730DC : USNM546434, USNM546436, USNM546437, USNM546379, USNM546403, USNM546365, USNM546364, USNM546369,USNM546370, USNM546373, USNM546386, USNM546385, USNM546418, USNM546419, USNM546361, USNM546360, USNM546366, USNM546363, USNM546362, USNM546371, USNM546367, USNM546368, USNM546375, USNM546372, USNM546374, USNM546383, USNM546382, USNM546384, USNM546388, USNM546380, USNM54640, USNM546433, USNM546429, USNM546426, USNM546423, USNM546412, USNM546448, USNM546425, USNM546417, USNM546420, USNM546421, USNM546439, USNM546440, USNM546442, USNM546443, USNM546378, USNM546435, USNM546441, USNM546401, USNM546427
SINAMOTELLA, WANKIE NATIONAL PARK , 18°35'S 26°18'E, 1826CB : NHMZ73584 NHMZ73588, NHMZ73593,NHMZ73615, NHMZ73585, NHMZ73611, NHMZ73590, NHMZ73623, NHMZ7361, NHMZ73617, NHMZ73605, NHMZ73583, NHMZ73610, NHMZ73586, NHMZ73600, NHMZ73607, NHMZ73597, NHMZ73601, NHMZ73613, NHMZ73614, NHMZ73598, NHMZ73624, NHMZ73594,NHMZ73622, NHMZ73599, NHMZ73618, NHMZ73582, NHMZ73604,NHMZ73620,NHMZ73609, NHMZ73606, NHMZ73589, NHMZ73581, NHMZ73616, NHMZ73621,NHMZ73595

M.coucha

NAMIBIA.

WINDHOEK, 22°34'S 17°06'E, 2217CA : TM32333, TM32334,TM32343, TM32346, TM32347 ,TM32335 ,TM32336, TM32337, TM32340

SOUTH AFRICA.

FORMER CAPE PROVINCE (includes the new Eastern Cape and Northern Cape Provinces) :

15 KM FROM DORDRECHT, 3126AC :TM32320
DORDRECHT, 3127AC : TM32319, TM32332, TM32326, TM32327
30KM N.TARKASTAD, 3226BD : TM32329
ABERDEEN, 3224AC : TM32369, TM32372, TM32351, TM32362, TM32368

ALIWAL NORTH, 3026DA : TM32358
BACKHOUSE (KIMBERLY - DOUGLAS AREA), 2923BB : TM32353, TM29204
BYRON, KURUMAN, 2723CA:TM29230
CROFTON SLABBERT, KURUMAN, 2723 BC : TM29214
DE BAD (BETWEEN KIMBERLY AND DOUGLAS), 2824CC : TM29215
GELUKPOINT, KIMBERLY, 2924AD : TM29234
KIMBERLY, 2824DB : TM29247
FARM VLEIPLAAS, KURUMAN, 27°26'S 23°28'E, 2723AD : TM44469
KURUMAN, 2723AD : TM29236, TM29195, TM29193, TM29238, TM29235, TM33302, TM29240
KURUMAN, MUNICIPAL BIRD SANCTUARY, 27°26'S 23°25'E, 2723AD: TM44466, TM44468, TM44465,TM44458
SELSDEN, ERF 97, KURUMAN, 27°25'S 23°26'E, 2723AD : TM44460 , TM44467,TM44459
MOFFAT CHURCH, KURUMAN, 2722BD : TM33303, TM33307
ULSTER, KURUMAN, 2723AC : TM29232 , TM29242
BARKLY EAST, 3027DC : TM32364, TM32366, TM32357, TM32371
BELVEDERE MOLOP RIVER, 2523DB : TM29243, TM29246, TM29245, TM29244, TM29208
BLACKHEATH, VRYBURG, 2624AA : TM29004
COLESBURG MUNICIPAL PUMPHOUSE, 3025CA : TM32331, TM32321
COURANS DRIFT, KIRKWOOD, 3325AD : TM29207
DE HOOP, VRYBURG, 2624AC : TM29202,TM29214
DEEPLAAGTE, VRYBURG, 2624AB : TM29210, TM29221, TM29198
HOOGMOED, VRYBURG, 2624AD : TM29194
KOLHAAR FARM, VRYBURG : TM29192 , TM29186, TM29220
FARM KLEINFONTEIN, 26 KM ENE NOUPOORT,31°07'S 25°12'E, 3125AB: TM34271 , TM34297
GLENCONNER, (BETWEEN ALICEDALE AND GRAHAMSOTOWN), 3325AC : TM32370 , TM32361, TM32363
GRAHAMSTOWN, 3326BC : TM32359
LADY GREY, 3027CA : TM32378, TM32376
ROODEBLOEM (GRAAF REINET), 3224BA : TM32360
STERKSTROOM, 3126DB : TM32350,TM32373,TM32375,TM323245,TM32318, TM32323,TM32348,TM32356,TM32367,TM32374,TM32377,TM32349
VAALHARTS ESTABLISHMENT, 2724DA : TM29028,TM29021,TM29188,TM29185, TM29013,TM29014,TM29011,TM29197,TM29022,TM29026,TM29187, TM29199,TM29189,TM29196,TM27002,TM29201,TM29229,TM29226,TM29222, TM29183

KWAZULU\ NATAL PROVINCE

NYEKAI, 2832AB : TM29016,TM29009,TM29020

FREE STATE PROVINCE

FICKSBURG, 2827DD: TM29231,TM29237,TM29228,TM29233

FARM SANDVLEI, BETHLEHEM, 2828AC : TM32322
ODENDAALSRUS, KENTUCKY, 2726DC : TM32354,TM32365
SHOW GROUNDS, FICKSBURG, 28°53'S 27°53'E,2827DD : TM41593

FORMER TRANSVAAL PROVINCE (includes the new Northern , Gauteng and Mpumalanga Provinces and parts of the new Northwest Province):

SATARA CAMP, KRUGER NATIONAL PARK, 24°24'S 31°47'E, 2431BD : TM38803, TM36670,TM36673
BERGUS FARM, THABAZIMBI, 24°30'S 27°37'E, 2427BC : TM34279I
BLYBERIVIERSPOORT, 24°01'S 30°04'E, 2430AA: TM43335
BOSKOPDAM NATURE RESERVE, NORTH OF POTCHEFSTROOM), 26°34'S 27°07'E, 2627CA : TM41857
DELMAS, 26°07'S 28°40'E, 2628BA : TM29203,TM29223,TM29225
DELMAS (FARM 225), 2628BB : TM29190,TM29255,TM29206,TM29212,TM29209, TM29217,TM29218,TM29227,TM29213
FARM NOOITGEDACHT,14K FROM POTCHEFSTROOM, 26°55'S 27°10'E, 2627CC : TM45016,TM45011,TM45010,TM45015,TM45013,TM45012,TM45017
GROOT MARICO NURSERY FARM, 25°35'S 26°25'E, 2526CB: TM33299,TM33431, TM33287,TM33260,TM33269,TM33298,TM33256,TM33257,TM33263,TM33264, TM33268,TM33271,TM33274,TM33280,TM33282,TM33284,TM33290,TM33296, TM33434,TM33435,TM33294,TM33252,TM33258,TM33259,TM33261,TM33267, TM33270,TM33275,TM33276,TM33277,TM33278,TM33283,TM33285,TM33286, TM33289,TM33293,TM33297,TM33433,TM33262,TM33272,TM33273,TM33279, TM33288,TM33295,TM33300,TM33432,TM33301,TM33254,TM33281
KOMATI POWER STATION, BETHAL, 26°06'S 29°29'E, 2629AB : TM42410, TM42413,TM42414,TM42408,TM42409,TM42411,TM42415,TM42416,TM42417, TM42412
LODWICHS LUST FARM, HECTORSPRUIT, 25°23'S 31°42'E, 2531BC : TM33326, TM33330,TM33332,TM33324,TM33327,TM33328,TM33329,TM33331,TM33333
MALMANIESOOG N. ZEERUST, 2526CC : TM42045
NOOITGEDACHTDAM NATURE RESERVE, 25°57'S 30°02'E, 2530CC : TM42429
RIETFONTEIN, JOHANNESBURG , 26°03'S 28°04'E, 2628AB : TM29007,TM29029, TM29005,TM29012,TM29224,TM29211
SPRINGS MUNICIPAL BIRD SANCTUARY, 26°13'S 28°27'E, 2628AB : TM42446, TM42447
SUIKERBOSRAND NATURE RESERVE, BRAKPAN, 26°32'S 28°12'E, 2628CA : TM41856 RIETVLEI DAM, IRENE (PRETORIA), 25°53'S 28°17'E, 2528CD : TM41122 , TM41121,TM41123,TM41125,TM41124
ROODEPLAAT DAM, 20K NE PRETORIA, 25°39'S 28°21'E, 2528CB : TM38801
FAERIE GLEN NATURE RESERVE, PRETORIA, 2547S 2817E, 2528CD : TM39263
FONTANA MILITARY AREA NEAR PETRONELLA, 2530S 2817E, 2528AD : TM41008,TM41009,TM41010
HARTEBEESHOEK PLOT 30, 25°40'S 28°05'E, 2528CA : TM44222,TM44180, TM44182,TM44186,TM44187,TM44178,TM44179,TM44183,TM44181,TM44184, TM44185

SOUTPAN AGRICULTURAL EXPERIMENTAL FARM, 25°23'S 28°05'E, 2528AC : TM34286,TM34281,TM34284,TM34285,TM34282,TM34280,TM34287,TM34283
UNIVERSITY EXPERIMENTAL FARM, PRETORIA, 25°46'S 28°15'E, 2528CC : TM44938,TM44941,TM44945,TM44928,TM44930,TM44931,TM44932,TM44933, TM44934,TM44942,TM44927,TM44929,TM44943,TM44944,TM44939,TM44940
VERWOEDBURG, CAMBRIDGE RD, 25°52'S 28°14'E, 2528CC : TM41024,TM41025
WAPADRAND X 5 (ERF 318), PRETORIA, 25°47'S 28°20'E, 2528CD : TM43338, TM43339,TM43337

ZIMBABWE

NORTON, NEAR HARARE, 1753S 3042E, 1730DC : USM546376, USM546390, USNM546394,USNM546395,USNM546397,USNM546396,USNM546432,USNM546428, USNM546406,USNM546422,USNM546445,USNM546381,USNM546393,USNM546398, USNM546399,USNM546400,USNM546431,USNM546430,USNM546424,USNM546411, USNM546409,USNM546407,USNM546413,USNM546414,USNM546416,USNM546438, USNM546444,USNM546446,USNM546402,USNM546391,USNM546389,USNM546392, USNM546410,USNM546415

2. **Previously unknown** ***Mastomys natalensis s.l.*** : identified as *Mastomys natalensis* and *Mastomys coucha* on the basis of the discriminant scores generated from the regional two group discriminant functions analyses. Calculating a discriminat score is described in Chapter 4, and standardized coefficients and overall means are given in Table 4.12 and Table 4.13. Question mark preceding specimen number denotes that the specimen was classified as ambiguous.

LESOTHO.

MASERU, 2927AD; *M.coucha*: ZM8237,ZM8235,ZM8236
BOTSOELA, MAFETENG, 2927DC; *M.coucha*: NMB8635
MARAKABEI, 2928CA; *M.coucha*: NMB7329,NMB7341,NMB7340
MALHANAPENG, THABA-ISEKA, 2928DA; *M.coucha:* NMB8154,NMB8153
MATEANONG, MOKHOTLONG,2929AC; *M.coucha*:NMB6972,NMB6915,NMB6889 NMB6896, NMB6918; *M.natalensis:* NMB6940, NMB6919, NMB6941, NMB6917, NMB6939, NMB6921, NMB6922, NMB6970, NMB6930
MT. MOOROSI, QUTHING,3027BB; *M.natalensis:*NMB8216

SOUTH AFRICA

FORMER CAPE PROVINCE (includes the new Eastern Cape, parts of the Western

Cape and Northern Cape Provinces)

KING WILLIAM'S TOWN AND BLUE QUARRIES\GOLF COURSE, KWT 32°52'S 27°23'E, 3227CD; *M.coucha*: KM7094,KM24415,KM18429,KM10996,KM18385, KM18439, KM18387,KM20887,KM20884,KM10995,KM18384,KM18445,KM18399, KM20889,KM20888,KM20892,KM20891,KM20890,KM7022,KM7044,KM7076,KM7095, KM7091,KM9335,KM7079,KM7078,KM6769,KM5692,KM7030,KM7047,KM7061, KM7059,KM7063,KM7058,KM7066,KM7065,KM7073,KM7083,KM7081,KM7090, KM7104,KM7096,KM7610,KM7609,KM7613,KM7021,KM7050,KM7070,KM7069, KM7085,KM7103,KM6772,KM9336,KM7084,KM7612,KM7106,KM7071,KM7082, KM18419,KM18410,KM18408,KM18432,KM18430,KM20893,KM18413,KM18406,KM20886,KM18342,KM18466,KM18465,KM18383,KM18535,KM7606; *M.natalensis:* KM18440,KM18438,KM7031,KM7026,KM7043,KM7042,KM7041,KM7611,TM6774, KM7088,KM18407,KM18462, KM7064
FARM GLENGLENIFFER, KING WILLIAM'S TOWN, 32°42'S 27°34'E, 3227DA; *M.coucha:* KM33269; *M.natalensis:* KM33272
COFIMVABA\ENGCOBO, 32°00'S 27°35'E, 3227BA; *M.coucha:* KM12710
MIDDELBURG,25°46'S 29°28'E, 2529CD; *M.coucha*: KM11110, KM11109
NEAR UPINGTON,28°26'S 21°14E, 2821AC; *M.coucha*: KM5610
ABERDEEN, 32°27'S 24°03'E, 3224AC; *M.coucha:* KM1855, KM18555
VAALHARTS ESTABLISHMENT, 2724DA; *M.natalensis*: TM29001
MATOPPIE FARM,2523BC, *M.natalensis:* TM21833
40MI W. OF THE WAR GRAVE\ MALOPO RIVER, 2523CA; *M.coucha*: TM7019,TM6893
LANGEDRAAI, 17MI PADDON, MAFEKENG, 2524CA; *M.coucha*: TM21809 *M.natalensis*: TM21776,TM21804,TM21807
FARM VILLA FRANCA,FRQ 11-32,22K VRYBURG,26°52'S 24°52'E, 2624DD; *M.coucha*: TM28215,TM28206
FARM MARTHAVILLE, KURUMAN,27°21'S 23°08'E, 2723AC; *M.natalensis*: TM28180
KURUMAN, 2723AD; *M.coucha*: ZM8217
FARM GROOTFONTEIN,19K SW REIVILO, 27°39'S 24°01'E, 2724CA; *M.coucha*: TM35856
FARM VERGENAL, LOUWSBERG, 27°29'S 31°42'E, 2731BC; *M.coucha*: KM19687, KM19685; *M.natalensis*: KM19688,KM19673,KM19674,KM19749
AUGRABIES FALLL NATIONALPARK, 28°35'S 20°20'E,2820CB; *M.coucha:*TM27441, TM27442; *M.natalensis:* TM27420,TM27421,TM27440
WARRENTON,2824BA; *M.coucha:* TM32342
KEIKAMSPOORT FARM,20K SE PRIESKA, 29°50'S 22°47'E, 2922DD; *M.coucha:* TM27266,TM27186,TM27219; *M.natalensis:* TM27267,TM27220
HOPETOWN,29°46'S 23°30'E,2923CD; *M.coucha:* KM23126,KM23125,KM23122 KM23124,KM23123; *M.natalensis:* KM23127
ALFALFA,HOPETOWN, 2924CA; *M.coucha:* NMB8578,NMB8562,NMB8575, NMB8566,NMB8573,NMB8558,NMB8561,NMB8571,NMB8553,NMB8552
DE AAR,30°45'S 23°54'E, 3023DB, *M.coucha:* KM23121,KM23119,KM23120
DEELFONTEIN, RICHMOND, 3023DD; *M.coucha:* ZM7154,ZM7152,ZM7151,ZM7150 *M.natalensis:* ZM7157,ZM7156

PHILLIPSTOWN,30°10'S 24°46'E, 3024BB; *M.coucha*: KM23137,KM23138,KM23133, KM23136,KM23135,KM23134; *M.natalensis*: KM23131

BRANDLAAGTE, LADY GREY, 3027CC; *M.coucha:* NMB4352

GLEN GYLE, GLEN GREY, 3027DA; *M.coucha*: NMB4478,NMB44477

HALSTONE, BARKLY EAST, 3027DB; *M.coucha:* NMB4947,NMB4949,NMB4956

DRUMBO, BARKLY EAST, 3027DC; *M.coucha*: NMB4534,NMB4539,NMB4536, NMB4533,NMB4538,NMB4537,NMB4535

BRUCEDELL, RHODES,3027DD; *M.coucha:* NMB4226

MILTON, MACLEAR, 3028CB; *M.coucha*: NMB5833

WOODCLIFFE, MACLEAR, 3028CC; *M.coucha*: NMB5916, NMB5913

RISLER, MACLEAR, 3028CD; *M.coucha*: NMB5856,NMB5859,NMB5857,NMB5860 NMB5847,NMB5864,NMB5862, NMB5861,NMB5863,NMB5865

FLAGSTAFF, 30°54'S 29°32'E, 3029DC; *M.coucha:* KM33286, KM33285

PORT ST JOHNS, 30°44'S 30°28'E, 3030CB; *M.coucha*: KM5655,KM5666,KM5658,KM5665,TM12398,TM12407,TM12409,TM12401,TM12410; *M.natalensis*: KM33299KM33300,TM12406,TM12397,TM12400,TM12399, TM12405,TM12411,TM12412,TM12413,TM450,TM452

UMZIMKHULU, EAST GRIQUALAND,30°16'S 29°56'E, 3029BD; *M.natalensis:* TM6777,TM6773

VICTORIA WEST, 31°19'S 23°09'E, 3123AC;*M.coucha*: KM23321,KM23329,KM23334, KM23333,KM23323,KM23339,KM23328,KM23340,KM23320,KM23332,KM23326 KM23338,KM23327,KM23331,KM23325,KM23335,KM23322;*M.natalensis*:KM23337

MURRAYSBURG, 31°54'S 23°36'E, 3123DC; *M.coucha:* KM23129,KM23128

HANOVER, 3124AB; *M.coucha:* ZM7369, ZM7370,ZM7365,ZM7337,ZM7334,ZM7364 *M.natalensis:* ZM7371

FARM KLEINFONTEIN, ENE NOUPOORT, 31°07'S 25°12'E, 3125AA; *M.coucha:* TM34075,TM34088,TM34043; *M.natalensis:*TM34119

FARM RIETFONTEIN, STEYNSBURG\STEYNSBURG, 31°14'S 25°31'E, 3125BA; *M.coucha*: KM25711,KM25709,KM25708,KM25707,KM25706,KM25713,KM25714 KM2571, KM25712,TM32328,TM32345; *M.natalensis*:KM25705,KM25716,KM25715

MODDERFONTEIN,JAMESTOWN, 3126BA; *M.coucha*: NMB5599,NMB5611,NMB5629,NMB5633

GLORIA, JAMESTOWN, 3126BB; *M.coucha*: NMB5033,NMB5043,NMB5053

MARIBA, DORDRECHT, 3126BD; *M.coucha*: NMB5097

KALKOENKRANTZ, STERKSTROOM, 3126DB; *M.coucha:* NMB5403,NMB5449, NMB5446,NMB5401,NMB5425,NMB5445,NMB5471,NMB5441,NMB5404,NMB5448 NMB5455,NMB5402,NMB5492,NMB5408,NMB5405

CARNARVON, STERKSTROOM,3126DC;*M.coucha*: NMB5534,NMB5519,NMB5538, NMB5546,NMB5525,NMB5563,NMB5515,NMB5520,NMB5526,NMB5502,NMB5533 NMB5537,NMB5532,NMB5510,NMB5521,NMB5562,NMB5524

SKOORSTEENMANTEL, JAMESTOWN, 3127AA; *M.coucha*: NMB4850,NMB4812, NMB4844,NMB4841, NMB4810,NMB4849,NMB4832; *M.natalensis:* NMB4847

BARKLY EAST, 31°02'S 27°21'E, 3127AB; *M.coucha:* KM25105,KM25102,KM25104, KM25101,KM25098, KM25097,KM25106,KM25103,KM25095,KM25094,KM25100

FARM VREDEHOEK, BARKLY EAST, 31°02'S 27°21'E, 3127AB; *M.coucha:* KM25096

KIEWIETSHOEK, ELLIOT, 3127AD; *M.coucha:* NMB6147

ROODEKRAAL, INDWE, 3127AD; *M.coucha:* NMB4613,NMB4614,NMB4615
DENORBEN, BARKLY EAST, 3127BA; *M.coucha:* NMB4925
HEIMAT, INDWE, 3127BC; *M.coucha*: NMB4182, NMB4191,NMB4171, NMB4168,NMB4170, *M.natalensis*: NMB4169
ROOIPOORT, ELLIOT, 3127BD; *M.coucha:* NMB4660,NMB4658
SNOWHILL, DORDRECHT, 3127CA; *M.coucha:* NMB5803
GOODSTONE, INDWE, 3127CB; *M.coucha:* NMB6246,NMB6251,NMB6250
BOLOTWA, NEAR QUEENSTOWN, 31°58'S 27°12'E, 3127CC; *M.coucha:* KM10993
QAMATAPOORT, NEAR QUEENSTOWN, 31°59'S 27°26'E,3127CD; *M.coucha*: KM12712, KM12711
KEVITSHOEK, ELLIOT, 3127DB; *M.coucha:* NMB5996,NMB6158
HIGHFIELD, MACLEAR, 3128AB; *M.coucha*: NMB5327,NMB5319,NMB5297,NMB5306,NMB5305,NMB5299,NMB5301,NMB5310,NMB5303,NMB5303B,NMB5322,NMB5300,NMB5298,NMB5304
BENBECULA, ELLIOT, 3128AC; *M.coucha:* NMB4916; *M.natalensis*: NMB4890
ROCKY PARK,UGIE, 3128AD; *M.coucha*: NMB5380,NMB5369,NMB5383
SPRINGS,UITENHAGE, 3128AD; *M.coucha*: TM29265,TM29268
BASHEE RIVER MOUTH,TRANSKEI, 3128CD; *M.natalensis:* TM13434
UMTATA,31°36'S 28°45'E, 3128DA; *M.coucha:* KM26197,KM26196,KM26195 *M.natalensis:* TM6775,TM6776
NGQELENI, 31°40'S 29°02'E, 3128CA; *M.natalensis*: TM384,KM33298,KM33289, TM385,TM1178,TM1179,TM382,TM379,TM380,TM451,TM378
BIG UMGAZI, PONDOLAND, 31°37'S 29°27'E, 3129CB; *M.coucha:* KM5670 *M.natalensis:* KM5669
CULLENDALE, BEDFORD, 3220CA; *M.coucha:* TM21818
KAROO NATIONAL PARK, 9K NW BEAUFORT WEST, 32°20'S 22°33'E; *M.coucha:* KM33267,TM29577,TM29578,TM29593,TM29594,TM29587,TM29591,TM29576, TM29551,TM29552; *M.natalensis:* TM29603,TM29592,TM29553
MIDDELPLAAT, KAROO NATIONAL PARK, 3228BC; *M.coucha:* TM38732
MURRAYSBURG, 32°18'S 23°24'E, 3223AD; *M.coucha*: KM23314,KM23318,KM23319 *M.natalensis:* KM23317
KENDREW, 3224DA; *M.coucha:* TM32344
MOUNT ZEBRA NATIONAL PARK, CRADOCK, 3225BA; *M.coucha:* TM17641,TM17639;*M.natalensis:* TM17638
COOKHOUSE COMMONAGE, 3225BD; *M.natalensis:* TM21802
3K W. TARKASTAD, 3226AA; *M.coucha:*TM32330
TARKASTAD, 3226AB; *M.natalensis:* TM32352,TM32339
HURTLY GLEN, BEDFORD, 32°24'S 26°06'E, 3226AC; *M.coucha:* KM18645
QUAGGASKRAAL, TARKASTAD, 32°19'S 26°14'E, 3226AC; *M.coucha:* KM9004 *M.natalensis:* KM9005,KM9008
QUEENSTOWN, 32°08'S 26°30'E, 3226AB; *M.natalensis:* KM24793
STOCKENSTROOM, 32°29'S 26°42'E, 3226BC; *M.coucha*: KM24940,KM24942,KM24941, KM8922
LOWESTOFFE, CATHCART, 32°27'S 26°54'E, 3226BD; *M.coucha:* KM18696 *M.natalensis:* KM18690
BEDFORD, 32°41'S 26°06'E, 3226CA; *M.coucha:* KM5685,KM5684,KM5682

LYNDOCH, BEDFORD, 32°31'S 26°01'E, 3226CA; *M.coucha:* KM18646, KM18647
POST RETIEF, 32°34'S 26°32'E, 3226DA; *M.coucha:* KM18812,KM18594
TAMBOOKIESVLEI, STOCKENSTROOM, 32°36'S 26°43'E, 3226DA; *M.coucha:* KM8921,KM8918,KM8920; *M.natalensis:* KM8924
FORT BEAUFORT, 3226DC; *M.coucha:*TM21777
THORN GROVE, CATHCART, 32°13'S 27°08'E, 3227AA; *M.coucha:* KM18888,KM18883,KM18893
ST MARKS RAILWAY STATION, 3227AB; *M.coucha:* TM21805; *M.natalensis:* TM21834
HAPPY VALLEY, CATHCART, 32°29'S 27°04'E, 3227AC; *M.coucha:* KM18670
STUTTERHEIM, 32°40'S 27°17'E, 3227CB;*M.coucha:* KM31901,KM31301,KM31295, KM31306,KM31294,KM31305,KM31298,KM31296,KM31307,KM31302,KM31304, KM31299
M.natalensis: KM20220,KM19591,KM31297,KM31300
FARM VAN SCHALKWYK, LADISMITH, 33°28'S 21°01'E, 3321AC; *M.coucha*: KM29564
PRINCE ALBERT, 33°21'S 21°58'E, 3321BD; *M.coucha*: KM30559; *M.natalensis:* KM30577,KM30568
UNIONDALE, 33°37'S 23°10'E, 3323CA; *M.coucha:* KM24012, KM24008,KM24005, KM24004,KM24003,KM24007,KM24013; *M.natalensis:* KM24001,KM24000,KM24010, KM24009,KM24006
STEYTERVILLE, 33°14'S 24°22'E, 3324AB; *M.coucha*: KM23982,KM23985,KM23984, KM23983,KM23981,KM23980,KM23990,KM23996,KM23989,KM23988,KM23997 KM23987,KM2399,KM23991,KM23998,KM23992; *M.natalensis*: KM23986,KM23993, KM23995; ?: KM23999
HANKEY, 33°49'S 24°53'E, 3324DD; *M.coucha:* KM21188,KM21189,KM21187
ADDO NATIONAL PARK, 33°30'S 25°45'E, 3325BC;*M.coucha*: KM19867,KM20346, KM20345,KM20254,KM20023,KM19898,KM19894,KM20255,KM19891,KM20262 KM20256,KM20431, KM20400, KM20462, KM2078, KM20477, KM20460, KM20459, KM20457, KM20463, KM20480, KM20491, KM20499, KM19917, KM19926, KM19921, KM20430,KM20479,KM20455,KM20390,KM20318,KM20388,KM20320; *M.natalensis:* KM20268,KM20281,KM20432,KM20399,KM20387
ALEXANDRIA, 4K W. OF PATTERSON,33°18'S 25°44'E,3325BC; *M.coucha:* KM25703, KM25708, KM25704; *M.natalensis:* KM25701, KM25702
GROENDAL WILDERNESS AREA, UITENHAGE, 33°43'S 25°19'E, 3325CB; *M.coucha:* TM28801,TM28523,TM28803,TM28804, TM28524; *M.natalensis:* TM28525,TM28527,TM28509
KRUIS RIVER, PORT ELIZABETH AREA, 3325CB; *M.coucha:*TM21808
GAMTOOS RIVER, 3325CC; *M.natalensis:* TM12879
EMMAVILLE, KRUISRIVER\MIMOSADALE\SPRINGS, UITENHAGE,3325CD; *M.coucha:*TM21874,TM21811,TM29263,TM29264
AMANZI ESTATES, UITENHAGE, 3325DA; *M.coucha:* TM21792; *M.natalensis:* TM21796
NEW BRIGHTON, PORT ELIZABETH\PORT ELIZABETH, 33°53'S 25°37'E,3325DC; *M.coucha:* KM5690,TM5458
HOWISON'S PORT, GRAHAMSTOWN, 33°21'S 26°29'E, 3326AD, *M.coucha:* KM33233,

KM33160,KM33238,KM33236; *M.natalensis:* KM33237
ORINWAY FARM, ALBANY, 33°19'S 26°29'E, 3326AD; *M.coucha*: KM14717,KM14721, KM14724; *M.natalensis:* KM14719
LAKESIDE, GRAHAMSTOWN, 33°11'S 26°45'E, 3326BA; *M.coucha:* KM33148
ALBANY DISTRICT\ KLEINPOORT, GRAHAMSTOWN, 33°15'S 26°31'E,3326BA; *M.coucha:* KM33215,KM33214; *M.natalensis*: KM33185
GLEN CRAIG\ GOWESKLOOF, GRAHAMSTOWN, 33°15'S 26°35'E, 3326BA *M.coucha:*KM33190,KM33177,KM34175,KM33210,KM33216,KM33227,TM6705 KM5687,KM33212,TM6703,KM6704,TM6702; *M.natalensis:* KM33152,KM33210,KM33217, KM34174,TM1479,KM33199; ?: KM33161
CAXTON TRAPPES VALLEY, BATHURST, 33°25'S 26°58'E, 3326BD; *M.coucha:* KM33249,KM33257,KM33253,KM33254,KM33252
ALEXANDRIA, 33°41'S 26°00'E, 3326CA; *M.coucha:* KM25440,KM25847,KM25846, KM25439,KM31708,KM25543; *M.natalensis*: KM25442,KM25441
BATHURST STATE FOREST, 33°32'S 26°47'E, 3326DB; *M.coucha:* KM31147
EAST SIDE PORT ALFRED, 33°35'S 26°55'E, 3326DB; *M.natalensis*: KM33261
PORT ALFRED, 33°30'S 26°50'E, 3326BD; *M.coucha:* KM33260,KM33259,KM33262
KEISKAMMA MOUTH, 33°16'S 27°29'E, 3327AD; *M.coucha:* KM5673; *M.natalensis*: KM5671
KIDDS BEACH, EAST LONDON, 33°06'S 27°44'E, 3327BA; *M.coucha:* KM31766, KM31768
PEDDIE, 33°10'S 27°41'E, 3327BA; *M.natalensis*: KM19602
EAST LONDON, 33°00'S 27°55'E, 3327BB; *M.coucha:* KM5678
FARM HAZE FLAKTE, BREDASDORP\ BREDASDORP, 34°37'S 19°51'E, 3419CB *M.coucha*: KM30616,KM30614,KM30613,KM30610,KM30612,KM30609,KM30615, KM30611; *M.natalensis*: KM30617
FARM BERGHOEK, BREDASDORP, 34°32'S 19°57'E, 3419DB; *M.coucha*: KM30608
DE HOOP NATURE RESERVE, BREDASDORP, 34°29'S 20°25'E, 3420AD *M.coucha:* KM30964
HUMANSDORP, 34°12'S 24°50'E, 3424BB; *M.coucha:* KM21203,KM21204, KM21201,KM21200, KM21207,KM21197,KM21202, KM21199,KM21198,KM21208, KM2496

KWAZULU\NATAL PROVINCE

"FOUR WINDS", THORNVILLE JUNCTION,29°44'S 30°23'E, 2930CB; *M.coucha:* TM33127
2MI FROM PIETERMARITZBUG\THE GRANGE,29°36'S 30°23'E, 2930CB; *M.coucha:* TM33125; *M.natalensis:* TM24476,TM331224
CARTER'S NURSERY \DARVILLE SEWAGE\PIETERMARITZBURG, 29°36'S 30°23'E,2930CB; *M.coucha:*TM25434,TM26071,TM26067,TM26024, TM26043,TM26093,TM26221, TM26023,TM26027,TM26031,TM26035, TM26038,TM26082,TM26090,TM26166,TM24379,TM24382 , *M.natalensis:*TM26084, TM33138,TM24474,TM25441,TM25442,TM430,TM429,TM431 TM434,TM436,TM26018,TM26066,TM26034,TM26052,TM26054,TM26055,TM26058, TM26065,TM26075,TM26076,TM26081,TM26088,TM26094,TM26095,TM26167,

TM33140TM20431, TM20434,TM20436,TM20438,TM20440,TM20442,TM20443
TM26022,TM26021,TM26018,TM26026,TM26029,TM26033,TM26037,TM26048,
TM26049,TM26051,TM26053,TM26059,TM26064,TM26068,TM26073,TM26074,
TM26079,TM26087,TM26089,TM26092,TM26019,TM26021,TM26164,TM26165,
TM26168,TM26169,TM26171,TM33139,TM33141,TM20439,TM20444,TM24381,
TM26057,TM26085,TM24369,TM38486,TM26030
HILLSIDE FARM, ASHBURTON OR ASHBURTON, 29°40'S 30°27'E, 2930CB
M.coucha: TM26069,TM33142,TM26060; *M.natalensis:* TM26222,TM26220,TM26078,
TM26044,TM26047,TM26080,TM26040,TM26041,TM26046,TM26061,TM26086,
TM33143,TM33144,TM33145
KWALIWENI FOREST\OTOBOTINI, NGWAVUMA, 27°23'S 32°03'E,2732AC; *M.coucha:*
TM7210,TM7211; *M.natalensis*: TM27148,TM7213,TM7214,TM7212
MIDMAR,HOWICK,2930AC \DARGLE FOREST PLANTATION,29°28'S 30°07'E,
2930AC,*M.coucha*:TM33108,TM33186,TM33112,TM33118,TM33119,TM33121,TM33120
TM33122,*M.natalensis*:TM20435,TM20437,TM33116,TM33111,TM33105,TM33114,
TM33104,TM33106,TM33107,TM33109,TM33113,TM33115,TM33123,TM33110,
TM21799
NGOYE FOREST, MTUNZINI,28°51'S 31°41'E, 2831DC; *M.natalensis*: TM27489
UMLALAZI NATURE RESERVE,28°58'S 31°43'E, 2831DC; *M.coucha:* TM33154
*M.natalensis:*TM33146,TM33147,TM33155,TM33148,TM33150,TM33151,TM33152,
TM33153,TM33149,TM33168
SANTA SUZANNA SANCTUARY, NCEMANE, 28°04'S 32°18'E, 2832AB; *M.natalensis*:
TM33129,TM33128
THEUNIS BESTER, LOWER MKUZI GAME RANCH, 27°40'S 32°12'E,2732CA;
M.natalensis: TM24365,TM24367
PONGOLA RIVER, NE ZULULAND, 27°40'S 32°05'E, 2732CA; *M.coucha:*TM6137
M.natalensis: TM6136
UBOMBO, 27°34'S 32°03'E, 2732CA; *M.coucha:* TM5658,TM5657;
M.natalensis: TM5659,TM5655,TM5656
UMFOLOZI GAME RESERVE, 28°27'S 32°10'E, 2832AC; *M.coucha:* TM10320
M.natalensis: TM35415,TM10313,TM10318,TM10315,TM10319
CRAIGADAM FARM, ITALIA NATURE RESERVE, 27°31'S 31°21'E, 2731CB;
*M.coucha:*TM28827,TM31703,TM29000; *M.natalensis:* TM28811,TM31733,
TM28814,TM28819,TM31694,TM31706,TM31663,TM31688,TM31738,TM28812
TM28816,TM31657,TM28815,TM29390
NRC NDUMU CAMP\NDUMU\GUMEDE'S KRAAL, 26°52'S 32°21'E, 2632CD;
M.natalensis: TM21780,TM21779,TM21786,TM21819,TM21787,TM21775,TM21773,
TM21815
INGWAVUMA,27°08'S 32°02'E, 2732AA; *M.coucha*: TM7216,TM7219,TM7218,TM7215
M.natalensis: TM7217
MAPUTA, NE ZULULAND, 2632DD; *M.coucha:* TM6124; *M.natalensis:* TM6123
BUMBENI, ZULULAND,27°48'S 32°18'E, 2732CD; *M.natalensis:* TM12375
LOT 6, MAGUT,27°30'S 31°30'E, 2731AD; *M.natalensis:* TM17424
NGOME FOREST RESERVE, 27°50'S 31°24'E, 2731CD; *M.natalensis:* TM39144,TM39145
MANABA, NE ZULULAND, 27°15'S 32°26'E,2732AB; *M.natalensis:* TM6127,TM6126
MKUZI BRIDGE, CANDOVER,27°53'S 32°29'E, 2732CD; *M.coucha:*

TM21663,TM21685,TM5461; *M.natalensis:* TM21660,TM5654,TM5653
POTTERSHILL FARM, NEWCASTLE, 27°29'S 29°47'E, 2729BD; *M.natalensis:* TM668
3MI FROM NEWCASTLE- RD TO DUNDEE, 2729DD; *M.coucha:* TM21674
M.natalensis: TM21696
CHELMSFORD N.R., NEWCASTLE, 27°49'S 29°55'E, 2729DD; *M.coucha:* DM1368
DM1356, DM1375, DM1447, DM1359,DM1448; *M.natalensis:* DM1419, DM1458,
DM1395, DM1397, DM1457, DM1448, DM1470
ITALIA NATURE RESERVE, 27°30'S 30°12'E, 2730AC; *M.coucha:* TM31768
M.natalensis: TM31789,TM31790,TM31791,TM31783,TM31781,TM25877,TM10312
VRYHEID NATURE RESERVE,27°45'S 30°47'E, 2730DB; *M.coucha:* DM1403, DM1475
M.natalensis: DM1480, DM1481; ?: DM1837
FLAGSTONESPRUIT, 55MI S OF LADISMITH, 2829DA; *M.natalensis:* TM21648
WEENEN NATURE RESERVE, 28°51'S 29°59'E, 2829DD; *M.coucha:* DM2351,DM2346,
DM2343;*M.natalensis:*TM38487,DM2360,DM2356,DM2349,DM2330,
DM2326,DM2344,DM2402, DM2358,DM2363,TM38100
WEENEN, 28°51'S 30°05'E, 2830CC; *M.coucha:* TM4868; *M.natalensis:*
TM4922,TM4869,TM4923
MFONGOZI, 28°43'S 30°48'E, 2830DB; *M.natalensis:*
DM237,DM221,DM228,DM222,DM3233
JAMES DRIFT, TUGELA RIVER,28°46'S 30°55'E, 2830DD; *M.natalensis:* TM21659
0.81 MI FROM UMPUMLO, KRANSKOP, 28°58'S 30°52'E, 2830DD; *M.natalensis:*
TM21770
BLACK UMFOLOZI BRIDGE, NONGOMA, 28°03S 31°32E, 2831BA; *M.coucha:*
TM21656, *M.natalensis:* TM21691
HLUHLUWE GAME RESERVE, 28°16'S 31°44'E, 2831BC; *M.natalensis:*
TM32380,TM32381
MTUBATUBA FUTULU RESEARCH STATION, 28°27'S 32°17'E, 2832AD; *M.natalensis:*
TM43298
MTUBATUBA, 6K NNE DUKUDUKU, 28°22'S 32°21'E, 2832AD; *M.coucha:* TM40365
M.natalensis: TM43298,TM40356,TM40394,TM40364,TM41887
EASTERN SHORES, LAKE ST LUCIA, 28°20'S 32°24'E, 2832AD; *M.natalensis:*
TM24385, TM24389
COLESFORD NATURE RESERVE, 2732CC; *M.natalensis:* TM38394
2MI NW OF BOSTON SAP, 2930CA; *M.natalensis:* TM21820
KARKLOOF N.R, HOWICK, 29°18'S 30°13'E, 2930AB; *M.coucha:* DM1734, DM1812,
DM1758, DM1769, DM1753, DM1749, DM1756, DM1754, DM1749,
DM1752, *M.natalensis:* DM1793, DM1951, DM1750, DM1754
BROUGHTON, CRAMOND, 2930AD; *M.coucha:* TM33134,TM33131
M.natalensis: TM33132,TM33137,TM33133
ALBERT FALLS RESORT, 29°28'S 30°23'E, 2930AD; *M.natalensis:* TM38586,TM38585
KARKLOOF TWO STREAMS, 29°17'S 30°23'E, 2930AD; *M.natalensis:* TM41892,
TM41889,TM41893
KILGOBBIN DARGLE, 29°28'S 30°23'E, 2930AD; *M.natalensis:* TM8941
LINWOOD FOREST, 29°33'S 30°05'E,2930CA; *M.coucha:* DM3383,DM3382
M.natalensis: DM3384
RINGWOOD PARK\UNIVERSITY OF NATAL,DURBAN, 29°50'S 31°00'E, 2931CC

M.coucha: DM488,TM13752,TM38667,DM645,DM646,DM255,DM247,DM747,DM248
*M.natalens*is: DM644,TM12782,TM38660,TM38673,TM38669,TM38672,TM38670, DM750, DM749,DM744,DM748
RINGWOOD, INCHANGA, 29°40'S 30°40'E, 2930DA; *M.natalensis:* TM24430,TM24432
MALVERN, 29°53'S 30°56'E, 2930DD; *M.natalensis:* TM422
UMHLANGA NATURE RESERVE, STANGER, 29°44'S 31°05'E, 2931CA; *M.natalensis:* DM858,DM859,DM857
16MI N OF IXOPO RD TO BULWER, 3039BD; *M.natalensis:* TM21797
VERNON CROOKES NATURE RESERVE, 30°17'S 30°36'E, 3030BC; *M.coucha:* DM837, *M.natalensis:* DM840,DM841, TM38700; ?: DM836
ORIBI GORGE NATURE RESERVE, 3030CA; *M.coucha:* TM33380,TM34140
M.natalensis: TM33377,TM33381,TM33382
IAN ELLIS NATURE RESERVE, PORT SHEPTONE, 30°46'S 30°24'E, 3030CD
M.coucha: DM2215,DM2210,DM2218,DM2216,DM2208,DM2203,DM2207
M.natalensis: DM2209,DM2220,DM2217,DM2204,DM2205,DM2221,DM2202,DM2206

FREE STATE PROVINCE

15 K W OF SENEKAL RD TO BETHLEHEM, 28°14'S 28°18'E, 2828AB; *M.coucha*: TM25453,TM25454,TM25455; *M.natalensis*: TM25452,TM25456
BOTHAVILLE, 27°24'S 26°37'E, 2726BC; *M.coucha*: TM2701, KM5642, *M.natalensis:* TM2702
BRAKVLAKTE, PARYS \ PARYS,26°52'S 27°22'E, 2627CD; *M.coucha:* TM3532,TM3534,TM3728
HELENA VREDEFORT BASIN,2627CD; *M.coucha*: NMB3169,NMB3173,NMB3168, NMB3165,NMB3161,NMB3166,NMB3167
GLASGOW, HOOPSTAD,2725DA; *M.coucha:* NMB3851,NMB3854,NMB3853
LEEUKRAAL,HOOPSTAD,2725DD; *M.coucha*: NMB3922,NMB3924,NMB3925
DEELFONTEIN, BOTHAVILLE, 2726BA; *M.coucha*: NMB2544
GRASPAN, KROONSTAD, 2726DD; *M.coucha:* NMB3255,NMB3253,NMB3252
KOPJES, 27°14'S 27°34'E, 2727BA; *M.coucha*: KM5641,TM6298
HENNINGSPRUIT, 27°25'S 27°15'E, 2727AC; *M.coucha:* KM5647,KM5648,KM5646
M.natalensis: KM5644,KM5645
13MI N OF KOPPIES, 2727BA; *M.coucha:* TM21669,TM21746,TM21687,TM21831
11.5MI \17MI FROM HEILBRON,WOLWEHOEK\ MOSCOW, HEILBRON,2727BB
*M.coucha:*TM21662,TM21689,TM21665,TM21661,TM21667,TM21669,NMB3808, NMB3804,NMB3806,NMB3805,NMB3803,NMB3801,NMB3809; *Mnatalensis:* NMB3802
FISCHER\SCHURWEPOORT\STILLEWONING, KOPPIES, 2727BC; *M.coucha*: NMB8779,NMB8761, NMB3390,NMB3384,NMB3385,NMB3386,NMB3389,NMB8763 NMB8778,NMB8722,NMB8776,NMB8773,NMB8764,NMB8765,NMB8766,NMB8781 NMB8775
17,6 MI HEILBRON, EDENVILLE\GOEDEHOEK\VAALBANK\HEILBRON, 27°17'S 27°58'E, 2727BD; *M.coucha*: TM21673,TM21741,TM3678,TM3682,TM3679, TM3681,TM6297,KM5640,TM3683,TM3684,TM3600; *M.natalensis:* TM6296
ERFDEEL,KROONSTAD,2727CB;*M.coucha:* NMB7790,NMB6555,NMB3097,NMB3092, NMB6554,NMB7789

VAALBANK, LINDLEY,2727DB; *M.coucha*: NMB3498, NMB3501, NMB3499
EUREKA, SENEKAL, 2727DC; *M.coucha:* NMB3287,NMB3289; *M.natalensis*: NMB3290
VAALBANK, EDENVILLE, 2727DD; *M.coucha:* NMB3500
6MI FROM ORANJEVILLE, HEILBRON, 2728AA; *M.coucha*: TM21657,TM21672
ALMA, FRANKFORT, 2728BA; *M.coucha:* NMB3872
HELENA, VREDE, 2728BD; *M.coucha:* NMB3304,NMB3301,NMB3298
SUSSANASKOP, REITZ,2728DA; *M.coucha:* NMB2091,NMB2087,NMB2079,
NMB2083,NMB2085,NMB2088,NMB2080,NMB2089; *M.natalensis:* NMB2094
UITVLUGT, VREDE, 2729AD; *M.coucha:* NMB3552,NMB3551
ALLANDALE, VREDE, 2729DA; *M.coucha:* NMB2585
K(L)IPPIESPAN, BOSHOF, 2824DD; *M.coucha:* NMB2618,NMB2621
BUITENHOOP, BOSHOF, 2825AA; *M.coucha:* NMB3826,NMB3825,NMB3771
SPITSKOP, BOSHOF 2826AD; *M.coucha*: NMB2816,NMB2818,NMB3198
VEEKRAAL, HERTZOGVILLE, 2825BA; *M.coucha:* NMB3055,NMB3048
M.natalensis: NMB3054
LAVIERA, BULTFONTEIN, 2826AB; *M.coucha:*NMB1428,NMB1425,NMB1426,
NMB1424
VIERKANT, BRANDFORT, 2826DA; *M.coucha*: NMB2861
DE POORT, VENTERSBURG, 2827AA; ?: NMB2926
WILLEM PRETORIUS N.R, VENTERSBURG, 2827AC;*M.coucha:* NMB2022,
NMB9116,NMB8962,NMB9117,NMB9073,NMB9122,NMB9048,NMB8975,NMB8964
NMB8979,NMB9012,NMB9013,NMB9082,NMB9086,NMB9014,NMB9092,NMB8954
NMB8963,NMB8965,NMB9001,NMB8989,NMB9000,NMB9005,NMB8982,NMB8983
NMB8986,NMB8985,NMB8987,NMB8977,NMB9009,NMB9006,NMB8955,NMB9091
NMB8966; *M.natalensis*: NMB8959,NMB8999,NMB9004,NMB8996,NMB8947
?: NMB9114
LARALA, SENEKAL, 2827BB; *M.coucha:* NMB2715,NMB2714,NMB2706,NMB2724
ASEM, SENEKAL, 2827BC; *M.coucha*: NMB3135; *M.natalensis:* NMB3132
BAKENFONTEIN, WINBURG, 2827CA; *M.coucha*: NMB2672
THE MINE, MARQUARD, 2827CD; *M.coucha:* NMB6637
FARM FRANSHOEK, SENEKAL (FICKSBURG), 2827DD; *M.coucha*: KM19860
M.natalensis: KM19861
LOCH ATHLONE, BETHLEHEM, 2828AB; *M.coucha:* TM25457,TM25458
BOSHOEK, BETHLEHEM, 2828AD; *M.coucha:* NMB3338,NMB3316,NMB3341,
NMB3337,NMB3336
BELLEVUE, BETHLEHEM, 2828BA; *M.coucha*: NMB2980, NMB2975
KORFSHOEK, HARRISMITH, 2828BD; *M.coucha:* NMB3360,NMB3358'
MALUTIZIGHT, FICKSBURG, 2828CA; *M.coucha*: NMB3471,NMB3464,
NMB3484,NMB3486,NMB3463,NMB3472
17MI S.CLARENS ON FOURIESBURG, 2828CB; *M.natalensis*: TM21647
LETSOAMASTAD, FOURIESBURG, 2828CB; *M.coucha:*NMB4145,NMB4142,
NMB4148
GOLDEN GATE NATIONAL HIGHLANDSPARK, 28°31'S 28°37'E, 2828BC; *M.coucha*:
TM16743,TM16741,TM16742,TM16744,TM16745
KOEBERG, BETHLEHEM, 2828DA; *M.coucha*: NMB2748
M.natalensis: NMB2753,NMB2756

FARM WESSELSHOEK, 1 MI S. MOLLEN, 2829AB; *M.coucha:* TM21676
HARRISMITH, 2829AC; *M.coucha*: TM12856
SPITSKOP, FAURESMITH, 2925AD; *M.coucha*: NMB3695, NMB3693
KOPPIESDAM, PETRUSBURG, 2925BA; *M.coucha*: NMB1755,NMB1754,NMB1757, NMB1756,NMB5407
ZUURFONTEIN, FAURESMITH, 2925CD; *M.coucha:* NMB3652 *M.natalensis:* NMB3654
BIRD SANCTUARY\ BOTANICALGARDENS\NAVAL HILL\ SYDENHAM\ VOLHARDING, BLOEMFONTEIN, 2926AA; *M.coucha:* NMB2577,NMB6550,NMB6559,NMB6551,NMB2194,NMB6553,NMB6552, NMB6549,NMB6548,NMB6556,NMB6557,NMB6547,NMB2193,NMB6558,NMB6560.NMB7658,NMB7652,NMB7656,NMB7651,NMB7655,NMB6628,NMB6629,NMB7653
LEEUKOP, DEWETSDORP,2926AD; *M.coucha:* NMB3577
HEX RIVER, REDDERSBURG, 2926CD; *M.coucha:* NMB3608
NOVA, LADYBRAND, 2927AB; *M.coucha:* NMB1686,NMB1791
ROSENDAL, LADYBRAND, 2927AC; *M.coucha*: NMB3226,NMB3227
VERZAMELKOP, LADYBRAND, 2927AD; *M.coucha:* NMB4035,NMB4039, NMB4038,NMB4040,NMB4036,NMB4034; *M.natalensis*: NMB4037
LEMOENBOORD, PHILIPPOLIS, 3024BB; *M.coucha*: NMB2383,NMB2410
MIDDELBRON, PHILLIPOLIS, 3025AA; *M.coucha:* NMB1488
KONNETJIESFONTEIN, SPRINGFONTEIN, 3025BA; *M.coucha:* NMB2898,NMB2899, NMB2894
EENDRAG\KLEINFONTEIN, BETHULIE, 3026AC; *M.coucha:* NMB3742,NMB3751 NMB1836,NMB1868,NMB1839,NMB1860,NMB1841,NMB1835; *M.natalensis:* NMB1847, NMB1867
SEBASTOPOOL, ZASTRON, 3027AC; *M.coucha:* NMB1952; *M.natalensis:* NMB1953
ALLAMANSKRAAL DAM, 28°18'S 27°09'E, 2827AC; *M.coucha:* TM13705, TM13707, TM13704, TM13706, TM13709, TM13710; *M.natalensis:* TM13711
ZOUTPANSDRIFT, LUCKHOFF, 2924CD; *M.coucha:* NMB1997

TRANSVAAL PROVINCE (includes the new Northern Province, Gauteng Province Mpumalanga Province and part of the Northwest Province):

MMABOLELA ESTATES, 14K NW MAASSTROOM, 22°46'S 28°28'E, 2228CA *M.coucha:* TM19754,TM19753,TM19710,TM19740,TM19698,TM19739,TM19711
FARM ALTEVER, 1K SSE MAASSTROOM, 22°46'S 28°28'E, 2228CD; *M.coucha:* TM26601,TM26489,TM26546,TM26554,TM26679,TM25519B,TM26542, TM26543,TM26544,TM26545,TM26547,TM26552,TM26556,TM26579,TM26580, TM26593,TM26594,TM26595,TM26596,TM26597,TM26598,TM26600,TM26614, TM26615,TM26616,TM26617,TM26618,TM26619,TM26624,TM26625,TM26626, TM26641,TM26642,TM26643,TM26644,TM26646,TM26647,TM26648,TM26649, TM26475,TM26488,TM26549,TM26550,TM26551,TM26581,TM26582,TM26645; *M.natalensis:* TM26553,TM26519,TM26591,TM26599
Z (S)OUTPANSBERG, HAPPY REST N.R, 23°00'S 29°40'E, 2329BA; *M.coucha:* TM3634,TM44609,TM3633; *M.natalensis:* TM3635,TM44613,TM44614
STERKSTROOM, MALARIA CAMP, 2229DC; *M.natalensis:* TM30388

FARM WINDHOEK, LOUIS TRICHARDT - MESSINA, 22°50'S 29°54'E, 2229DD
M.natalensis: TM21654
MESSINA NATURE RESERVE, 22°21'S 30°03'E, 2230AC; *M.coucha:* TM42351, TM44516
MABILINGWE \MADIMBO ON LIMPOMPO, 22°26'S 31°04'E, 2231AC; *M.coucha:* TM24653,TM24626,TM24627,TM40895; *M.natalensis:*TM40735,TM40736, TM40738, TM40739
SANDPAD, LIMPOMPO, 20K W OF PAFURI, 22°25'S 31°09'E, 2231AC; *M.natalensis:* TM29866
MOTALE RIVER, 22°30'S 30°50'E, 2230BD; *M.coucha:* TM7528, TM7524
M.natalensis: TM7526
ENTABENI STATE FOREST, ZOUTPANSBERG,23°03'S 30°13'E, 2330AA; *M.coucha:* TM25839
LEVHUVHU RIVER, VENDA, 22°40'S 30°53'E, 2230DB; *M.natalensis:* TM25488, TM25496,TM25509,TM25485; ?: TM25508
MALUKWA DAM, 5K NW PUNDA MILIA, 22°41'S 30°57'E, 2231CA; *M.coucha:* TM29919
SEBASA, 22°58'S 30°29'E, 2230CD; *M.coucha:* TM7522
PAFURI CAMP, 22°26'S 31°19'E, 2231AD; *M.coucha:* TM21726,TM21745,TM21714
MAHOGANY DRIVE, 2K N. OF PUNDA MILIA\MARIA,KNP, 22°41'S 31°01'E, 2231CA
M.coucha: TM29913; *M.natalensis:* TM29907
PUNDA MARIA, KNP, 22°41'S 31°02'E, 2231CA; *M.coucha:* TM21849,TM21739, TM12991,TM30643,TM30640
NWAMBIA PAN, 22°41'S 31°23'E, 2231CB; *M.coucha:* TM21705,TM21814
STANGENEDOM, KNP, 22°48'S 31°17'E, 2231CD; *M.coucha:* TM30556
M.natalensis: TM21695
HUWI PRIVATE NATURE RESERVE, 23°45'S 27°50'E, 2327AD; *M.coucha:* TM19974, TM19975,TM19973
D'NYALA NATURE RESERVE, ELLISRAS, 2327DB; *M.coucha:* TM40940,TM40933
TAMBPTKIELOOF, ELLISRAS, 23°55'S 27°44'E, 2327DC; *M.natalensis:* TM15207
MOTLATENG- BLOUBERG, 23°05'S 28°58'E, 2328BB; *M.coucha:* TM14926,TM14925, TM14727
FARM WELGEVONDEN, 16K W.STEILLOOP, 23°32'S 28°25'E, 2328CB; *M.coucha:* TM23303,TM23313,TM23306
FARM DORDRECHT, 20K S.MARKEN, 23°50'S 28°23'E, 2328CD; *M.coucha:* TM24666,TM24697,TM24685,TM24686
FARM URK, BLOUBERG, 13K W.VIVO, 23°02'S 29°07'E, 2329AA; *M.coucha:* TM24077,TM24076,TM24060; *M.natalensis:* TM24058
LOUIS TRICHARDT\ E. LOUIS TRICHARDT, 23°03'S 29°54'E, 2329BB; *M.coucha*: TM21772,TM7517,TM7512,TM7518,TM7519,TM7520,TM10421,TM10422,TM10418,TM 10425,TM10429,TM10427; *M.natalensis*: TM7513, TM7515,TM7530,TM10430
DERY P.O, SKEENSHOEK, MALARIA CAMP, 23°21'S 29°25'E, 2329AD; *M.coucha:* TM21816
BLIDJSCHAP PNR, BANDOLIERSKOP, 23°15'S 29°46'E, 2329AA; *M.coucha:* TM24168, TM24169, TM24170
FARM FORT KLIPDAM, 27K N. OF PIETERSBURG, 23°42'S 29°33'E, 2329DA;

M.coucha: TM24141, TM24159,TM24139

WOODBUSH VILLAGE, HOUTBOSDORP, 23°49'S 29°54'E, 2329DD; *M.coucha:* TM1462

SHANGONI RANGERS HOUSE, KNP, 23°14'S 30°59'E, 2330BB; *M.coucha:* TM21709, TM21711

BIRTHDAY MINE LETABA, 23°22'S 30°52'E, 2330BD; *M.coucha:* TM2705

BLINKWATER, ZOUTPANSBERG, 23°27'S 30°04'E, 2330AC; *M.coucha:* TM2703

FARM NARINA, 8K W. DUIWELSKLOOF, 23°42'S 30°16'E, 2330CB; *M.coucha:* TM25273,TM25270; *M.natalensis:* TM25275,TM25271

MOEKETSI, DUIWELSKLOOF, ZOUTPANSBERG, 23°40'S 30°05'E, 2330CA; *M.coucha:* TM4045; *M.natalensis:* TM4049

SAMA FARM, LEYDSDORP, 23°31'S 30°02'E, 2330CA; *M.coucha:* TM8711

H. MERENSKY PNR, 30K NE LETSITELE, 23°40'S 30°41'E, 2320DA;
M.coucha: TM24567, TM24548,TM41155,TM24551,TM24568; *M.natalensis*: TM24549

TZANEEN ESTATE, 23°48'S 30°11'E, 2330CC; *M.coucha:* TM414,TM412,TM404, TM407,TM409,TM411; *M.natalensis:* TM418,TM415,TM420,TM421,TM403

DE HOEK FOREST, MAGOEBASKLOOF, 23°50'S 30°02'E, 2330CC; *M.coucha:* TM40770

GRAVELLATTE, TZANEEN, 23°57'S 30°36'E, 2330DC; *M.coucha:* TM4051, TM4052, TM4053,TM4054; *M.natalensis:* TM4050

MAMARANGA, GREAT LETABA, 23°52'S 30°37'E, 2330DC; *M.coucha:* TM2120

FARM RHODA, 13K S. PHALABORWA, 24°03'S 31°06'E, 2431AA; *M.coucha:* TM24237, *M.natalensis:* TM24236

SHINGWEDZI NEAR REST CAMP, KNP, 23°06'S 31°27'E, 2331AB; *M.coucha:* TM21734,TM2431,TM2434,TM2433; *M.natalensis:* TM21712,TM2432

LETABA RANCH, 40K N. OF PHALABORWA, 24°43'S 31°05'E, 2431CA; *M.coucha:* TM25578,TM25587,TM25585

MALOTENE REST CAMP, 2331CC; *M.natalensis:* TM21755

FARM MOOIGENOEG, 8K S. DERDEPOORT, 24°43'S 26°17'E, 2426CB; *M.coucha:* TM23533,TM23568,TM23555,TM23567,TM23532,TM23534,TM23531

FARM MOOIPLAAS, 18K SSE OF DERDEPOORT, 24°43'S 26°17'E, 2426CB
M.coucha: TM20450,TM20452,TM20465,TM20505,TM20464,TM20475,TM20491, TM20492,TM20502

ATHERSTONE N.R, THABAZIMBI, 24°34'S 26°47'E, 2426DB; *M.coucha:* TM44536

MARICO RIVER, 24°12'S 26°52'E, 2426BB; *M.coucha:* TM4150

FARM PLATBOS, 32K NW VAALWATER, 24°13'S 27°52'E, 2427BB; *M.coucha:* TM24746,TM24761,TM24780,TM24781,TM24762

THABAZIMBI, 24°35'S 27°40'E, 2427DA; *M.coucha:* TM34291

FARM DONKERPOORT, 24K E. THABAZIMBI, 24°35'S 27°40'E, 2427DA; *M.coucha:* TM20626,TM20627,TM20640,TM20591

RISSIK P.N.R., 8K E. WARMBATHS, 24°53'S 28°27'E, 2428CD; *M.coucha:* TM20668, TM20675,TM20660,TM20680,TM20682,TM20683,TM20669,TM20676,TM20678, TM20679,TM20681,TM20685,TM20696,TM20695; ?: TM20677

MAGALAKUIN, POTGIETERSRUS, 23°36'S 28°36'E, 2328CB; *M.coucha:* TM4152

MOKERENG DISTRICT, WITVINGER N.R, 2428BB; *M.coucha:* TM44587

NABOOMSPRUIT, 24°30'S 28°43'E, 2428BC; *M.coucha:* TM13325,TM17426,TM17427

NOORDDRIFT, WATERBERG, 24°17'S 28°57'E, 2428BD; *M.coucha:* TM1461,TM1459

POTGIETERSRUS, DOORNDRAAIDAM N.R, 24°17'S 28°45'E, 2428BC; *M.coucha:* TM44527

NYLSTROOM, 24°47'S 28°25'E, 2428CD; *M.coucha:* TM2387,TM2388,TM2804 *M.natalensis:* TM2386

RUITSPRUIT, NYLSTROOM, 24°40'S 28°27'E, 2428CB; *M.coucha:* TM3884

NYLSVLEI DISTRICT, NABOOMJSPRUIT, 24°40'S 28°43'E, 2428DA; *M.coucha:* TM25794, *M.natalensis:* TM25793

NYLSVLEI NATURE RESERVE, POTGIETERSRUS, 24°40'S 28°41'E, 2428DA; *M.coucha:* TM44551

GELUK FARM 1113, CRECY CAMP, 24°38'S 28°52'E, 2428DB; *M.coucha:* TM21708

VLAKPAN, NABOOMSPRUIT, 24°31'S 28°52'E, 2428DB; *M.coucha:* TM18967

MOSDENE PRIVATE NATURE RESERVE, 24°36'S 28°46'E, 2428DB; *M.coucha:* TM19908,TM19900,TM19918,TM19902,TM19909,TM19936,TM19941; *M.natalensis:* TM19935

1K\2K\5K FROM SETTLERS, 24°58'S 28°33'E, 2428DC; *M.coucha:* TM28494,TM28492,TM28924,TM28926,TM28535,TM28921,TM28922,TM28925, TM28942,TM28943,TM28941,TM27587; *M.natalensis:* TM27589,TM27492,TM27498

MARIBASHOEK, 2429AA; *M.coucha:* TM4285

PERCY FYFE NATURE RESERVE, 24°03'S 29°07'E, 2429AA; *M.coucha:* TM18877

FARM GROOTHOEK, 25K FROM POTGIETERSRUS, 24°21'S 29°08'E, 2429AC; *M.coucha:* TM23376,TM23358,TM23359,TM23360,TM23361

5 MI FROM ROEDTAN STATION, ZEBEDIA, 24°31'S 29°09'E, 2429CA; *M.coucha:* TM21771

ACORNHOEK, 24°53'S 31°05'E, 2431CA; *M.coucha:* TM4286,TM4380, TM4381,TM4377,TM4378

SCHUINDRAAI N.R. GROBLERSDAL, 24°49'S 29°21'E, 2429CD; *M.coucha:* TM44751,TM44732,TM44783,TM44784,TM44785,TM44786,TM44787,TM44788, TM44781; *M.natalensis*: TM25381,TM25380

DE HOOP P.N.R., 40K N. OF ROOSSENEKAL, 24°57'S 29°57'E, 2429DD; *M.coucha:* TM25369,TM25337,TM25338,TM25371,TM25370; *M.natalensis:* TM25381,TM25380

MALTA FARM, 2409S 3013E, 2430AA; *M.coucha:*TM27555,TM27552,TM27556, TM27548; *M.natalensis:* TM27524

THE DOWNS, 24°08'S 30°11'E, 2430AA; *M.coucha:* TM27501,TM27586 *M.natalensis*: TM27544,TM27546,TM27502

WOLKBERG WILDERNESS, 24°01'S 30°04'E, 2430AA; *M.coucha:* TM43404

FARM CYPRUS, 13K SW OFCALACO, 24°12'S 30°17'E, 2430AB; *M.coucha:* TM25295 *M.natalensis:* TM25319

SEKORORO, PILGRIMS REST, 24°15'S 30°24'E, 2430AB; *M.coucha:* TM3432,TM3435, TM3433,TM3434,*M.natalensis:* TM3431

PILGRIM'S REST, 24°31'S 30°48'E, 2430DB; *M.coucha:* TM44718,

MARIEPSKOP, 24°41'S 30°54'E, 2430DB; *M.coucha:* TM4633,TM4525,TM4527, TM4529,TM4532,TM4533,TM4535,TM4536,TM12702,TM12703,TM12704,TM12705, TM12706,TM12736,TM4526,TM4530,TM12735,TM4531, *M.natalensis:* TM12737, TM4634,TM12732,TM12733,TM12734

PONIESKRANTZ (PILGRIM'S REST), 24°53'S 30°45'E, 2430DC; *M.coucha:* TM44718

OLIFANTS RIVER, KNP, 24°05'S 31°44'E, 2431BA; *M.coucha:* TM7339,TM7340, TM6330,TM21754,TM21718

SHEILA, LEYDSDORP,24°05'S 31°44'E, 2431BA; *M.coucha:* TM6330

THORNEYBUSH, LETABA, 2431AC; *M.natalensis:* TM44910

SATARA CAMP, KNP, 24°23'S 31°47'E, 2431BD; *M.natalensis:* TM37061,TM21729

SANDRINGHAM P.N.R, ACORNHOEK, 24°30'S 31°14'E, 2431AC ; *M.coucha:* TM23733,TM23747,TM23743; *M.natalensis:* TM23744,TM23745

7MI N OF NEWINGTON, 24°45'S 31°25'E, 2431CB; *M.coucha:* TM17264,TM17262,TM17261

FARM OTHAWA, NEWINGTON, 24°25'S 31°26'E, 2431AD; *M.coucha:* TM24013,TM24014,TM24033,TM24036,TM24037,TM24914,TM24917, TM24925,TM24935,TM24936,TM24946,TM24947,TM24948,TM28992,TM28991 *M.natalensis*: TM24015,TM24016,TM24023,TM24924,TM24934,TM24937,TM24945

SABIE RIVER, 3K NNE SKUKUZA, 24°58'S 31°40'E, 2431DC; *M.natalensis:* TM39707

SKUKUZA CAMP, KNP, 24°59'S 31°35'E, 2431DC; *M.coucha:* TM21716, TM21707,TM13496,TM13488,TM13492,TM13494,TM13495,TM13490, TM13491; *M.natalensis:* TM21703,TM21704,TM13497,TM13489; ?:TM21731

TSHOKWANE, 24°47'S 31°52'E, 2431DD; *M.natalensis:* TM21706

NELSPRUIT, 25°32'S 30°57'E, 2530DB; *M.coucha:* TM41546

FARM RYKVOORBY, 9K N OF ZEERUST, 25°29'S 26°04'E, 2526AC; *M.coucha:* TM20547 *M.natalensis:* TM20551

FARM ZANDSPRUIT, 63K NNW OF RUSTENBURG, 24°35'S 27°40'E, 2427DA; *M.coucha:* TM23608,TM23607,TM23609,TM23610,TM23618,TM23630; ?: TM23628

FARM FERNDALE, 8K SE GROOT MARICO, 25°38'S 26°26'E, 2526CB, *M.coucha:* TM23297,TM23296,TM23419

PANFONTEIN, BLOEMHOF, 27°35'S 25°27'E, 2725CB; *M.coucha:* TM12539, TM12536,TM12542,TM12538,TM12537,TM12425,TM12534,TM12533

KOSTER, 2552S 2654E, 2526DD; *M.coucha:* TM2982,TM2983

FARM BUFFELSPOORT, 3MI NE ASSEN, 25°07'S 27°37'E, 2527BA; *M.coucha:* TM20025,TM20030,TM20035,TM20051,TM20027,TM20028,TM20029,TM20036, TM20043,TM20037

ROB OVERVAAL, 25°12'S 27°52'E, 2527BB; *M.coucha:* TM44213

ROODEHUIL, BRITS, 25°15'S 27°49'E, 2527BB; *M.coucha:* TM1943,TM1945

VAALKOP DAM NATURE RESERVE, 25°23'S 27°28'E, 2527AD; *M.coucha:* TM40968 TM40973,TM40964,TM40965,TM40967,TM40971,TM40963,TM40966,TM40962

BUFFELSPOORT DAM, RUUSTENBURG N.R, 25°47'S 27°29'E, 2527CD; *M.coucha:*TM21843

ROODEPLAAT, BRITS, 25°37'S 28°22'E, 2528CB; *M.coucha:* TM1944,TM42336

OLIFANTSHOEK, 25°40'S 27°15'E, 2527CA; *M.coucha:* TM4151

FARM OLIFANTSPOORT, 12MI S. RUSTENBURG, 25°46'S 27°16'E, 2527CD; *M.coucha:* TM19661,TM19677,TM19622,TM19623,TM19638,TM19655,TM19659, TM19660; *M.natalensis:* TM19624

DAMELSRUST, KRUGERSDORP, 25°58'S 27°43'E, 2527DC; *M.coucha:* TM12189

UITKOMST FARM, 35MI W. OF PRETORIA, 25°55'S 27°45'E, 2527DC; *M.coucha:*TM13333,TM21826,TM21749,TM21806,TM13317

HENOPS RIVER ON HARTEBEESPORT DAM, JOHANNESBURG, 25°46'S 27°59'E, 2527DD; *M.natalensis:* TM30386

KROMDRAAI, KRUGERSDORP, 25°58'S 27°47'E, 2527DD; *M.coucha:* TM21675,TM21842,TM21838,TM21645,TM21740; *M.natalensis:* TM21839
SCHUNWEBERG, PRETORIA, 25°48'S 27°59'E, 2527DD; *M.coucha:* TM4154
WELGELEGEN, KRUGERSDORP, 2527DD; *M.coucha:* TM21846
PLOT 56, BON ACCORD, PRETORIA, 25°11'S 28°11'E, 2528AA; *M.coucha:* TM18961,TM18962
PIENAAR RIVER, 45K N. OF PRETORIA, 25°12'S 28°23'E, 2528AB; *M.natalensis:* TM41167,TM41168,TM41169
Z(S)OUTPAN AGRIC EXP FARM, 25°23'S 28°05'E, 2528AC; *M.natalensis:* TM34292
Z(S)OUTPAN, 25°24'S 28°06'E, 2528AC; *M.coucha:*TM19383,TM19377, TM19382,TM19381,TM19391,TM1425
VAALKOP DAM NATURE RESERVE, 25°23'S 27°28'E, 2527AD; *M.coucha:* TM40972,TM34312
33 MARE' STREET, PRETORIA, 25°44'S 28°11'E, 2528CA; *M.coucha:* TM396
BROOKLYN, PRETORIA, 25°45'S 28°14'E, 2528CA; *M.coucha:* TM3727
DASSPOORT, PRETORIA, 25°43'S 28°10'E,2528CA; *M.coucha:* TM5454
L.C DE VILLIERS ST, 25°45'S 28°10'E, 2528CA; *M.coucha:* TM17431
PRETORIA, 25°44'S 28°11'E, 2528CA; *M.coucha:*TM17485,TM5249,TM6325,TM5250, TM5251,TM6237,TM2455,TM2457,TM18964,TM18959; *M.natalensis:* TM2456,TM29098
PRETORIA NORTH, 25°40'S 28°11'E, 2528CA; *M.coucha:* TM29093
RIETONDALE, PRETORIA, 25°43'S 28°13'E, 2528CA; *M.coucha*: TM34153, TM1222,TM29102,TM29120,TM29125,TM29114,TM29123,TM29104,TM29110, TM29126,TM29129,TM29105,TM29094,TM40634,TM34154,TM29092
M.natalensis: TM29119,TM29099,TM29109,TM29112,TM29118,TM40633,TM29097
?: TM29106
TOWNSLAND, PRETORIA WEST, 25°43'S 28°09'E, 2528CA; *M.coucha:* TM4937,TM4936
WATERKLOOF, PRETORIA, 25°47'S 28°15'E, 2528CC; *M.coucha:* TM3862
WITFONTEIN, PRETORIA, 2528CA; *M.coucha*: TM44754
ALBEARY FARM, 15MI NE OF PRETORIA, 25°36'S 28°25'E, 2528CB; *M.coucha:* TM18966
DERDEPOORT RADIO STATION, PRETORIA, 25°43'S 28°18'E, 2528CB; *M.coucha:* TM17510,TM17593,TM17509
FONTANA MILITARY AREA, PETRONELLA, 25°30'S 28°17'E, 2528AD; *M.coucha:* TM41002,TM41003
LYNWOOD, 25°45'S 28°19'E, 2528CB; *M.natalensis:* TM12647
FOUNTAINS VALLEY, PRETORIA, 25°57'S 28°12'E, 2528CC; *M.coucha:*TM3873, TM17430,TM3874,TM17433
ISIS ESTATES, 2528CD; *M.coucha:* TM21649,TM21753,TM21822
HALFWAY HOUSE, 25°59'S 28°08'E, 2528CC; *M.coucha:* TM21752
ISCOR WORKS, PRETORIA, 25°46'S 28°09'E, 2528CA;*M.coucha:* TM12233
RIETVLEI DAM. PRETORIA, 25°53'S 28°17'E, 2528CD; *M.coucha:*TM29282, TM29292,TM29291,TM29290; *M.natalensis:* TM29288
VAN RIEBEECK NATURE RESERVE, PRETORIA, 25°53'S 28°17'E, 2528CD; *M.coucha:* TM19610
RENOSTEPOORT P.N.R, BRONKHORSPRUIT, 22°45'S 28°56'E, 2228DB;

M.coucha: TM23845,TM23825

SCHURWEBERG, PRETORIA, 25°48'S 27°59'E, 2527DD; *M.natalensis:* TM4156

LOSKOPDAM NATURE RESERVE, 25°25'S 29°20'E, 2529AD; *M.coucha*: TM19803, TM19822,TM19846,TM19849,TM19856,TM19899,TM19802,TM40898; *M.natalensis:* TM19848

RIETFONTEIN, 12K SW MIDDELBURG, 25°47'S 29°24'E, 2529CD; *M.coucha*: TM18968

LYDENBURG PROVINCIAL FISHERIES, 25°08'S 30°32'E, 2530BA; *M.coucha:* TM17480 ; *M.natalensis:*TM17479,TM17484,TM17478,TM17481,TM17482,TM17483, TM44037

SPITZKOP, 25°09'S 30°49'E, 2530BB; *M.coucha:* TM10345,TM10295

DULLSTROOM, 25°25'S 30°06'E, 2530AC; *M.coucha:*TM23762,TM23767,TM23777, TM23778,TM23796; *M.natalensis:* TM23763,TM23794,TM23795

9K SW RIVERSIDE PICNIC SITE, KNP, 25°16'S 31°30'E, 2531AD; *M.coucha:*TM39731,TM39742

GLADDESPRUIT, CARDINA WEST, 2530DB; *M.coucha:* TM1393

SERKSPRUIT, BARBETON, 25°55'S 30°42'E, 2530DC; *M.natalensis:* TM1999

KHANDIZWE PLATEAU, 9K W. OF MALELANE, KNP, 25°28'S 31°25'E, 2531AD *M.natalensis:* TM30018

LOWER SABIE, 2531BB; *M.coucha:* TM21697

HECTORSPRUIT, 25°26'S 31°41'E, 2531BC; *M.coucha*: TM1725

MALELANE CAMP, 25°28'S 31°31'E, 2531BC; *M.coucha:* TM1509,TM21692,TM21670 ?: TM1508

CROCODILE REST CAMP. KNP, 25°21'S 31°54'E, 2531BD; *M.coucha:*TM29976, TM29981,TM29982; *M.natalensis:* TM29975,TM29980

KOMATIPOORT,25°26'S 31°58'E, 2531BD; *M.coucha:* TM1727,TM29997 *M.natalensis:* TM1726

KROK BRIDGE CAMP, 25°21'S 31°55'E, 2531BD; *M.coucha:* TM21655; *M.natalensis:* TM21821

TEN BOSCH ESTATES, NE OF HECTORSPRUIT, 25°20'S 31°50'E, 2531BD; *M.coucha:* TM24868,TM24869,TM24870,TM24871; *M.natalensis:* TM24867,TM24882

PARANIE P.N.R, 12MI SSE OF NELSPRUIT, 25°36'S 31°07'E, 2531DA; *M.coucha:*TM20067,TM20103,TM20129

WORCESTER MINE, BARBETON,25°49'S 31°01'E, 2531CC; *M.coucha:* TM1396 *M.natalensis*: TM1398,TM1395B,TM1399,TM1397

BARBETON, 25°47'S 31°03'E, 2531CC; *M.coucha:*TM29433,TM29430,TM29446, TM29454,TM29449B,TM29435,TM29451,TM29450,TM29460; *M.natalensis*: TM29431,TM29436,TM29437,TM29438,TM29439,TM29443,TM29444,TM29449, TM29452,TM29453,TM29455,TM29456,TM29457,TM29458

TONGA BRIDGE, KOMATI RIVER, 25°50'S 31°51'E, 2531DD; *M.natalensis:* TM21832

BARBERSPAN PROVINCIAL NATURE RESERVE, 26°35'S 25°35'E, 2625CB; *M.coucha:* TM23443,TM23442

PRETORIA ZOO FARM, 6K NE OF LICHTENBURG, 26°07'S 26°12'E, 2626AA; *M.coucha:* TM27655,TM27654,TM27669

FARM ROTSEGAAI, 13K W OF VENTERSDORP, 26°22'S 26°32'E, 2626BC; *M.coucha:* TM27702,TM27745,TM27728,TM27729

DAMELSRUST, KRUGERSDORP, 2627AC; *M.natalensis*: TM12196

FILMANS FARM, SWARTKRANZ, KRUGERSDORP, 26°01'S 27°42'E, 2627BA
M.coucha: TM12187; *M.natalensis:* TM21778
HEUNINGKLIP, STERKFONTEIN, 26°01'S 27°44'E, 2627BA; *M.coucha:*TM21688, TM12188,TM21817
2MI S. ROODEPOORT, 26°11'S 27°52'E, 2627BB; *M.coucha:* TM17439,TM17438
FOUNTAIN BLUE, JOHANNESBURG, 26°06'S 27°58'E, 2627BB; *M.coucha:* TM1914
HONEYDEW, 26°05'S 27°56'E, 2627BB; *M.coucha:* TM17437
KLIPFONTEIN, 4.12MI NW OF JOHANNESBURG, 26°05'S 27°59'E, 2627BB; *M.coucha:* TM21789,TM21794
FARM VLAKFONTEIN, SW OF ROODEPOORT, 26°12'S 27°50'E, 2627BB; *M.coucha:* TM17435
THE WILLOWS, KRUGERSDORP, 2627BA; *M.natalensis:* TM21845
ZUURBEKOM, WESTONARIA, 26°18'S 27°47'E, 2627BD; *M.coucha*:TM18969, TM18970,TM18973
POTCHEFSTROOM, 26°44'S 27°04'E, 2627CA; *M.coucha:*TM392,TM1279, TM1285,TM391,TM1281,TM1282,TM2080; *M.natalensis:* TM1287
FARM NOOIDTGEDACHT, POTCHEFSTROOM, 26°55'S 27°10'E, 2627CC; *M.coucha*: TM44949,TM44951,TM44965,TM44948,TM44950; *M.natalensis:* TM44952
HOUGHTON ESTATE\SANDHURST, JOHANNESBURG, 26°05'S 27°59'E,2627BB; *M.coucha:*TM394,TM15509
RIETFONTEIN, 26°07'S 28°07'E, 2628AA; *M.coucha:*TM29003,TM29006,TM29008, TM29010,TM29017,TM29018,TM29025,TM29027,TM29030,TM29024; *M.natalensis*:TM29015
BOKSBURG, 26°14'S 28°15'E, 2628AA; *M.coucha*: TM29122,TM29095
SUIKEBOSCHRAND NATURE RESERVE, 26°30'S 28°13'E, 2628AC; *M.coucha:* TM41684,TM41683,TM25086,TM25093,TM25079,TM25083,TM25090, TM25092,TM25142,TM25174,TM25078,TM25175,TM25072,TM25075,TM25077, TM25088,TM25165; *M.natalensis:* TM25094
BLOEMDAL FARM, 26°20'S 28°35'E, 2628BC; *M.coucha:* TM17465
FARM ROLSPRUIT, 7K E. LESLIE, 26°25'S 29°00'E, 2629AC; *M.coucha*: TM27112,TM27039,TM27043,TM27075,TM27110,TM27132,TM27037 TM27038,TM27040,TM27042,TM27076,TM27078,TM27095,TM27096,TM27097, TM27130,TM27131,TM27036, TM27133,TM27113; *M.natalensis:* TM27109,TM27041
FARM MOORDRIFT, WATERBERG, 24°17'S 28°57'E, 2428BD; *M.coucha:* TM4288
FARM GOEDEHOOP, 11K S. OF BETHAL, 26°32'S, 29°27'E, 2629CB; *M.coucha:*TM26955TM27006,TM26958,TM26957,TM26952,TM26979,TM26981 *M.natalensis:* TM26951,TM26954,TM27003,TM26980
FARM ROODEPOORT, 15K E. STANDERTON, 26°55'S 29°23'E, 2629CD; *M.coucha:* TM24984,TM24991,TM25033,TM25054,TM25067,TM24986,TM24989, TM24997,TM25001,TM25011,TM25012,TM25031,TM25032,TM25055,TM25066, TM25068,TM25069,TM24973; *M.natalensis:*TM24987,TM24983,TM25002,TM24992 TM24999,TM25008,TM24988,TM25003,TM25022,TM25028,TM25034,TM25065
13MI \16MI FROM STANDERTON,26°46'S 29°29'E, 2629CD; *M.coucha:* TM21783,TM21743
ARAHEMBURG, CAROLINA, 26°03'S 30°50'E, 2630BB; *M.coucha:* TM1796, TM1801,TM1794B,TM1795,TM1802; *M.natalensis:* TM1797,TM1800

DOORNHOEK, KOMATI RIVER, 26°01'S 30°53'E, 2630BB; *M.coucha:*TM8719, TM1721,TM1717,TM1718
JOSHUA MOOLMAN P.N.R, AMSTERDAM, 26°41'S 30°36'E, 2630DA; *M.coucha:*TM20264,TM20172,TM20176,TM20212,TM20245,TM20247,TM20248,TM20249,TM20261,TM20171,TM20250; *M.natalensis*:TM20175,TM20177,TM20238,TM20263, TM20244,TM20173
POKWANI, GROBLERSDAAL, 24°54'S 29°44'E, 2429DC; *M.coucha:* TM6770
FARM WELGEDAAN, CHRISTIANA, 27°41'S 25°14'E, 2725CA; *M.coucha:*TM20788, TM20765,TM20789,TM20795,TM20797,TM20798,TM20814,TM20815, TM20816,TM20817,TM20791,TM20786; ?: TM20822
BLOEMHOF, 27°39'S 25°31'E,2725DA; *M.coucha:* TM5456,TM5455,TM5457
FARM BRANDHOEK, LEEUDORINGSTAD, 27°17'S 26°26'E, 2726AD; *M.coucha:*TM20718, TM20727,TM20751
KLIPSPRUIT 8, BARAGWANATH, 26°16'S 27°58'E, 2627BD; *M.coucha:* TM21765
? : TM21848
MAJUBA KRAGSTASIE RESERVE, 27°27'S 29°52'E, 2729BD; *M.coucha:* TM42428
FARM LANGFONTEIN, ENE OF WAKKERSTROOM, 27°13'S 30°08'E, 22730AA; *M.coucha:* TM25200,TM25227,TM25241; *M.natalensis:* TM25226,TM25218,TM25250
1\2 WAKKERSTROOM, VOLKRUST, 2730AC; *M.coucha:* TM21666
PIET RITIEF COMMONAGE, 27°00'S 30°48'E, 2730BB ; *M.coucha*: TM21652,TM21694
WAHLERSTROOM, 27°21'S 30°09'E, 2730AC ; *M.natalensis:* TM1221
20MI FROM PIET RITIEF, 2730DB; *M.coucha:* TM21750
FARM LEEUWSPOOR, ON PONGOLA RIVER, 27°25'S 31°52'E, 2731BD; *M.natalensis*: TM24965
PONGOLA NATURE RESERVE, PONGOLA, 27°42'S 31°52'E, 2731DB; *M.coucha:* TM42386 *M.natalensis:*TM42382

SWAZILAND

MANTENGA RANCH, 2531DC ; *M.coucha*: TM13429,TM13430 ; *M.natalensis*:TM13431,TM13432
P.O ERANCHI, 2531DC; *M.coucha*: TM21847; *M.natalensis*: TM21671,TM21824
MALOLOTJA NATURE RESERVE, 26°10'S 31°05'E, 2631AA; *M.coucha*: TM44838 *M.natalensis:* TM42064
RANCHES LTD, 26°02'S 31°42'E, 2631BA; *M.coucha:* TM8491A,TM8491B, TM8494,TM8495,TM8509,TM8511,TM8480,TM8488,TM8496,TM8507,TM8510,TM8513, TM8516,TM8521,TM8477,TM8505,TM8506,TM8525,TM8470A; *M.natalensis:* TM8486,TM8481,TM8492,TM8500,TM8468,TM8474,TM8475,TM8478,TM8478B, TM8489,TM8493,TM8465,TM8466,TM8467,TM8469,TM8472,TM8473,TM8476 TM8482,TM8497,TM8498,TM8501,TM8508,TM8522,TM8523,TM8487,TM8499,TM8504, TM8515,TM8520,TM12707
NCABANENI, 26°37'S 31°08'E, 2631BD; *M.natalensis*: TM44982
ALLEN'S FARM, 26°33'S 31°16'E, 2631CA; *M.natalensis:* TM44839,TM44841

ZIMBABWE

SEICHE TOWERS,KARIBA,1828DA; *M.coucha:* TM31168; *M.natalensis:* TM31170,TM31169

NAMPINI,CAPRIVI,17°51'S 25°20'E,1725CD; *M.natalensis*: TM12301

UMFULI DISTRICT,17°30'S 29°23'E,1729AD; *M.natalensis:* ZM4151

EDMONDS FARM HARARE OR HARARE,17°50'S 31°04'E,1731CC; *M.coucha:*ZM3043,ZM4070,ZM4079,ZM4078,ZM4074,ZM3258,ZM3259,ZM4080; *M.natalensis:* ZM4077

SENGWA WILDLIFE RESEARCH STATION OR ST PAULS MISSION 18°10'S 28°23'E,1823AB; *M.natalensis*: TM35883,TM34959,TM34960,TM34962, TM34867,TM34868,TM34882,NHMZ73699,NHMZ73698

INYANGA,18°12'S 32°45'E,1832BA; *M.natalensis*: TM11411

RHODES INYANGA NATIONAL PARK,18°17'S 32°46'E,1832BD; *M.natalensis:* TM34727

HANDI VALLEY,1832DA; *M.natalensis:* TM11412

VUMBA,19°05'S 32°44'E,1932BA; *M.natalensis:* TM8645

SPRINGVALE, BULAWAYO OR BULAWAYO,20°10'S 28°35'E,2028BA; *M.coucha:* KM33301; *M.natalensis:* KM33302

RHODES MATOPOS NATIONAL PARK,20°23'S 28°28'E,2028AD; *M.natalensis:* TM35003,TM35004,TM35005,TM35019,TM35002,TM35016

NSESI RIVER,48MI FROM BULAWAYO,2029AC; *M.natalensis:* TM21908

RUSITO FOREST,20°02'S 32°59'E,2032BB; *M.coucha:* TM34774; *M.natalensis:* TM34773,TM34775,TM34799

MOUNT SELINDA,CHIRINDA FOREST,20°27'S 32°40'E,2032BC; *M.natalensis:* TM7755,TM7756,TM34630,TM7757,TM8646,TM34645

BUMBOOSIE,18°33'S 26°13'E,1826CA; *M.coucha:* NHMZ73830,NHMZ73859; *M.natalensis:NH*MZ73840,NHMZ73841,NHMZ73842,NHMZ73845,NHMZ73846, NHMZ73847,NHMZ73848,NHMZ73851,NHMZ73851B,NHMZ73852,NHMZ73853, NHMZ73855,NHMZ73856,NHMZ73858,NHMZ73821,NHMZ73822,NHMZ73823, NHMZ73824,NHMZ73826,NHMZ73827,NHMZ73828,NHMZ73829,NHMZ73833, NHMZ73835,NHMZ73837,NHMZ73839,NHMZ73849,NHMZ73850,NHMZ73852, NHMZ73852B,NHMZ73831,NHMZ73832,NHMZ73834,NHMZ73836,NHMZ73863

SABI-LUNDI,21°18'S 32°24'E,2132AD; *M.natalensis*: TM10761,TM10758,TM10757

MATETSI,18°17'S 25°56'E,1825BD; *M.natalensis*: NHMZ73865,NHMZ73868

KAZUNGULA,17°47'S 25°16'E,1725CD; *M.natalensis*: NHMZ73869,NHMZ73867

NKAI AIRFIELD,19°00'S28°54'E,1928BB; *M.coucha:* NHMZ73656; *M.natalensis:*NHMZ73658,NHMZ73660,NHMZ73659,NHMZ73662,NHMZ73655

ST LUKES MISSION,1927BA; *M.natalensis:* NHMZ73697,NHMZ73700, NHMZ73703, NHMZ73704,NHMZ73723,NHMZ73724,NHMZ73702

ROBIN'S CAMP,1825DB; *M.natalensis:* NHMZ73844, NHMZ73854,NHMZ73860, NHMZ73862,NHMZ73838, NHMZ73861

KAZUMA,1825BC; *M.natalensis*: NHMZ73871

PANDAMATREWA,1825DA; *M.natalensis*: NHMZ73872, NHMZ73870

MAFUBAGUSI FOREST,1828BD; *M.natalensis:* NHMZ73657,NHMZ73661, NHMZ73664

BINQA =(BENGA),17°40'S 27°40'E,1727DA; *M.natalensis:* NHMZ73726,NHMZ73727

KARYANGWE MISSION,1727DC; *M.coucha:* NHMZ73733; *M.natalensis:* NHMZ73729, NHMZ73732, NHMZ73731

LUSULU,1827BB; *M.coucha*: NHMZ73738,NHMZ73740,NHMZ73741,NHMZ73748, NHMZ73734; *M.natalensis*: NHMZ73750,NHMZ73735,NHMZ73736,NHMZ73737, NHMZ73739,NHMZ73742,NHMZ73743,NHMZ73744,NHMZ73746,NHMZ73751
DEKA,18°05'S 26°40'E,1826BA; *M.natalensis*: NHMZ73752,NHMZ73754,NHMZ73753
NAMBIDZWA,1729CA; *M.coucha*: NHMB73580, *M.natalensis:* NHMZ93592; ?: NHMZ73602
MAIN CAMP,WANKIE NATIONAL PARK,1826DB; *M.natalensis:*NHMZ73608, NHMZ73591, NHMZ73612, NHMZ73587