

Identification of cryptic species of rodents (*Mastomys*, *Aethomys*, *Saccostomus*) in the Kruger National Park

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Mastomys natalensis, *Aethomys chrysophilus* and *Saccostomus campestris* are three common and widespread rodent species in the Kruger National Park. Chromosomal and protein electrophoretic investigations reveal that these species are in fact complexes of morphologically similar, though genetically distinct, species. Their respective distributions in the Kruger National Park are reported and species diagnostic characters (diploid chromosome number, genitalia, spermatozoa and electromorphs) are presented for the practical identification of the cryptic species. The value of a genetical approach to resolving cryptic species is emphasized with respect to ecological studies.

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Mastomys natalensis, *Aethomys chrysophilus* en *Saccostomus campestris* kom algemeen en wydverspreid in die Nasionale Krugerwildtuin voor. Chromosoom- en elektroforetiese studies dui aan dat hierdie spesies in werklikheid komplekse van morfologies eenderse maar geneties onderskeibare spesies is. Hul verspreidingsgebiede binne die Krugerwildtuin en hul diagnostiese kenmerke (diploïede chromosoomgetal, genitalia, spermatozoa en elektromorfe) word gegee om die identifikasie van die onderskeie sibbespesies te vergemaklik. Die nut van 'n genetiese benadering word beklemtoon, veral ten opsigte van ekologiese studies.

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Faunal surveys of National Parks are considered of prime importance as the basis for effective conservation and management of animal populations (Pienaar 1984). In the case of large mammals, species identification does not present a particular problem. By contrast, small mammals and especially rodents pose numerous unresolved questions concerning their distribution, ecology and species identification despite the fact that they have been the subject of a number of major taxonomic and zoogeographic studies in southern Africa (Pienaar, Rautenbach & De Graaff 1980; De Graaff 1981; Rautenbach 1982; Lynch 1983; Smithers 1983).

Accurate identification of rodent species is problematical because a phenetic approach which traditionally involves the comparison of morphological features such as pelage colour or cranial features may not resolve the differences between cryptic, but genetically distinct, species. The problem may be resolved if a genetic concept of species is considered.

In this sense, species are defined in terms of positive assortative mating between conspecifics or in population genetic terms, as a gene pool or 'field for gene recombination' (Carson 1957). Recent studies on southern African rodents have revealed that the taxa *Aethomys chrysophilus*, *Mastomys natalensis* and *Saccostomus campestris*, are species complexes (Gordon & Rautenbach 1980; Green, Keogh, Gordon, Pinto & Hartwig 1980; Gordon in press). In the case of *Mastomys*, the nomenclatural problems have been resolved with reference to the genetic species: *M. coucha* is characterized by a diploid chromosome number ($2n$)=36 and 'fast' double-banded haemoglobin electromorph and *M. natalensis* by $2n$ =32 and 'slow' haemoglobin electromorph relative to a human standard. The distribution of these cryptic species is known generally for southern Africa but not specifically for Kruger National Park. *Aethomys chrysophilus* was recognized as a species complex based on chromosomal data from sympatric populations; no intermediate chromosomal forms were detected in nature. Gordon & Rautenbach (1980) proposed that *A. chrysophilus* specimens with $2n$ =50 be referred to *A. chrysophilus sensu stricto* and those with $2n$ =44 as *A. chrysophilus* sp. B. Systematic and nomenclatural aspects of the *Aethomys* group are currently under study by D. Visser (in prep., M.Sc. thesis). On the basis of chromosomal, biochemical and zoogeographic data, the taxon *S. campestris*, formerly recognized as a monotypic genus in southern Africa, is now considered to comprise at least two genetic species which are tentatively referred to as sp. A and sp. B (Gordon in press).

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In this report we describe chromosomal, electrophoretic and morphological markers of cryptic rodent species which occur in the Kruger National Park. The significance of accurate species identification with regard to ecological studies and taxonomy is stressed.

Materials and Methods

A total of $N=10$ *Aethomys*, $N=510$ *Mastomys* and $N=23$ *Saccostomus* specimens were used in this study from six localities near Luvuvhu Hippo Pool (quarter degree grid reference 2231AB), Pretoriuskop Camp (2531AB), Punda Maria Camp (2231CA) and Satara Camp (2431BD) in the Kruger National Park. Transvaal Museum voucher specimen numbers and localities are given in Appendix A.

All animals were captured with Sherman live-traps and processed in the laboratory with the exception of the majority of *Mastomys* which were captured, marked and released as part of an ecological study in the Satara Camp area (Watson, C.R.B. in prep., M.Sc. thesis). Each new *Mastomys* specimen found on the trap lines was toe-clipped for individual recognition and a blood sample taken for electrophoresis.

The combination of techniques given below was used to identify the genetic species.

Electrophoresis of haemoglobin. One to two drops of whole blood were taken up in a heparinized capillary tube from a tail snip or into a syringe from a cardiac puncture. Separation of plasma and erythrocytes was not necessary. Samples were lysed with an equal volume of water and applied to a 5 mm × 5 mm chromatography paper insert. Samples were electrophoresed in a 12,5% horizontal starch gel and Tris-EDTA-Borate buffer system (Harris & Hopkinson 1976) for approximately 4 h at 350 V. No staining was needed to visualize the haemoglobin electromorphs.

Chromosome preparation. Karyotypes were prepared by the standard *in vitro* method from bone marrow after treating the animal with yeast-dextrose (Lee & Elder 1980).

Spermatozoa preparation. Spermatozoa were expressed into HBSS and slides prepared and stained with silver-nitrate and Giemsa according to Elder & Hsu (1981). Measurements of spermatozoa tail parts, the mid-piece and principal piece, were taken with a measuring eyepiece.

Preparation of genitalia. Penes were removed from freshly killed specimens and preserved in AFA (a solution of ethyl alcohol, formalin and acetic acid). Anatomical terminology of the phallus follows that of Lidicker (1968).

Results

The following species were found in the Kruger National Park: *M. natalensis*, *M. coucha*, *A. chrysophilus* s.s., *A. chrysophilus* sp. B and *S. campestris* sp. A.

Mastomys natalensis and *M. coucha*

Karyotyped specimens showed similar $2n$'s ($2n=32$ and 36) and associated haemoglobin electromorph (Figure 2) to that described by Green, Gordon & Lyons (1978).

In the two-year study by Watson (in prep., M.Sc. thesis) of the rodent communities of the Marula/Knobthorn and *Acacia welwitschii* landscapes (Gertenbach 1983), the haemoglobin marker was used extensively to identify *Mastomys* specimens. *Mastomys coucha* was widespread and abundant in both habitats. *Mastomys natalensis* occurred primarily west of Satara, including the *Acacia welwitschii*

habitat under study. Its presence was sporadic and transitory for most of the study, though in the latter part, a more stable population did appear following good rains. The main areas of sympatry then occurred west of Satara with *M. coucha* being numerically dominant. However, Satara Camp and its immediate vicinity (< 500 m) appeared to support only *M. natalensis* as no *M. coucha* were ever identified there. Sampling near Pretoriuskop Camp in the southern portion of the Kruger National Park revealed *M. natalensis*.

Spermatozoa morphology represents an easy means to discriminate between the two genetic species (Figure 1). Although the sperm head shape and differential staining pattern are very similar between *M. coucha* and *M. natalensis* (Figure 3E, F), differences in tail structure length are apparent. The tail mid-piece (MP) and principal piece (PP) are larger in *natalensis* (range & mean): MP 50–52,5, & 51,2; PP 101–107 & 104,1) than in *coucha* (MP 44–46,5 & 45,5; PP 96–101 & 98,8).

In addition to spermatozoa morphology, Gordon (1984) found that *coucha* and *natalensis* could be distinguished on the basis of internal morphology of the phallus, in particular the shape of the lobed urethral lappets which form the distal surface of the urethra. Although the *coucha* specimens used in this study were immature males, it was possible to examine three of the four *natalensis* males (TM 37173, 37174, 37061). The urethral lappet shape was trilobed, each lobe being of equal size.

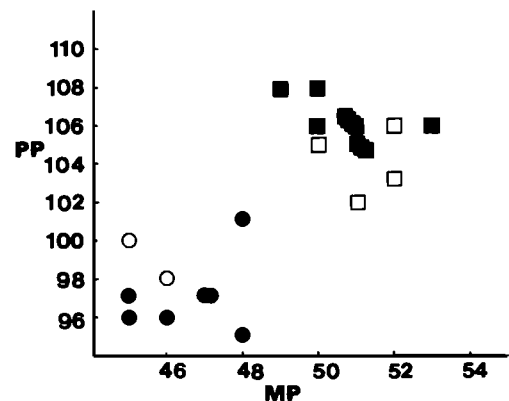


Figure 1 Bivariate plot of *Mastomys* spermatozoa tail principal piece (PP) length versus midpiece (MP) length. A complete separation of the cryptic species, *M. natalensis* (squares) and *M. coucha* (circles) is evident. Specimens from the Kruger National Park (open symbols) cluster centrally with respect to specimens from other areas of South Africa.

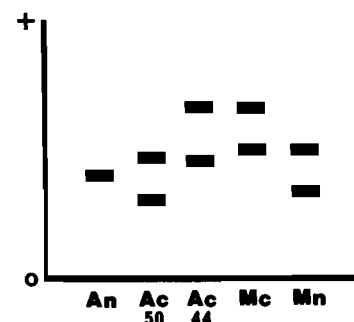


Figure 2 Species-specific electromorph pattern of haemoglobin from *Aethomys namaquensis* (An), *A. chrysophilus* (Ac, $2n=50$), *A. chrysophilus* sp. B (Ac, $2n=44$), *Mastomys coucha* (Mc) and *M. natalensis* (Mn).

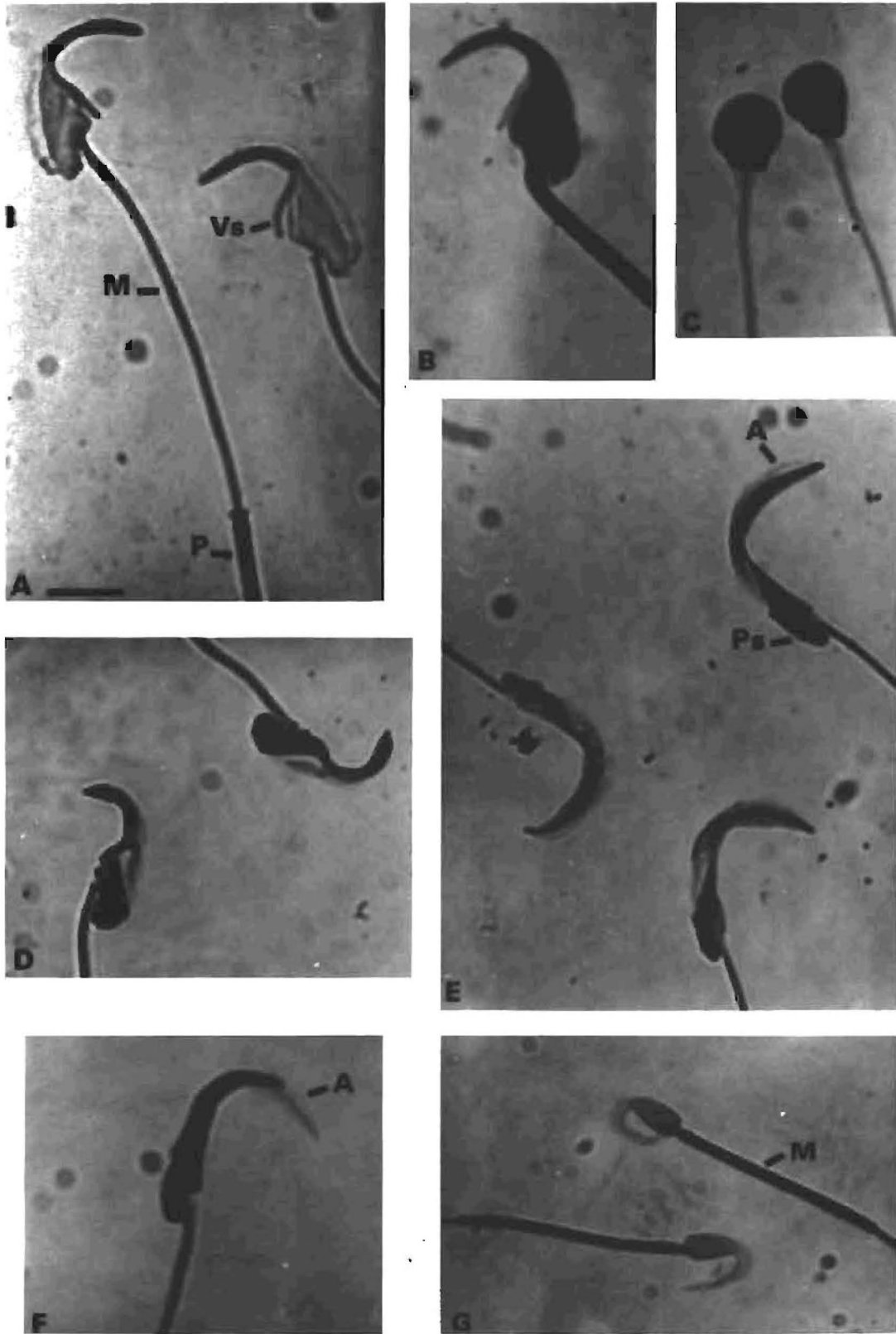


Figure 3 Spermatozoa of *Aethomys*, *Mastomys* and *Saccostomus* specimens from the Kruger National Park. (A) Silver-nitrate stained spermatozoa of *A. chrysophilus* ($2n=50$) showing differentially stained head with ventral spike (Vs), tail midpiece (M) and part of principal piece (P). (B) Giemsa-stained head of *A. chrysophilus* ($2n=50$). (C) Giemsa-stained spermatozoa head of *A. chrysophilus* sp. B ($2n=44$) which shows the extreme shape difference between the *chrysophilus* cryptic species. (D) Silver-nitrate stained spermatozoa head of *A. namaquensis*. (E) Silver-nitrate stained head of *M. natalensis* showing acrosome (A) and postacrosomal sheath (Ps). (F) Giemsa-stained head of *M. natalensis*. (G) Giemsa-stained head of *S. campestris*. Scale bar in A is approximately $10\ \mu\text{m}$ for A-F.

Aethomys spp.

Initial cytogenetic analysis of *A. chrysophilus* s.l. in the *Acacia welwitschii* area showed that specimens were *A. chrysophilus* sp. B ($2n=44$), the widely distributed South

African species (Gordon & Rautenbach 1980). *A. chrysophilus* s.s. ($2n=50$) and *A. namaquensis* were subsequently found in sympatry in the Klopperfontein area north of Punda Maria Camp. Although specimens with $2n=50$ had

been described previously by Gordon & Rautenbach (1980) from Tzaneen and Mulder's Drift, their identity is in doubt; no voucher specimens or karyotype slides are available and further attempts to trap *Aethomys* in Tzaneen resulted only in *A. chrysophilus* sp. B (Visser, D. 1984, per. comm.). The Klopperfontein population therefore represents at present the only positively known location of *A. chrysophilus* s.s. in South Africa.

Both spermatozoa morphology and haemoglobin electromorphs can be used to differentiate between these species (Figures 2 & 3). The spermatozoa of *A. chrysophilus* s.s. and *A. chrysophilus* sp. B are strikingly different in head shape. The former has hook-shaped sperm head with a ventral spike while the latter is essentially spatulate shaped. The sperm head of *A. namaquensis* is also hook-shaped though the apex is shorter and no ventral spike is present; differential staining with silver nitrate shows a different silver-positive post acrosomal sheath shape to that of *A. chrysophilus*.

Haemoglobin electromorph patterns are species specific. *A. namaquensis* has a single band while *A. chrysophilus* has a slow double band and *A. chrysophilus* sp. B has a fast double band (Figure 2).

A preliminary examination of penile morphology indicates that there are relative size and shape differences in the urethral lappets, bacular mound and baculum between *A. chrysophilus* and *A. chrysophilus* sp. B. However, a larger sample size and an analysis of geographic variation are needed in order to assess the diagnostic value of these characters.

S. campestris

Karyotypes from widely distributed populations in southern Africa have revealed extensive variation in $2n$ (16 variants ranging from $2n=28-50$) and in chromosomal arm morphology within $2n$ form (Gordon in press). Specimens trapped in the Satara, Luvuvhu Hippo Pools and Punda Maria areas all have $2n=46$ and can be referred to sp. A. Their identity is consistent with the overall distribution of the $2n=46$ species in the Southern Savanna Woodland Biotic Zone in the northern Transvaal, northern Natal coastal area, western coastal Cape and southern Zimbabwe.

A comparison of phallus and spermatozoa morphology between the species is at present under study.

Discussion

The presence of cryptic rodent species in the Kruger National Park serves to emphasize two important points. Firstly, the use of informal designations such as sp. A or B draws attention to the need for an appropriate taxonomy and nomenclature that accurately reflects the nature of genetic species. Secondly, unequivocal species identification is a prerequisite to meaningful epidemiological, ecological, distributional and management studies. Apposite examples from medical zoological studies which concern insect vectors or disease reservoir hosts demonstrate the insight gained into disease biology and zoonoses; examples include the resolution of the *Anopheles* species complex with regard to malaria transmission (Paterson 1963) or the *Mastomys* complex in plague studies (Taylor, Gordon & Isaacson 1981). In the latter study, the distribution of human plague cases correlated positively with the distribution of *M. coucha*; subsequent plague susceptibility tests on both *Mastomys* species demonstrated that only *coucha* was susceptible and therefore played a role in the maintenance

of the zoonosis (Isaacson, Taylor & Arntzen 1983).

Within the Kruger National Park, unequivocal species identification is an essential prerequisite for meaningful rodent ecological research, particularly in the case where sympatric species occur or for comparison of species diversity within various habitats or localities in the Park. There have been two studies on rodent ecology to date in the Kruger National Park: the study by Kern (1981) on the influence of fire on small mammal species composition, population density and species diversity and the study by Watson (in prep., M.Sc. thesis) in the Satara area. The latter represents the first attempt to take the occurrence of cryptic rodent species in the Park into account.

Incisive questions may now be asked regarding the habitat preferences and population dynamics of the *Aethomys* species and of *M. coucha* and *M. natalensis*. The latter species have been described as 'the most common and widely spread rodent in the Park' (Pienaar *et al.* 1980:33). Since both species are now known to occur in the Park further sampling and identification of genetic species is needed to assess their respective distributions. Although electrophoretic methods are the most efficient tool for identification, the morphological markers described in this study represent an alternative inexpensive means to the same end.

The occurrence of sympatric populations of *Mastomys* in the Satara area provides a convenient locality (enhanced by the ecological data base provided by Watson (in prep., M.Sc. thesis) for investigating the commensal behaviour of *Mastomys* as well as the host specificity and ecology of their ectoparasites. Questions are also forthcoming with respect to *Saccostomus* population genetics. Preliminary evidence shows that the $2n=46$ form in the Kruger National Park is far more variable chromosomally than the $2n=46$ populations in Hluhluwe Game Reserve; the significance of these contrasting degrees of variation is not yet clear. It is probable that further genetic investigations of the small mammals of Kruger National Park will reveal hitherto unrecognized complexes of cryptic species and thus enable research workers to gain a better insight into rodent biology and management.

The approach used in this study has merit beyond its specific application to the Kruger National Park. More faunal surveys have been prepared for southern Africa than perhaps any other region in Africa. There is, therefore, adequate basic data on distribution, variation in habitat preferences and morphology of rodent species to highlight which taxa should be re-examined. This study emphasizes the advantages of reassessing zoogeographic and ecological conclusions from a genetic perspective.

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Appendix A

Gazetteer of *Mastomys*, *Aethomys* and *Saccostomus* specimens examined from the Kruger National Park. Diploid chromosome number ($2n$) and sample size (N) are given in brackets; voucher specimens deposited in the Transvaal Museum Mammal Department are prefixed with TM. *Mastomys* trapped by C.R.B. Watson for an ecological study are not included.

M. natalensis ($2n=32$) ($N=9$): Satara Camp (TM 36655, 36656, 36669, 36671, 36673, 37061, 37173, 37174); 2 km W of Pretoriuskop Camp (TM 36668).

M. coucha ($2n=36$) ($N=2$): Satara Camp (TM 36670, 36673).

A. chrysophilus sp. B ($2n=44$) ($N=2$): 10 km W of Satara Camp (TM 35954).

A. chrysophilus ($2n=50$) ($N=3$): 14 km ENE of Punda Maria Camp (TM 35959, 36657, 37172).

A. namaquensis ($N=5$): 14 km ENE of Punda Maria Camp (TM 36648, 36649, 36651, 36672); 2 km NW of Pretoriuskop Camp (TM 36652).

S. campestris sp. A ($2n=46$) ($N=23$): 12 km ESE of Satara Camp (TM 35949, 35950, 35951, 35952, 35953, 36650, 36653, 36659, 36661, 37050, 37053, 37054, 37056, 37058, 37182, 37183, 37186, 37189); Punda Maria Camp (TM 37044, 37045, 37051, 37188); Luvuvhu Hippo Pool (TM 30523).