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The *Lophuromys flavopunctatus* THOMAS 1888 s.l. species complex : a craniometric study, with the description and genetic characterization of two new species (Rodentia - Muridae - Africa)

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Abstract

We revised the taxonomy of the East African *Lophuromys flavopunctatus* species complex using craniometric data of nearly 3000 specimens grouped in 49 operational taxonomical units (OTU's) covering the whole of its geographical distribution.
Our study demonstrates that the differences in age and sex composition of OTU's are of no consequence for the branching of the obtained phenetic trees. This observation permitted us to screen the whole of the *L. flavopunctatus* s.l. species complex, to evaluate the validity of the already known taxa and to describe two species new to science.
Our study complements this traditional morphological approach with mitochondrial nucleotide sequences to characterize the two new species and several OTU's.

Keywords: Rodentia, East Africa, *Lophuromys*, taxonomy, craniometry, cytochrome b, genetics.

Résumé

La taxinomie du 'complexe d'espèces' *Lophuromys flavopunctatus* a été réévalué en nous basant sur les données craniométriques d'environ 3000 crânes groupés en 49 unités taxinomiques opérationelles (UTO's).
Notre étude a démontré que des différences de composition des UTO's, (âge et sexe) n'influencent pas la structure des arbres phénétiques (UPGMA) obtenus. Cette observation nous a permis d'évaluer la validité systématique des différents taxa et de décrire deux nouvelles espèces.
Nous avons employé des séquences mitochondriales afin de mieux charactériser nos deux nouvelles espèces et plusieurs UTO's.

Mots-clés: Rodentia, Afrique de l'Est, *Lophuromys*, taxinomie, craniométrie, cytochtome b, génétique.

INTRODUCTION

The "speckled brush furred" African rats have been grouped in the *Lophuromys flavopunctatus* THOMAS 1888 s.l. species complex by VERHEYEN *et al.* (1996). Representatives of this complex are widely distributed from North-Eastern Angola through Southern Congo, Northern Zambia, Malawi, Northern Mozambique, Tanzania, Burundi, Ruanda, Eastern Congo, Northern Congo, Uganda, Southern Kenya and Ethiopia. The distributional range of the Ethiopian populations is separated from that of the rest of the species complex by dry lowlands.
L. flavopunctatus s.l. prefers moist, marshy and grassy biotopes. Vegetation composition, structure and density seem not to be too important. In these optimal biotopes the "speckled brush furred" rats are generally by far the most dominant rodent. The actual geographical distribution of *Lophuromys flavopunctatus* s.l. seems to be solely determined by rainfall (density and pattern) and not by altitude, temperature or biotope structure (DIETERLEN, 1976).
Over the whole distributional range of this species group, fifteen taxa have been described based on the considerable variation in pelage colouration and external measurements. For a full listing of these taxa, their type-localities, geographical co-ordinates we refer to Appendix 1.1 and fig. 1. We do not include *Neanthomys giaquintoi* TOSCHI 1946 from Addis Ababa, which description was solely based upon an artifact: the absence of a tail in the type specimen (TOSCHI, 1963). The skull measurements of the type-specimens, as measured by us, are listed in Appendix 1.2.
In their general taxonomic review of the Murids of the world, MUSSER & CARLETON (1993) consider that most of these taxa fall into synonymy with *flavopunctatus* THOMAS 1888 adding however that "... the appreciable character variation among samples probably reflects more than one species" (ib., p.605).
A recent study on the Ethiopian *Lophuromys flavopunctatus* species complex (LAVRENCHENKO *et al.*, 1998) demonstrated that *Lophuromys chrysopus* OSGOOD 1936 and *Lophuromys brevicaudus* OSGOOD 1936 are clearly distinct Ethiopian endemic species. *Lophuromys melanonyx* PETTER 1972, a morphometrically well defined endemic species, was at that time intentionally not included in that analysis. For Southern Ethiopia, we mentioned the presence of what we provisionally considered to be representatives of *Lophuromys flavopunctatus brunneus* and even a fourth taxon classified tentatively as *Lophuromys* species D but possibly identical to typical *Lophuromys flavopunctatus* s.s.
The present study intends to complete the taxonomical revision of the *Lophuromys flavopunctatus* species group over its entire geographical range except for the "speckled brush

furred" rats from Mt Ruwenzori (our OTU 14) to be described elsewhere.

Our study illustrates the practical problems that stand between the many biological species concepts and the practical considerations of taxonomists that are faced with the task to describe taxa based on specimen collections. As in this study, the most often practiced approach is the description of rodent species based on morphometrical data, ideally taken from a sufficient large number of specimens. This approach enables us to deal with the disparities and inadequacies that characterize some of the early taxonomical descriptions.

The poor quality of many of the type specimens led us to implement multivariate cranio-metrical analyses for this revision. Because the cranium is often the only more or less usable part of a type specimen, we selected a number of reliable measurements which will allow us to compare between even severely damaged skulls. In view of the well-documented skull variability (growth and sexual dimorphism), we had to give a statistical dimension to our approach by including sufficiently large samples (OTU's = Operational Taxonomical Unit's) of measurable skulls, that cover the whole geographical range of the species group (in total 49 OTU's were used). An essential consequence of our approach is that we will try, through a number of specific canonical analyses and by subsequent plotting of the type-skulls, to discuss the status of the described taxa and to identify, for some of those taxa, OTU's which can be considered to represent these taxa.

We complement this traditional approach by the genetic characterization of a species new to science with partial mitochondrial cyt b sequences. These molecular data cannot substantiate the claim that the morphologically described species are biologically valid. However, whenever the studied OTU's possess fixed genetic differences, these nucleotide sequences can be used as an additional diagnostic feature to characterize newly described taxa. To test the hypothesis that observed differences between the studied taxa are meaningful, we first analyzed the nucleotide variation among specimens of some conspecific populations for which sufficient samples were available.

MATERIAL AND METHODS

The specimens

As the results of many fieldtrips in central Africa, realized between 1965 and 1995 by the Research group on African Rodents (RUCA, Department of Biology, University of Antwerp, Belgium), we established an extensive specimen collection. All specimens were prepared for study at the University of Antwerp before they were deposited at the Royal Africa Museum (Tervuren, Belgium). This study is largely based on these collections, but when necessary, our study material was completed with skulls from other collections. Our results are based on information from about 3150 skulls, of which 2.700 were suited for our analyses

In Appendix 2 we have grouped the specimens examined and measured per OTU. For each OTU the number of specimens, classified by sex and age, is provided. For the description of the acronyms, that identify the musea and institutions were these specimens are curated, we refer to VERHEYEN *et al.* (1996). Appendix 3 gives an alphabetical list of the collecting localities, followed by their geographical co-ordinates; between brackets is the OTU number into which the locality is included. Fig.2 describes the geographical distribution of the OTU's studied.

Craniometry

All skulls are grouped into age-classes using tooth eruption and tooth wear patterns as described in VERHEYEN *et al.* (1996). Also in the present study, the observed cusp structure of the upper cheekteeth and more especially in M^1 is very variable, which implies that the age-classifying method, with its well established theoretical and practical limitations, can at best be used to give a general impression.

The present study uses the same cranial and external measurements and the same acronyms as described in VERHEYEN *et al.* (1996). We draw special attention to p.246 where we discuss how we selected the used cranial measurements. The measurements were taken with callipers with digital reading graduated to hundreds of millimetres, but were recorded with a precision of 0,05 mm.

To facilitate the interpretation of our results, we supplied a full description of our measurements with drawings of a *Lophuromys flavopunctatus* skull (see appendices 4.1. and 4.2. in VERHEYEN *et al.* 1996). Table 1 (App. 4) briefly recapitulates the cranial measurements used here. Basic statistics, Student-t tests, One-way Analysis of Variance, Student-Newsman-Keuls a posteriori test (SOKAL & ROHLF, 1969), Multiple Discriminant or Canonical Analysis were performed on a PC with the statistical package STATISTICA 5.5 from StatSoft, Inc.

Statistical analyses were always carried out using the whole set of available data regardless of sex, but excluding data from specimens of age-classes 0 and 5. The metrical datasets of the operational units used in this study (see Appendix 2) are not fully published but can be obtained through e-mail (hulsel@ruca.ua.ac.be).

In order to clarify the multi-group graphs, we do not show the individual scores, but draw the 95% equiprobable ellipses. In certain cases we prefer to construct a tree diagram, based on the Mahalanobis squared distances between the centroids, using the Unweighted Pair Group Arithmetic Average method (SNEATH & SOKAL, 1973). This accounts for all the relevant axes in the canonical hyperspace. Sometimes missing data are replaced by group means.

DNA methods

The twenty four specimens that were used for this part of our study were collected during various expeditions in East Africa in 1984 (Ruanda), 1985 (D. R. Congo), 1989 (Tanzania), 1991 (Kenya), 1994 (Tanzania) (see listing of specimens in table 5). Tissues were stored at -80°C (or in 80% ethanol at 4°C). Samples from specimens from Ethiopia were supplied by Corti and Bekele.

DNA was isolated from ethanol-preserved muscle or liver tissues by standard proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation (SAMBROOK *et al.*, 1989) or by the Chelex method (WALSH *et al.*, 1991). We amplified and sequenced a 402 bp long segment of the cyt b gene (from position 14139 to 14540 on the mitochondrial DNA sequence of *Mus musculus* (BIBB *et al.*, 1981). The cyt b primers are L13724 [5'-cgaagcttgatat gaaaaaccatcgttg-3'] and H14139 [5'-aaactgcagcccctcagaat gatatttgtcctca-3'] (KOCHER *et al.*, 1989) and the PCR reaction was done using the protocols given in KOCHER *et al.* (1989). The samples were sequenced with the H14139-primer using a standard CycleSequencing protocol (Amersham Pharmacia Biotech), following the manufacturer's protocol, using 0.8µM primer, 2.5 units of Taq polymerase and approximately 0.15-0.20 µg of the PCR product. The cycle-sequencing reaction consisted of 30 cycles: 36 s at 94°C, 36 s at 52°C and 80 s at 72°C. Sequences were read and aligned by eye. The obtained nucleotide sequences were imported in Mega 2.1 for analyses (KUMAR *et al.*, 2001).

RESULTS

1. Sexual dimorphism and growth in the skull of *Lophuromys flavopunctatus*

Because previous studies considered that sexual dimorphism in *Lophuromys flavopunctatus* and *Lophuromys sikapusi* skulls is insignificant, it has become customary to lump sexes in the OTU's that were used in our previous studies (VERHEYEN *et al.*, 1996, 1997, 2000, LAVRENCHENKO, et al. 1998). Since the present data set is the first to contain certain OTU's with a sufficiently large number of skulls, we will be able to test statistically whether or not lumping data from both sexes affects the outcome of our canonical analyses.

The biggest OTU available for this purpose comprises 377 complete skulls (209M-168F) and was collected in and around Tshibati (see App. 2) mostly by our colleagues U. RAHM and F. DIETERLEN. We split the data of this OTU by sex and age classes and calculated the basic statistics of the skull measurements and of the in the field collected external measurements (Appendix 4 and Table 2).

Sexual differences seem to be nearly absent not only for the skull measurements but also for the external measurements. The only exceptions are in age class 3, where sexual dimorphism is highly significant for "choanae breadth" (M18) and "distance between coronoid and angular process in the mandibula" (M24). Consequently both measures were excluded in our further canonical analyses. We also note that in age-class 3 male animals are about 10% heavier than females.

Our data also suggest that the growth of the skull of a specimen, once it has fully erupted teeth, will remain rather limited. In both sexes, most measures show a small but steady growth from class 1 up to class 3. As expected, the molars and bullae do not increase in size whereas the upper incisor caliber (M21) shows a small size increase.

To evaluate if and how strong the sex and age of specimens influence the outcome of canonical analyses (graph. 1.1) we first compare the six subgroups distinguished in table 2. It is reassuring to observe that there is a huge overlap between sexes and also between age-groups; only root 1 is influenced by growth but in a very moderate way.

To investigate how results of multivariate analyses are influenced by OTU's with different age-sex composition, and whether the results can be used for taxonomical purposes we made a canonical analysis between three geographical clearly different OTU's (Uluguru, Mutura, Tshibati) in which we could also distinguish between age-sex subgroups. The resulting phenetic tree (graph. 1.2) shows clearly that differences in sex- and (or) age-composition of the compared OTU's have no influence on the outcome of our analysis and demonstrates that subtle shape differences in skull configuration, independent from sex and age, are effectively reflected by canonical graphs and phenetic trees.

Graph.1.3 goes a step further and adds the species dimension into the analysis by including OTU 4 (Kisangani RB) and OTU 37 (*L. brevicaudus*). Again we observe that the resulting phenetic tree is not influenced by sex or age composition. It is therefore safe to conclude that we can compare OTU's of different age and sex composition and still obtain results that we can use in a taxonomical context.

2. The taxonomical status of the type specimens of the *L. flavopunctatus* species-complex

Since the previous study on the Ethiopian representatives of the *Lophuromys flavopunctatus* species complex (LAVRENCHENKO *et al.* 1998) we have completed our craniometrical data-base to address some unresolved problems. One of our primary concerns is the allocation of the Ethiopian *Lophuromys* type-specimens to one of our OTU's, in order to allow a clear-cut taxonomical revision of the whole *flavopunctatus*-group.

However, first we have to establish that none of our Ethiopian OTU's belong to the *Lophuromys sikapusi*-species group represented in Eastern Africa by *Lophuromys ansorgei*. Graph. 2.1 depicts the results of a canonical analysis that involves an OTU representing *L. ansorgei* with the different OTU's we were able to compile of Ethiopian *Lophuromys*. The graph convincingly shows that the two species groups are clearly separated from each other and that the skull of the controversial type specimen of *L. major* (see App.1.1 and fig.1) pertains certainly to the *sikapusi* species group and is not related to any of the Ethiopian *Lophuromys* we studied.

Graph. 2.2 shows that OTU 37, OTU 38+39, and OTU 35 are directly identified by the type skulls as belonging to respectively the species *L. brevicaudus, L. chrysopus* and *L. melanonyx*. The type skulls of *L. brunneus* and *L. simensis* plot well within OTU 36, suggesting that *simensis* is possibly a synonym of *brunneus*. The type skulls of *L. flavopunctatus* and *L. zaphiri* fit within OTU 34, implying that the latter is synonymous with the former. Additional analyses (forward and backward) with other OTU's as outgroups confirm these conclusions.

In graph. 3.1 we introduced some non-Ethiopian OTU's

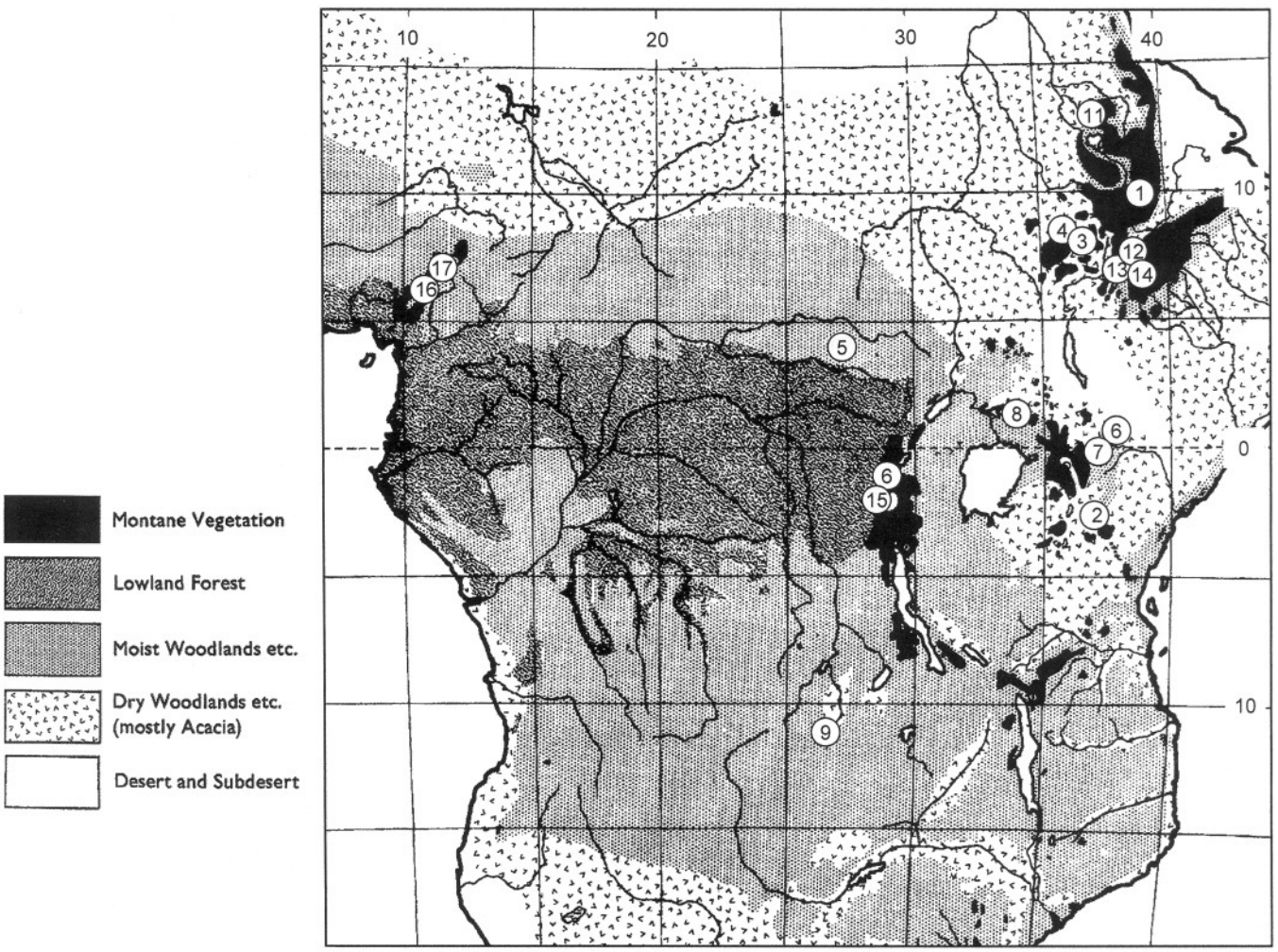


Fig. 1. Map showing the geographical situation of the type localities of the different taxa of the *Lophuromys flavopunctatus* species complex. For the co-ordinates and approximate altitudes we refer to Appendix 1.1. Based on Map B (p. XIII) of HALL & MOREAU (1970).

1. *flavopunctatus* THOMAS 1888
2. *aquilus* TRUE 1892
3. *zaphiri* THOMAS 1906
4. *brunneus* THOMAS 1906
5. *major* THOMAS *et al.* 1907
6. *laticeps* THOMAS *et al.* 1907
7. *zena* DOLLMAN 1909
8. *rubecula* DOLLMAN 1909
9. *rita* DOLLMAN 1910
10. *margarettae* HELLER 1912
11. *simensis* OSGOOD 1936
12. *brevicaudus* OSGOOD 1936
13. *chrysopus* OSGOOD 1936
14. *melanonyx* PETTER 1972
15. *cinereus* DIETERLEN *et al.* 1974
16. *eisentrauti* DIETERLEN 1978
17. *dieterleni* VERHEYEN *et al.* 1997

(OTU 6: Tshibati; OTU 20: Solai; OTU 26: Usambara E.) covering as much as possible the geographical variation of the taxonomically recognized non-Ethiopian taxa next to some relevant Ethiopian taxa such as *L. brevicaudus* (OTU 37) and *L. chrysopus* (OTU 38+39). We decided not to include the OTU's 34, 35 and 36 (respectively typical for *flavopunctatus, melanonyx* and *brunneus*) because we found that they tend to lump the non-Ethiopian OTU's, reducing seriously the usefulness of the graphs for the intended plotting of the non-Ethiopian type-specimens.

The critical type-specimen of *L. aquilus* TRUE 1892 (type locality: Mt Kilimanjaro) situates itself right in the centre of graph.3.1 but clearly outside OTU 37 (*brevicaudus*). On the other hand it might be concordant with OTU's 38 and 39 (*chrysopus*) as well as with the other OTU's. However, when we consider also the Kilimanjaro (Mweka) specimens, which are possibly topo-typical for *aquilus*, we have to conclude that *aquilus* identifies with one of the east African OTU's and not with OTU 38+39 (*chrysopus*). We will go further into this matter in the conclusions of this paper.

Most of the other east African type-specimens (*laticeps, zena, rita, margarettae)* fall within the east African OTU's (6, 20, 26) and are probably to be considered synonymous with *aquilus*. The only exception is *rubecula*, described in 1909 by DOLLMAN from Mt Elgon, which plots within *chrysopus* (OTU 38+39).

As to the two type-specimens from northern Cameroon (*eisentrauti, dieterleni*) they plot outside the east African and

Ethiopian OTU's and should probably be considered belonging to a different species group. Unfortunately, we do not possess the necessary skull series to investigate this issue further.

In graph. 3.2 we try to go into more detail by making a canonical analysis between Kenyan populations (OTU 19: Cherangani Hills; OTU 20: Solai; OTU 21: Aberdare Range; OTU 22: Mt Kenya) and the Ethiopian *chrysopus* (OTU 38: Harenna; OTU 39: Beletta). In this graph it becomes clear that the Aberdare Range and Mt Kenya series are characteristic for *zena* DOLLMAN 1909, whereas the Cherangani Hills population and Solai are concordant with *margarettae* HELLER 1912. It becomes also apparent that *rubecula* DOLLMAN 1909 (Mt Elgon) has to be allocated to the *chrysopus* species group and the same applies probably to *eisentrauti* DIETERLEN 1979 and to *dieterleni* VERHEYEN *et al.* 1997.

In graphs 4.1 and 4.2 we have tried to allocate the more or less damaged skulls of certain type specimens to some geographically selected OTU's by making specific canonical analyses. Graph.4.1 allows us not only to show that the type-specimen of *rita* DOLLMAN 1910 fits well, as expected, within OTU 11 (Congo S.) and *laticeps* THOMAS *et al.* 1907 within OTU 8 (Mutura). More importantly the crucial type skull of *aquilus* TRUE 1892 fits nicely within the Usambara E population (OTU 26), which is geographically the closest to the Mount Kilimanjaro from which we have unfortunately no adequate topo-typical series. Finally, we show in graph.4.2 that very probably *cinereus* is to be considered a synonym of *L. laticeps* confirming what was already suspected by DIETERLEN (1987).

Summarized, we can safely conclude that the five Ethiopian OTU's, identified on craniological and other morphological characters, represent respectively the following taxa: OTU 34: *L. flavopunctatus*, OTU 35: *L. melanonyx*, OTU 36: *L. brunneus*, OTU 37: *L. brevicaudus*, OTU 38+39: *L. chrysopus*. We have not found any specimens related to the *L. sikapusi* species group in the Ethiopian *Lophuromys* collection we were able to study. Moreover *L. zaphiri* is in our opinion synonymous with *L. flavopunctatus* and *L. simensis* is possibly also a synonym of *L. brunneus*. We did not attempt to determine the geographical distribution of each of the recognized taxa since we do not possess enough reliable data for the whole of Ethiopia.

Concerning the non-Ethiopian taxa of the studied species group we have demonstrated that the studied populations can be identified with the following taxa: OTU 26: *aquilus*, OTU 8: *laticeps*, OTU 11: *rita*, OTU 21-22: *zena*, OTU 19-20: *margarettae* .We also have shown that the severely damaged *cinereus* type skull falls clearly within OTU 8 and is thus to be considered synonymous with *laticeps*. The *rubecula* type skull from Elgonyi (Mt Elgon) is however situated consistently within the OTU's (38+39) typical for Ethiopian *chrysopus;* however, the other Mt Elgon specimens (OTU 18) that we could measure seem to be referable to *margarettae* (see following chapter).

Finally, we mention that both type skulls of *dieterleni* and *eisentrauti* appear to be near *chrysopus* (OTU 38+39). We are convinced that both belong to a clearly differentiated strain of *chrysopus* but for the moment we are not able to go deeper into this for lack of adequate series from N. Cameroon.

3. Craniometrical variation within the *L. flavopunctatus* s.l. species complex

We choose to tackle this problem by making canonical analyses including as many OTU's as possible distributed over the entire geographical range of the species complex and then visualizing the results by tree diagrams based upon the Mahalanobis squared distances between the obtained centroids (UPGA). The usefulness of this approach is limited by the size of the OTU's (numbers of adult and complete skulls) versus the number of cranial variables (measurements) taken per skull. Indeed, the number of observations per OTU has to be preferably greater than the number of variables (i.e. measurements) per skull, otherwise the matrix of within variation of this OTU tends to be singular.

Keeping in mind our results concerning the importance of sexual dimorphism and skull-growth we eliminated from the start the measurements M18 (CHOB) and M24 (PCPA).

Each OTU used in our analyses comprises only fully adult and complete skulls and contains a sufficient number of specimens to make statistically valid analyses. When necessary we maximalised OTU's either by eliminating some measures responsible for excluding some otherwise complete skulls or by replacing missing data by the mean of the group.

Fig. 2 shows schematically the geographic distribution of the OTU's over the Eastern and Central African region. By far most of these OTU's are homogenous as to the geographical origin of the included specimens. Only for Congo S (OTU 11) we had to group specimens from rather wide-spread or varied geographical origin to obtain statistically valid series. In total we compiled for this study 46 OTU's out of which 33 group twenty or more complete adult skulls and another 5 with at least 16 usable adult skulls; the 8 remaining OTU's are composed of about 10 skulls. All our OTU's were screened for measuring errors and recording oddities.

In a first approach (graph.5.1) we include only the 33 "major" OTU's which contain a minimum of 20 skulls each. In a second step we add another 5 OTU's grouping a minimum of 16 skulls each (graph.5.2). Finally, the 8 "minor" OTU's with about 10 skulls each (graph.5.3) are included in the analysis. Graph.5.1 is based upon the morphometrical comparison of 33 OTU's, using 20 skull measurements (see table 1). We also identified, hoping to facilitate the interpretation of the resulting phenetic tree, the OTU's that we found to be representative for the 5 major east African type-populations (see 2). Our first observation is the clear morphometrical separation between the Ethiopian species *melanonyx, brevicaudus, flavopunctatus* from all the other OTU's. This also applies to *chrysopus*; however, *chrysopus* W. and E. form a well defined clade not only with the Ethiopian species *brunneus,* but also with the *"zena"* populations of Mt Kenya and the Aberdare Range. The sister clade of the *chrysopus-zena-brunneus* group is formed by the totality of all the other non-Ethiopian OTU's that we examined. We note that both clades are linked at a rather high linkage distance of 100.

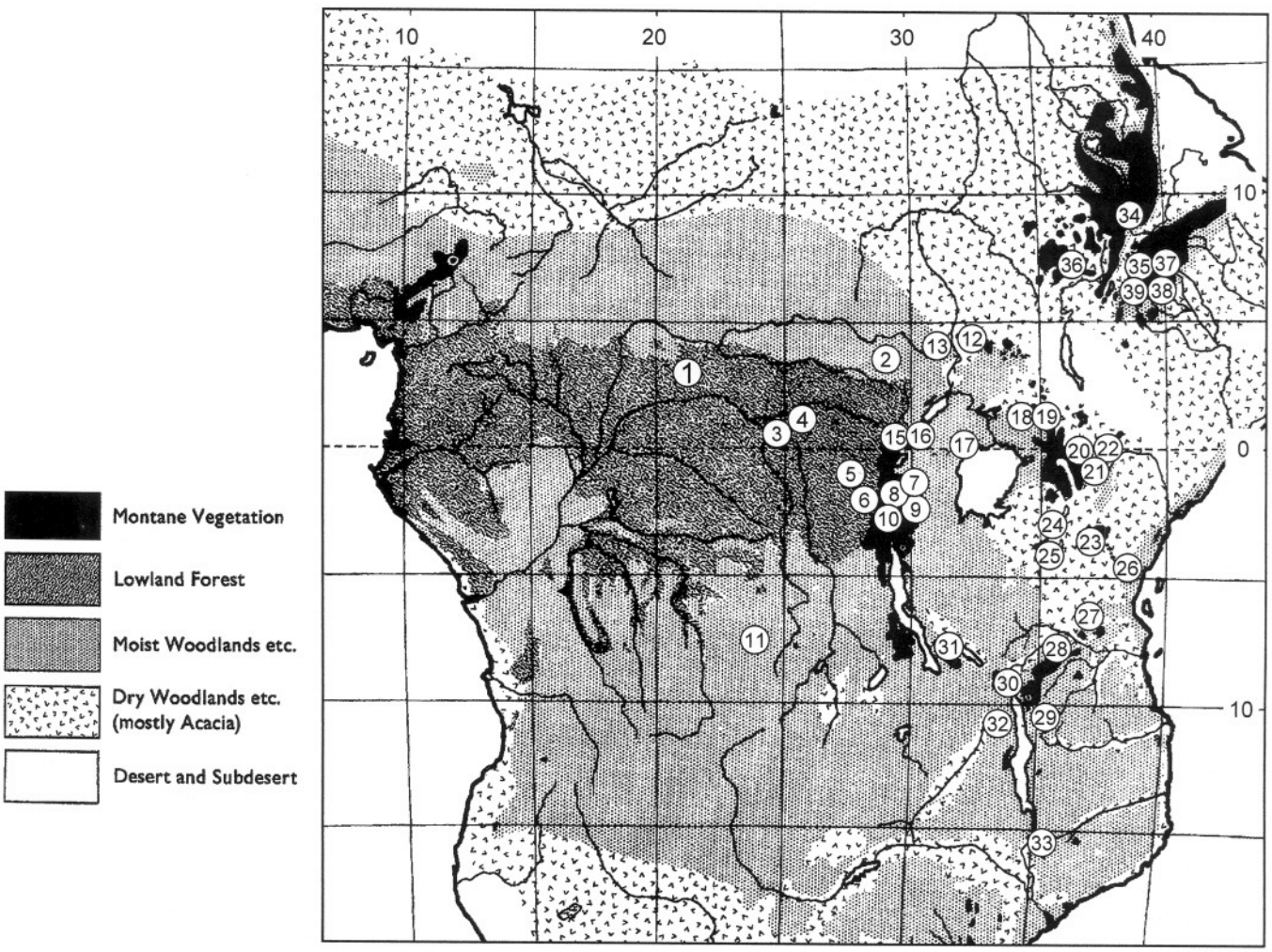


Fig. 2. The geographical distribution of the OTU's of the *L. flavopunctatus* species complex as used in this study.For the exact composition of the individual OTU's, we refer to Appendix 2 and for the co-ordinates of the collecting localities, see Appendix 3. Based on Map B (p. XIII) of HALL & MOREAU (1970).

1	Congo NW.	10	Uwinka (Nyungwe Forest)	20	Solai	30	Rungwe Mt
2	Congo NE.	11	Congo S.	21	Aberdare Range	31	Ufipa Plateau
3	Kisangani LB	12	Gilo (Imatong Mts)	21.1	Gatamaiyu	32	Nyika Plateau
4	Kisangani RB	13	Iwatoka	21.2	Aberdare Range (BMNH)	33	Zomba
5	Irangi	14	Ruwenzori Mt (p.m.)	22	Kenya Mt	34	***L. flavopunctatus*** s.s.
6	Tshibati	15	Rutshuru	22.1	Kenya Mt (BMNH)	35	***L. melanonyx***
6.1	Kahuzi Mt	16	Kigesi	23	Meru Mt	36	***L. brunneus***
7	Virunga Volcanoes	17	Uganda	23.1	Kilimanjaro Mt (Mweka)	37	***L. brevicaudus***
8	Mutura	17.1	Bugala Isl.	24	Ngorongoro Rim	38	***L. chrysopus*** E.
8.1	Kinigi	18	Elgon Mt	25	Hanang Mt	39	***L. chrysopus*** W.
8.2	Kidaho	19	Cherangani Hills	26	Usambara E. Range		
9	Butare	19.1	Kaptagat	27	Uluguru Range		
9.1	Ruanda E.			28	Mufindi		
				29	Peramiho		

Within the non-Ethiopian clade of graph. 5.1, two branches are quite distinct from all the others: first OTU 4 (Kisangani RB) and OTU 5 (Irangi), secondly OTU 23 (Mt Meru). The other non-Ethiopian OTU's group into geographically related clades such as (Mutura - Uwinka – Kinigi - Butare - Kidaho - Ruanda E), (Tshibati - Rutshuru - Virunga Volc. - Kigezi), (Congo S - Rungwe Mt - Nyika Plat.), (Cherangani Hills - Solai - Kenya Mt), (Ngorongoro Rim - Gilo) and (Usambara E. - Uluguru). However, in some cases OTU's, such as Kisangani LB and Ufipa Plateau, cluster in a rather unexpected way. We also found that the introduction or exclusion of one or more critical non-Ethiopian OTU's into the

analysis often resulted in a reshuffling of low-level branching between the observed geographical clades, but it never affected the clear branching and identity of OTU 23 (Meru Mt.) or OTU 4 (Kisangani RB) and OTU 5 (Irangi).
In graph.5.2 we introduced 38 OTU's, containing each at least 16 adult complete skulls and using 16 cranial measurements. The results of graph.5.1 and 5.2 are identical: the 5 newly introduced populations Entebbe (OTU 17), Hanang Mt (OTU 25), Peramiho (OTU 29), Congo NW (OTU 1), Aberdare Range (OTU 21.2) do not differ significantly from the other non-Ethiopian OTU's while some OTU's, e.g. Hanang (OTU 25) and Aberdare Range (OTU 21.2) fit geographically rather well with the already existing groups. In general however, we see a rather important exchange of OTU's between the geographical clades we could discern in graph.5.1 and thus a breaking down of their geographical identity.
Graph. 5.3 represents a tree-diagram for 46 OTU's based on a backward canonical analysis of 11 cranial measurements and on 8 new OTU's with each a minimum of 10 adult skulls. This analysis not only confirms the results of graphs 5.1 and 5.2, but also suggests that not one of the 8 newly introduced OTU's is clearly differentiated from the other non-Ethiopian populations. We note however, that the geographic clades in the non-Ethiopian OTU's that we clearly could discern in graph. 5.1, tend to dissolve in graph. 5.2 to disappear almost completely in graph. 5.3. This progressive loss of information can be explained by dwindling numbers of specimens per OTU, which entails a restriction of the number of used measures.
Summarized, we conclude that we can identify craniometrically from the examined non-Ethiopian populations at least 3 samples, viz. OTU 4 (Kisangani RB), OTU 5 (Irangi) and OTU 23 (Meru Mt), which can be considered for formal taxonomic recognition.

4. Genetic results

The observed intraspecific genetic variation in the studied cyt b fragment is significantly lower than the differentiation observed at the interspecific level (appendix 4, tables 5 and 6). Among eight specimens from three populations of *zena* we detect only 10 variable sites, 7 of which are substitutions at the third codon position, 3 are substitutions at the first codon position (data not shown). The number of parsimony informative sites in the context of the seven other studied OTU's is reduced to 6 sites. Both measures of variability are low in comparison with the differentiation observed among the other OTU's, including representatives of the taxa *flavopunctatus, laticeps, aquilus, rita, margarettae* and the OTU's 4 and 24 that are discussed further.
Among the 402 bp long cyt b sequences from *flavopunctatus, laticeps,* OTU 4 (Kisangani Right Bank), *aquilus, zena, margarettae, aquilus,* OTU 23 (Meru Mt), *rita, margarettae* and *zena* we observe 54 parsimony informative sites, 14 at the first codon postion, one at the second codon position and 39 at the third codon position (appendix 4, table 5).
The observed nucleotide changes result in 12 amino acid substitutions, all of which (except one leucine-isoleucine change in OTU 4 – Kisangani RB) are differences between taxa or OTU's (data not shown). The amount of nucleotide substitutions observed ranges between 2.3 and 8.1 %. In all cases, the sequences of (putative) conspecifics are considerably more similar than sequences of different OTU's (appendix 4, tables 5 and 6).
The OTU's and taxa represented by more than one partial cyt b sequence share a typical set of informative sites. The fact that little variation is observed among the eight *zena* specimens indicates that the observed differences, even when based upon a single specimen, are likely to be significant, and diagnostically reliable differences.

5. Description of *Lophuromys dudui* n.sp.

Holotype
RUCA D1739; adult female ; alc. specimen ; skull complete ; collected by DUDU AKAIBE (26 March 1986) in Masako (00.36 N - 25.13 E ; alt. 440 m) in the rainforest of the Tshoporiver ; collecting number D1739.

Paratypes
10 specimens from the same locality as the type-specimen and collected by DUDU Akaibe between 12 December 1985 and 23 August 1986.

RUCA D1170 (ad.male; skull + alc.spec.; coll.nr D1170)
RUCA D1290 (ad.male; skull + alc.spec.; coll.nr D1290)
RUCA D1315 (ad.male; skull + alc.spec.; coll.nr D1315)
RUCA D1332 (ad.male; skull + alc.spec.; coll.nr D1332)
RUCA D1649 (ad.male; skull + alc.spec.; coll.nr D1649)
RUCA D1734 (ad.female; skull + alc.spec.; coll.nr D1734)
RUCA D1771 (ad.female; skull + alc.spec.; coll.nr D1771)
RUCA D1902 (ad.female; skull + alc.spec.; coll.nr D1902)
RUCA D2322 (ad.female; skull + alc.spec.; coll.nr D2322)
RUCA D2974 (ad.female; skull + alc.spec.; coll.nr D2974)

For the craniometrical data of the type and paratypes we refer to App.1.3.

Type locality
The holotype and the paratypes were collected in the Masako forest (in primary as well as old secondary forest).

Etymology
We dedicate this new species to our colleague and friend Prof. Dr. DUDU AKAIBE in appreciation of his continuous efforts to promote scientific research on small mammals in extremely difficult circumstances.

Diagnosis
L. dudui is a new species of "speckled and short-tailed brush-furred rat" that belongs to the so called *Lophuromys flavopunctatus* species complex. Consequently it is clearly differentiated cranially and dentally from 1°/ the *Lophuromys nudicaudus-huttereri* species complex 2°/ the small *L. rahmi* endemic to the mountainous region of Kivu (Congo) 3°/ the *sikapusi-ansorgei-angolensis-roseveari* species complex.
Within the *flavopunctatus* species complex, it can easily be characterized a.o., by its (1) smallish skull, (2) short maxillary and mandibulary toothrow, (3) short ears and (4) short hind-

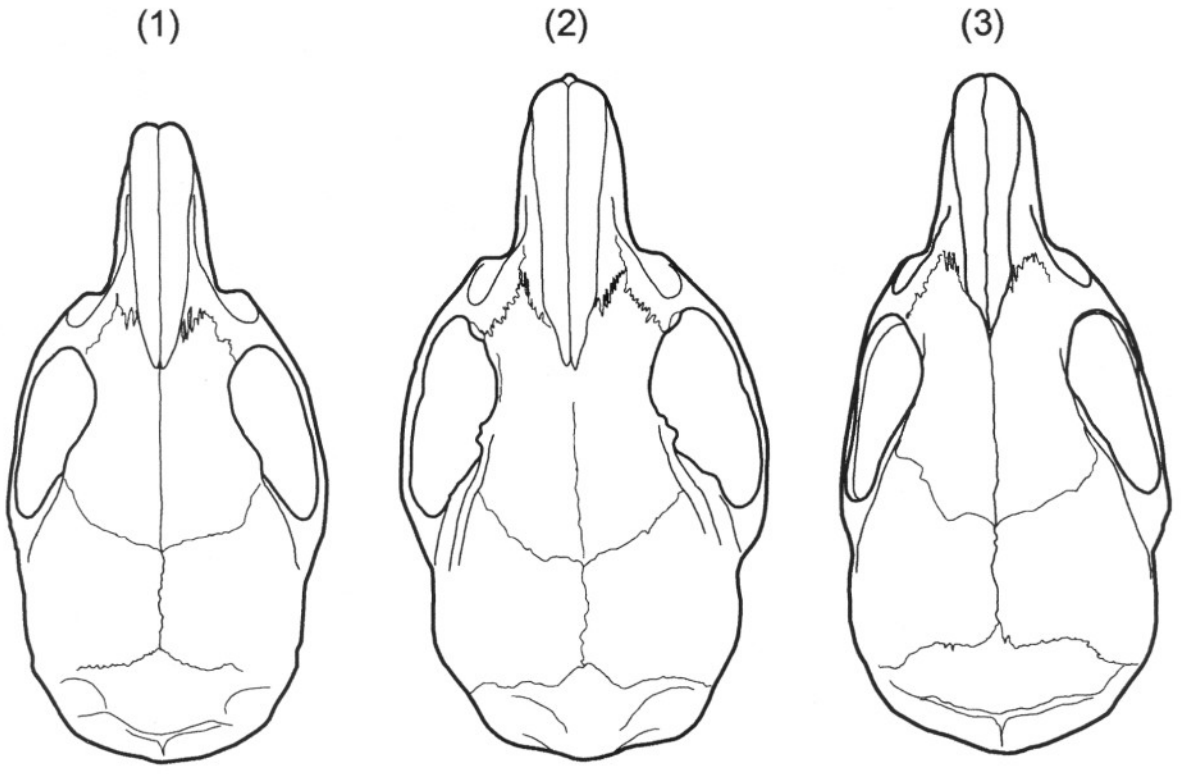


Fig. 3. Schematic representation of the dorsal views of representative skulls for (1) *Lophuromys dudui* (type: D1739), (2) *laticeps* (KMMA30366) and *(3) L. verhageni* (RUCA14327).

foot. For full details : see table 3 in App.4, graphs 6 and 8. For the genetic characterization see table 5 and 6 in App. 4

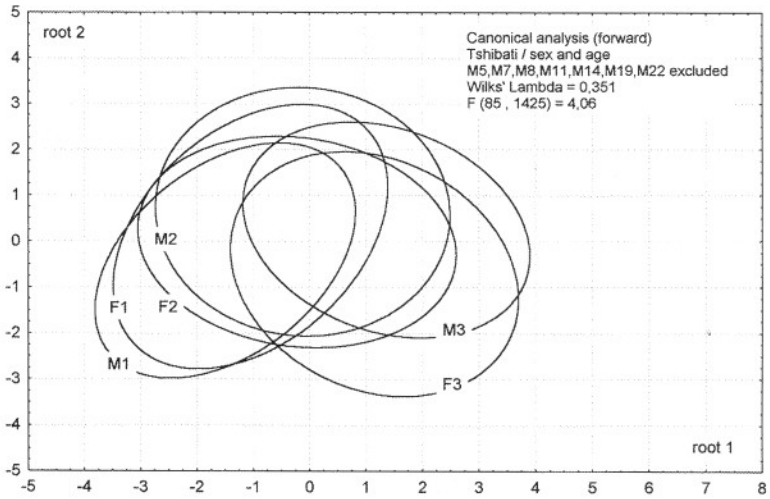
Discussion of the morphological characters
(table 3, fig. 3)

It is impossible to include in this chapter a description of the external characters such as the colouration of the pelage, the feet, ears etc... Indeed, the type-specimens, fixed in formalin and then stored and transported for a long period in inadequate containers, have taken a more or less rusty tinge.
In table 3 we have included statistics of the external measurements of our new taxon, but we stress that these data have to be used with caution. Indeed, these field measurements were taken by different research groups using slightly different measuring techniques. Nevertheless we can conclude that *L. dudui* is on the overall clearly smaller when compared with *laticeps* (Mutura: OTU 8): from 5% (HB) to 7,5% (TL) to 10% (HF) to 20% (EL) smaller.
The morphology of the skull (fig.4) situates this new taxon within the *L. flavopunctatus* species complex, but when compared with representative skulls of a topo-typical population of *laticeps* (OTU 8) we can characterize our new taxon in the following way:

(1) smaller, more slender skull with a more delicate rostrum;
(2) less pronounced supraorbital ridges and less prominent notches;
(3) smaller upper molars and relatively wider palate.

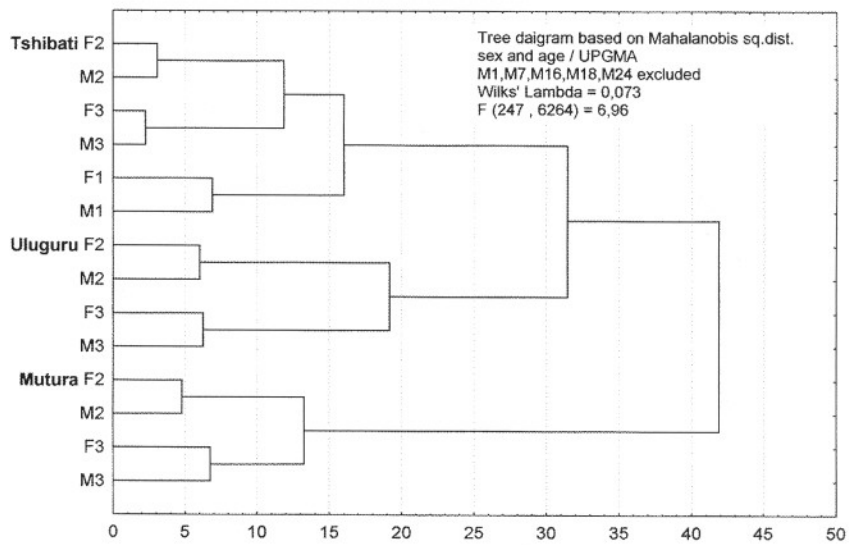
Univariate analysis *(table 3)*

The basic statistics show the variation coefficients to be far below 10% except for the "choanae breadth" (nr 18) which is a rather unreliable measure. When comparing the basic data of the skull-measurements of *dudui* to our standard population of *laticeps* (OTU 8: Mutura) we notice that the skull of *dudui* is about 5 to 10% smaller for length as well as breadth and height measures (with a maximum of 20% for DINC: depth of the upper incisor = M21). Only for measures of the skull basis, represented here by M10 (PALA), M18 (CHOB) and in a lesser degree by M12 (UPDA), we find that both taxa have about the same size.
The comparison between *dudui* and OTU 5 (Irangi) reveals that, although both are rather close in our multivariable analyses (graphs 5.1-5.2-5.3), they differ rather importantly in the univariate approach (table 3). Indeed, we see that in 9 out of the 24 cranial measures (M3, M4, M5, M6, M7, M10, M13, M14, M24) there is statistically no significant difference between both OTU's but that 9 out of the remaining 15 measurements are statistically highly different (M1, M8, M9, M11, M15, M16, M20, M21, M23). Summarizing, we can describe the skull of OTU 5 (Irangi) to be on the overall metrically closer to *dudui* than to our standard population of *laticeps* (OTU 8: Mutura) meaning that its skull is clearly smaller than the latter. However, for certain measures it is markedly less different (M1, M8, M9, M11, M16, M17, M19, M20, M21) from OTU 8 (Mutura). Finally, we see that Irangi (OTU 5) is well differentiated from *dudui* by two



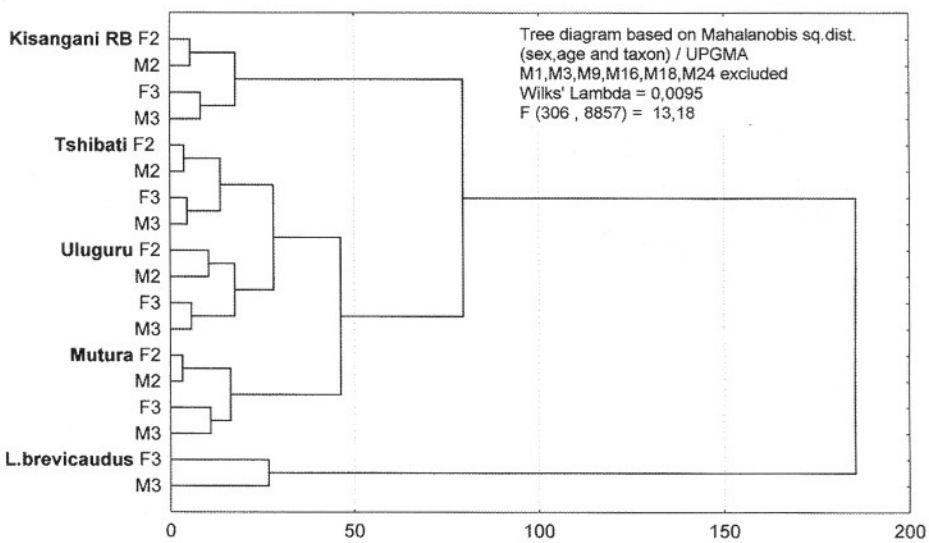
Graph.1.1

Graph. 1.1.
Graphical representation of a canonical analysis of the six subgroups of OTU 6 (Tshibati) as we recognized in table 2.



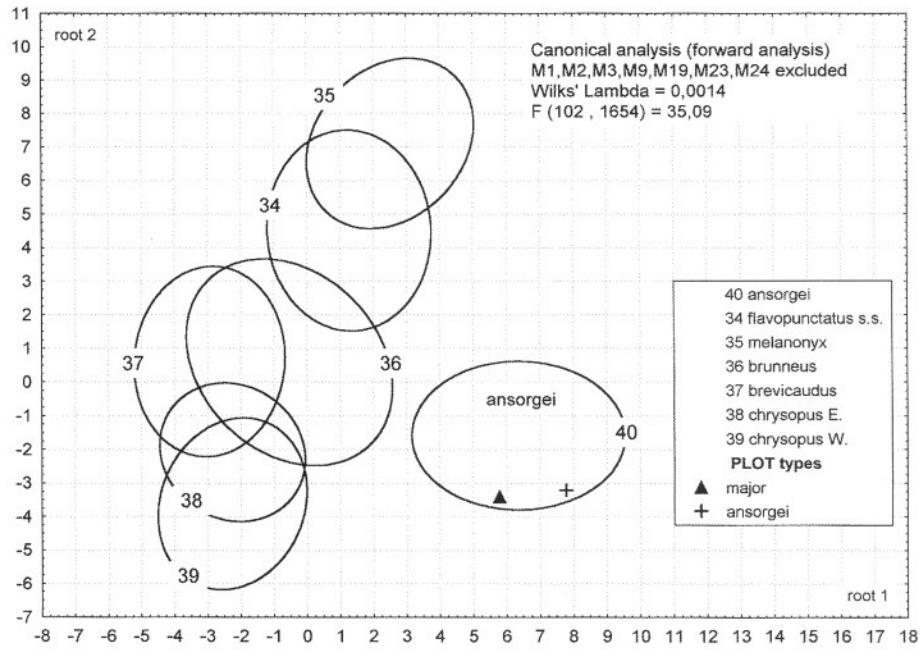
Graph.1.2

Graph. 1.2.
Phenetic tree based on Mahalanobis squared distances between the age-sex subgroups of three geographically different OTU's of taxonomically not different populations of "speckled" *Lophuromys*.



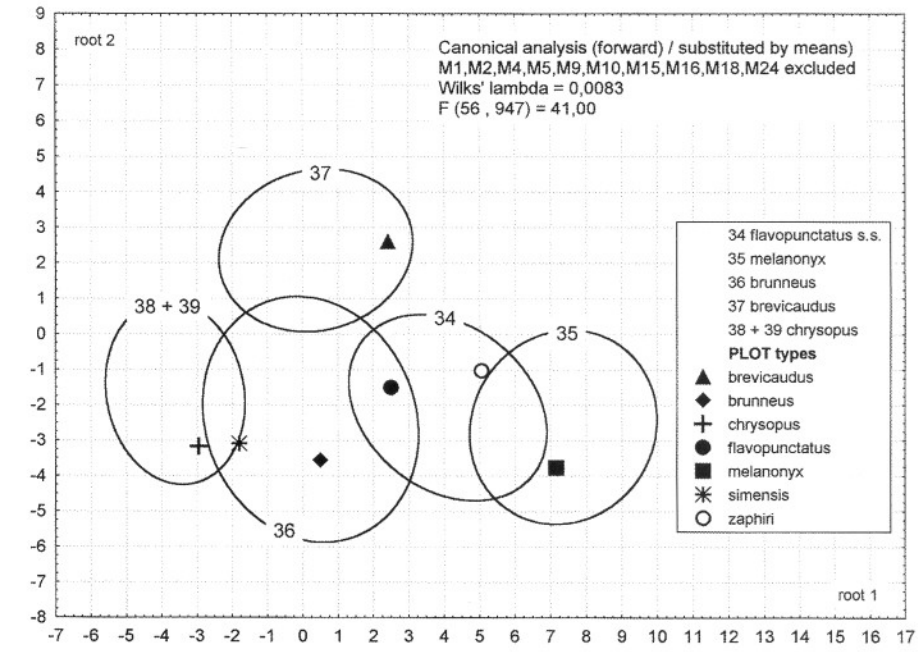
Graph.1.3

Graph. 1.3.
Phenetic tree based on Mahalanobis squared distances between age-sex subgroups of three geographically distant OTU's of the same taxon and two taxonomically different populations of "speckled" *Lophuromys*.



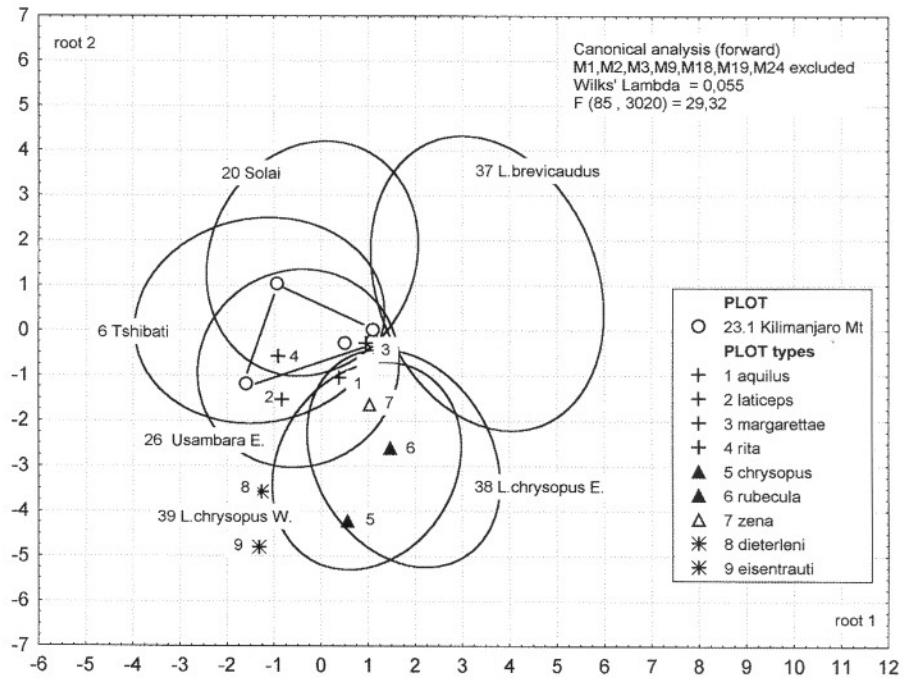
Graph.2.1

Graph. 2.1.
Graphic representation of a forward canonical analysis performed on the "speckled" Ethiopian *Lophuromys* OTU's (34, 35, 36, 37, 38, 39) compared with a representative of the *L. sikapusi* species - complex (OTU 40).

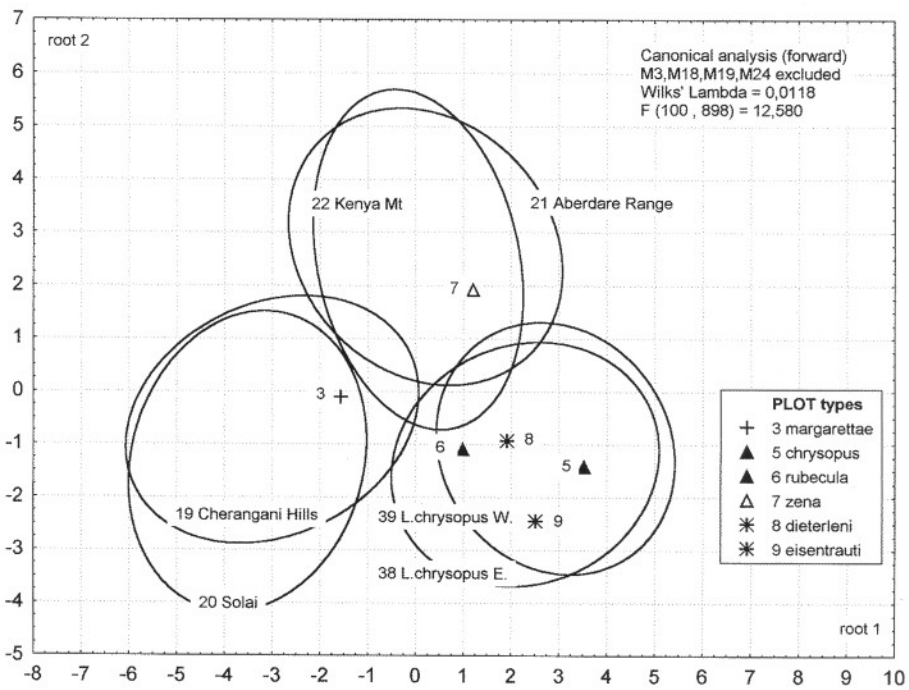


Graph.2.2

Graph. 2.2.
Graphic representation of a forward canonical analysis of five Ethiopian "speckled" *Lophuromys* OTU's (34, 35, 36, 37, 38, 39) providing the background to allocate by plotting the Ethiopian types of the species-group.

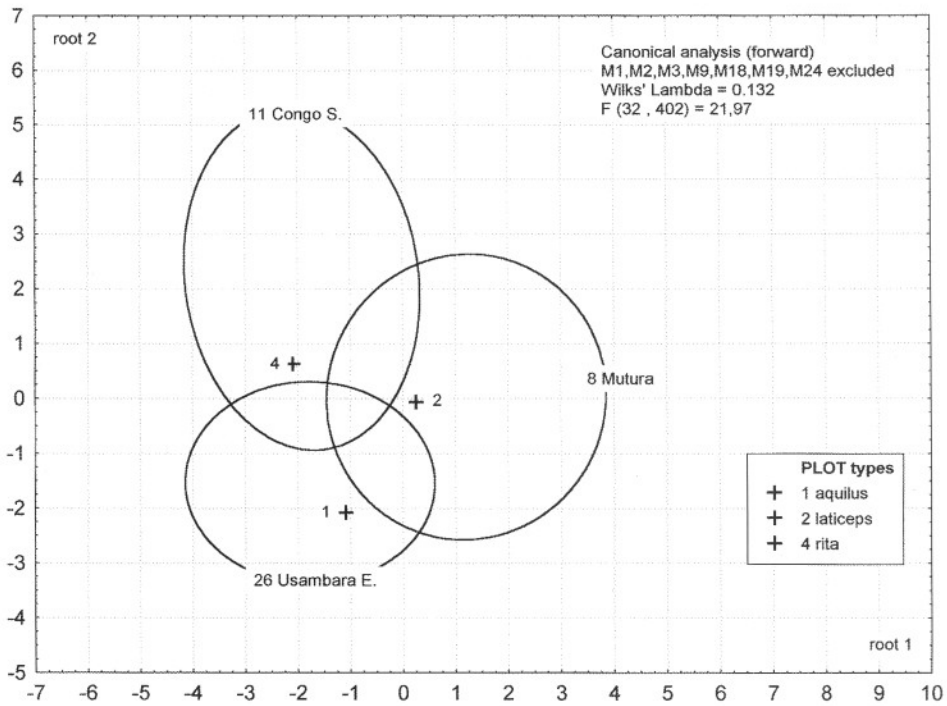


Graph.3.1

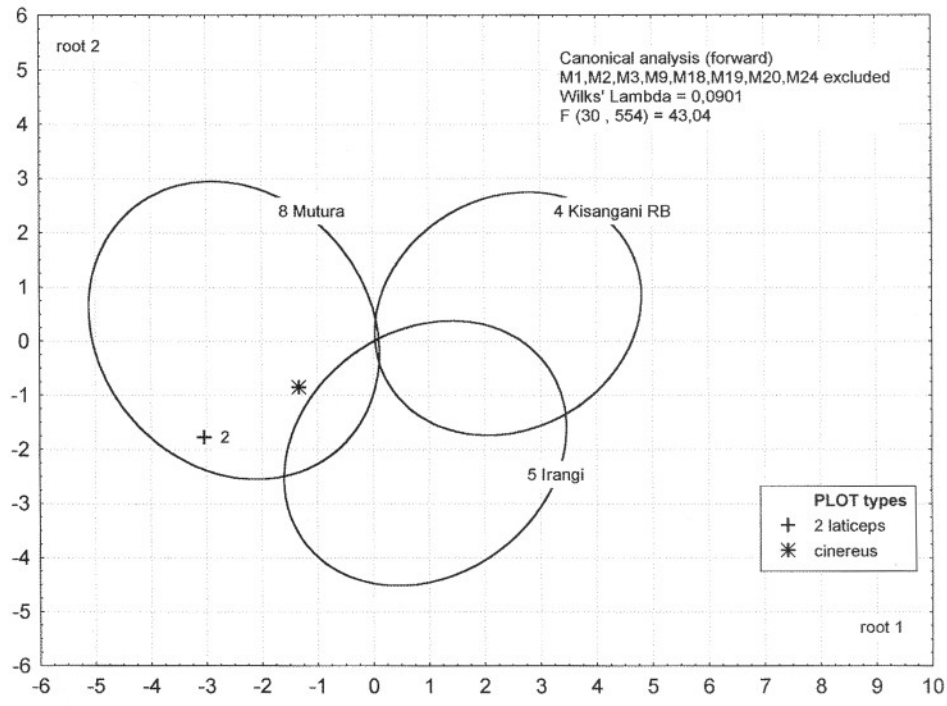


Graph.3.2

Graph. 3.1 and 3.2. Canonical analyses providing the necessary background to situate by plotting most of the non-Ethiopian type-specimens of the *L. flavopunctatus* species group (see text for discussion).



Graph.4.1



Graph.4.2

Graph. 4.1 and 4.2.
Canonical analyses attempting to link by plotting some of the non-Ethiopian types to some of our OTU's (see text).

measures (M15 and M23) representing the rostral width indicating that the average skull of OTU 5 (Irangi) is rather close to *dudui* but with a significantly more slender rostrum.

Discriminant analysis *(graphs 6 and 7)*

For diagnostic reasons we performed through forward analysis a discriminant function between our new taxon *dudui* (OTU 4: Kisangani RB) and the population of Mutura (OTU 8) representing the taxon *laticeps*. The results represented in graph 6 indicate that 100% of the individual skulls can be correctly classified by the obtained discriminant function using 15 out of the 24 available measurements. We note also that the most important discriminating measures are: M12 (UPDA), M7 (DIA2), M21 (DINC), M11 (UPTE).
In graph 7 we demonstrate further that *dudui* is also easily differentiated craniometrically from OTU 5 (Irangi) and that both populations can be identified 100% correctly through a discriminant function using 14 out of the 24 available measurements, the most important discriminating measures being M21 (DINC), M12 (UPDA), M13 (M1BR), M15 (BNAS).

Multiple discriminant analysis *(graph. 8)*

We performed a canonical analysis by comparing the population of *dudui* (OTU 4: Kisangani RB) with 1°/ the Mutura (OTU 8) series which is probably topotypical for *laticeps* and which represents the east African taxa 2°/ the Irangi-population (OTU 5) and 3°/ the Mt Meru population (OTU 23) representing the new east African taxon that we will describe next.
We see that in this graph *dudui* is not only clearly differentiated from topotypical *laticeps* (OTU 8) but also from the Mt Meru-population (OTU 23). As to OTU 5 (Irangi) it coincides with *dudui* slightly overlapping with OTU 8 and OTU 23. However, the results of the same analysis, but comparing roots 2 versus 3 show that Irangi identifies closely with *dudui*. Finally, plotting of the specimens of OTU 2 (Congo NE) demonstrates that *dudui* is probably present in the whole of the forested areas north-east of Kisangani.

Genetic characterization *(appendix 4, tables 5, 6)*

All OTU's and taxa represented by more than one partial cyt b sequence share a typical set of informative sites. Therefore, it is not surprizing that the comparison of the partial cyt *b* sequences of the two specimens representing *dudui* with sequences of relevant described taxa and OTU's from the same region show the utility of molecular markers to identify this new taxon.
The observation that only little variation exists among the eight *zena* specimens indicates that the differences for *dudui*, even when based upon only two specimens, are likely to be significant, and diagnostically reliable differences
Interestingly, these molecular data suggest that *dudui* is clearly distinct (average p distance = 0.054) from *flavopunctatus*, but also differs significantly from *aquilus* and *laticeps* (average p-distances are respectively 0.063 and 0.032). The implications of these data for the phylogenetic relationships between *dudui* and other *Lophuromys* taxa will be the subject of a separate study using more and other nucleotide sequences.

Additional information

Through some additional canonical comparisons including surrounding OTU's (o.a. 1, 3, 7, 11, 15) we acquired the certainty that the populations living in the mountainous rift region and on the left bank of the Congo river are distinctly different from *dudui*. By plotting we found also evidence that our new taxon is present in the northeastern region of Congo (OTU 2) as far as the Garamba-Blukwa-Djugu-region.
Furthermore, plotting of individual skulls (KMMA 38.734, 738, 739, 770) allowed us to allocate a small series of Visiki (00.23 N - 29.12 E. 1100 m alt) to *dudui* whereas all the measured skulls originating from localities on the western flank of the rift mountains seem to belong to *laticeps,* from which *dudui* is significantly genetically differentiated (7.8% uncorrected sequence divergence). On the other hand the series of Irangi (OTU 5) (alt 850 m) which can be considered to be more or less representative for the population of the south-eastern lowland rainforest of Congo separates itself as a rather well defined population (graph 8).
For the moment we consider *dudui* to be sufficiently craniometrically and genetically different from other OTU's and described taxa to be allocated the status of species whereas the Irangi population is probably a local form (subspecies) of *dudui.* However, it remains always possible that new data (e.g. caryology or additional genetic analyses) might change the taxonomic status of our new form.

6. Description of *Lophuromys verhageni* n.sp.

Holotype
KMMA 96-037-M-3848; adult female; alc.specimen; skull complete; collected by Ron VERHAGEN (9 March 1996) on Mount Meru (03.13'47" S - 36.41'34" E - alt.2600 m), collecting number RUCA 14.329.

Paratypes
22 specimens collected by Ronald VERHAGEN.

KMMA 96-037-M3832 (ad.male; alc. + cr.; col.nr.14.295)
96-037-M3833 (ad.fem.; alc. + cr.; col.nr. 14.307)
96-037-M3834 (ad.fem.; alc. + cr.; col.nr. 14.308)
96-037-M3835 (ad.male; alc. + cr.; col.nr. 14.309)
96-037-M3836 (ad.fem.; alc. + cr.; col.nr. 14.312)
96-037-M3837 (ad.fem.; alc. + cr.; col.nr. 14.313)
96-037-M3838 (ad.? ; alc. + cr.; col.nr. 14.319)
96-037-M3839 (ad.male; alc. + cr.; col.nr. 14.320)
96-037-M3840 (ad.male; alc. + cr.; col.nr. 14.321)
96-037-M3841 (ad.male; alc. + cr.; col.nr. 14.322)
96-037-M3842 (ad.fem.; alc. + cr.; col.nr. 14.323)
96-037-M3843 (ad.male; alc. + cr.; col.nr. 14.324)
96-037-M3844 (ad.male; alc. + cr.; col.nr. 14.325)
96-037-M3845 (ad.male; alc. + cr.; col.nr. 14.326)
96-037-M3846 (ad.fem.; alc. + cr.; col.nr. 14.327)
96-037-M3847 (ad.fem.; alc. + cr.; col.nr. 14.328)
96-037-M3849 (ad.male; alc. + cr.; col.nr. 14.347)
96-037-M3850 (ad.male; alc. + cr.; col.nr. 14.348)
96-037-M3851 (ad.male; alc. + cr.; col.nr. 14.352)

96-037-M3852 (ad.male; alc. + cr.; col.nr. 14.353)
96-037-M3853 (ad.male; alc. + cr.; col.nr. 14.354)
96-037-M3856 (ad.male; alc. + cr.; col.nr. 14.368)

For the craniometrical data of the type and paratypes: see App. 1.4.

Type locality

All specimens were collected on the 8 and 9 March 1996 on Mt Meru (03°13'47"S-36°41'34"E at the altitude of 2.600 m (grid 8) except for col.nrs 14.347 and 14.348; these were collected on 10 March 1996 in grid 10 (03°13'54"S-36°42'39"E at the altitude of 3.050 m).
The collector describes grid 8 as situated in a grassy glade of about 2.500 m^2, dotted here and there with low bushes and several small pools heavily surrounded by papyrus, the whole surrounded by moist mountain forest with little undergrowth and here and there small patches of open grassy spaces.

Etymology

We have the pleasure to dedicate this new species to our colleague and friend Ronald VERHAGEN, who has contributed greatly to our ecological knowledge on African small mammals in Tanzania.

Diagnosis

Lophuromys verhageni is a new species of "speckled and short-tailed brush-furred rat" of the *L. flavopunctatus* species complex and as such can be easily differentiated cranially and dentally from all the other species complexes of the genus *Lophuromys*.
Within the east African representative OTU's we can characterize our new species easily as a somewhat bigger animal with a slightly bigger but more slender skull with weak supraorbital ridges. (See also table 4, fig. 3, graph.8 and 9)

Discussion of morphological characters
(Appendix 4, table 4; fig. 3)

We have not found reliable colour or pattern differences in the pelage that would allow us to characterize this population. Indeed, our specimens have been fixed in formalin and conserved in alcohol so that comparisons with dry type skins become rather difficult and unreliable.
In table 4 we compare the basic statistics of the external measurements of the Mt Meru sample with similar data obtained by our research teams for the Mutura (OTU 8) population. We find that *verhageni* is about 10% bigger than what we found for our topo-typical *laticeps* population; only for the tail length (TL) there seems not to be a significant difference. We note however that the CV% are high for HB, TL and EL and that only the hindfoot length (HF) gives results which are reliable.
The morphology of the skull and teeth situates *verhageni* within the *L. flavopunctatus* complex. A morphological comparison with typical *laticeps* skulls (OTU 8) shows that the skull of *verhageni* is slightly bigger but with a more slender aspect and with less pronounced supraorbital ridges (fig. 3).

Univariate analysis *(Appendix 4, table 4)*

A comparison with the topotypical population of *laticeps* (Mutura: OTU 8) shows that *verhageni* is for most breadth measures significantly smaller than the latter (M8, M9, M15, M18, M20, M23) but bigger for most length-measures (M1, M2, M3, M4, M6, M7). The most significant measures characterizing *verhageni* are however M5 (PAFL), M14 (ZYPL), M21 (DINC) and M22 (ROHE).
We note that for some teeth measures (M11, M13) *verhageni* is not significantly different as well as for bullae length (M9: BULL) and nasal length (M16: LNAS). Finally, it is remarkable that for M21 (DINC) *verhageni* is significantly smaller and for M17 (LOTE) significantly bigger.
Summarizing we can characterize *verhageni* as having a somewhat larger but more slender skull with a finer rostrum, a weaker zygomatic plate and a rather heavier set of lower molars than *L. laticeps*.

Discriminant analysis *(graph. 9)*

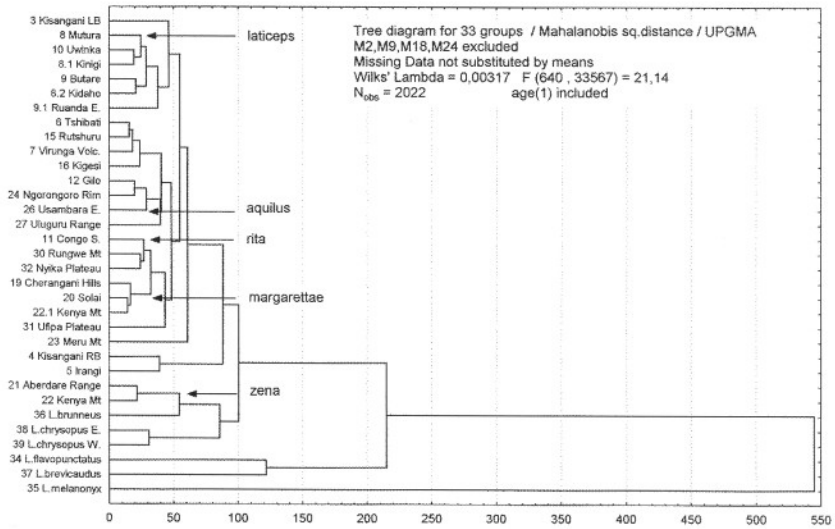
In this graph we attempt to characterize *verhageni* by comparing it to our topotypical population of *laticeps* (Mutura: OTU 8). The results show that close to 100% of the individual skulls can be correctly identified by the discriminant function (forward analysis) by using 13 out of the 24 available measurements. We note also that the most important discriminating measures are: M14 (ZYPL), M13 (M_1BR), M23 (ROBR), M12 (UPDA) and M17 (LOTE).

Multiple discriminant analysis *(graph. 8)*

We refer to graph.8 where we show that *verhageni* (OTU 23) is not only well differentiated from a population topotypical for *laticeps* (Mutura: OTU 8) but also from *L. dudui* (Kisangani RB: OTU 4).

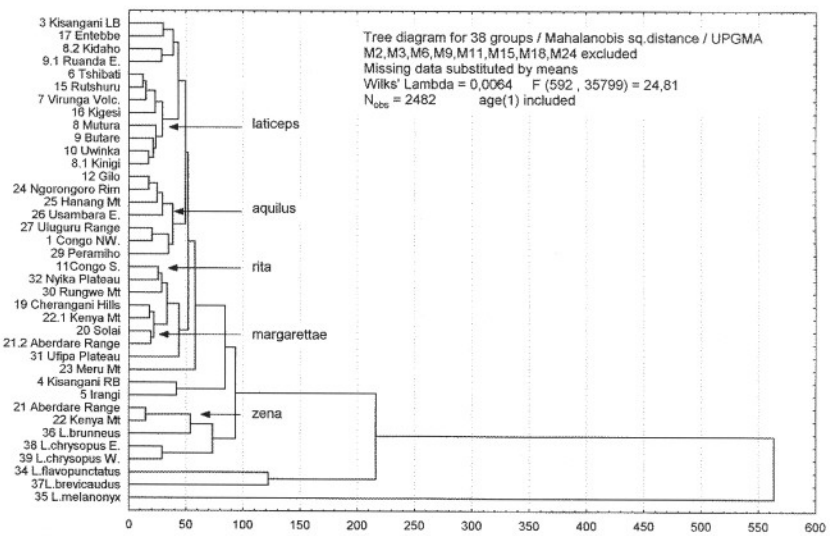
Genetic characterization *(Appendix 4, tables 5 and 6)*

As observed above, all OTU's and taxa represented by more than one partial cyt b sequence share a typical set of informative sites. The comparison of the partial cyt b sequences of the two specimens representing *verhageni* with sequences of relevant described taxa and OTU's from the same region (i.e. *laticeps, aquilus* and *dudui*) show the utility of molecular markers to identify this new taxon.
Since only little variation exists among the conspecifics tested here (tables 5 and 6), we suggest that the differences observed between *verhageni* and related taxa, such as the geographically closely situated *aquilus* (separated by approximately 60 km) are meaningfull (p distance = 0.045). It is important to note that the genetic distance observed between *verhageni* and *aquilus* is considerably higher than p distances between the assayed *zena* populations (average p distance = 0.001) that are separated by approximately the same geographic distance [approximately 50-60 km separate Aberdares from Mount Kenya]. Also the genetical differences between *verhageni* with *laticeps* and *dudui* (p-dis-



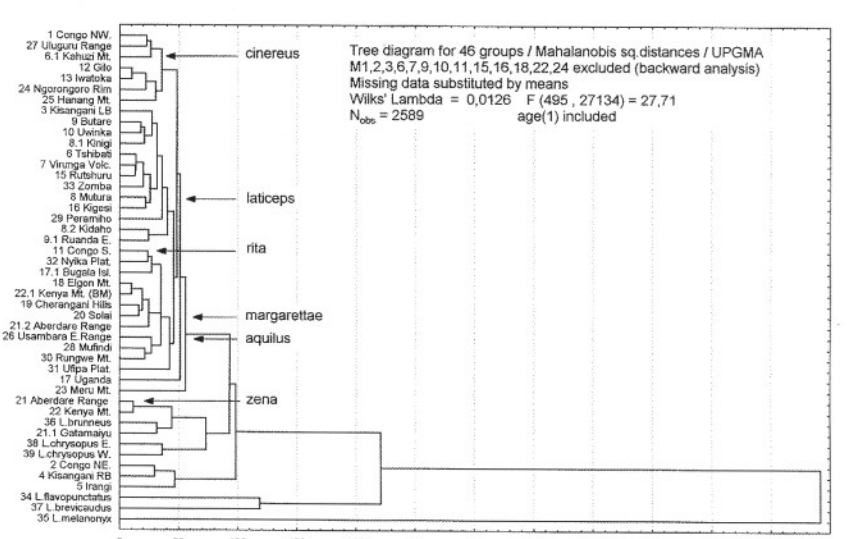
Graph.5.1

Graph. 5.1.
UPGMA dendrogram based upon the craniometric comparison of 33 OTU's, using a minimum of 20 skull measures.



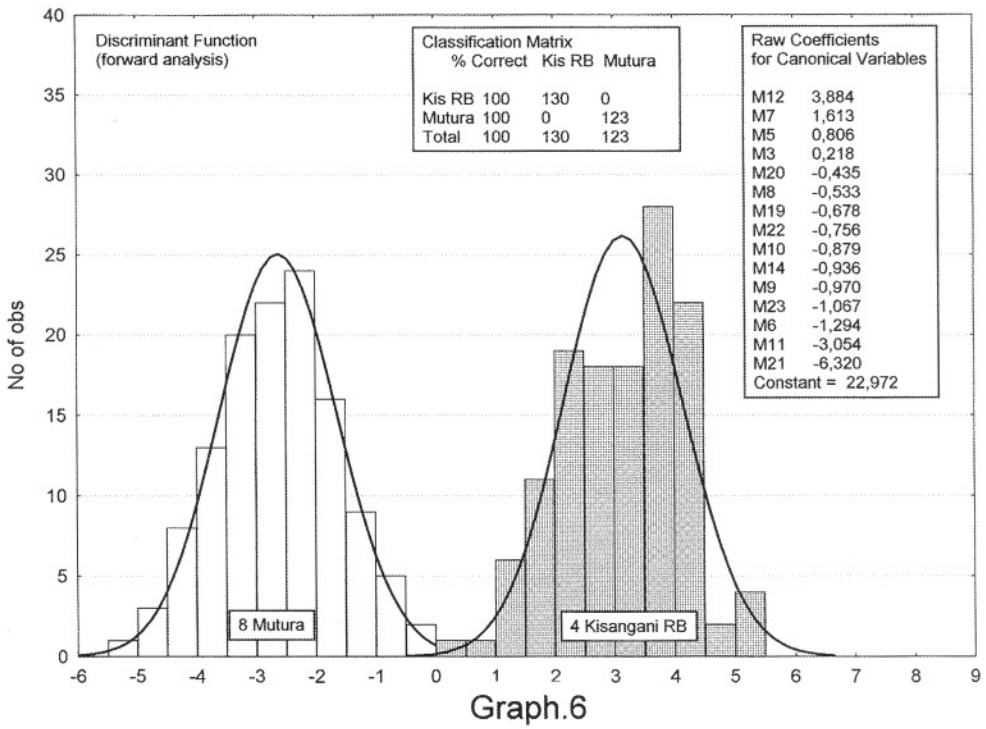
Graph.5.2

Graph. 5.2.
UPGMA dendrogram based upon the craniometric comparison of 38 OTU's, using a minimum of 16 skull measures.

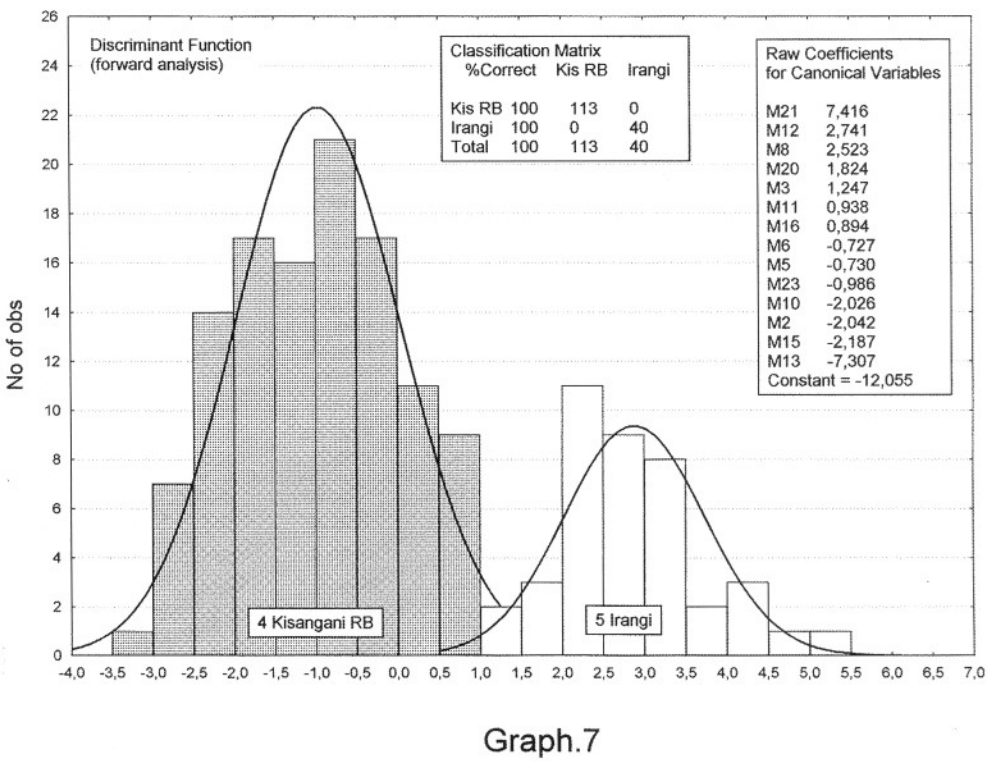


Graph.5.3

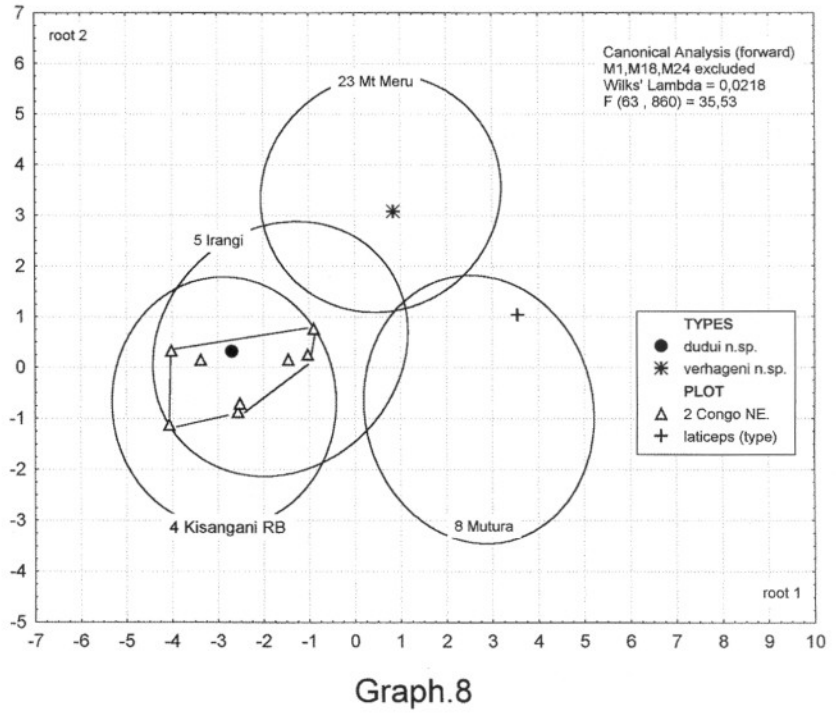
Graph. 5.3.
UPGMA dendrogram based upon the craniometric comparison of 46 OTU's, using a minimum of 10 skull measures.



Graph. 6.
Graphic representation of the discriminant function allowing the characterization of *L. dudui* n.sp. versus a topo-typical sample of *laticeps* (OTU 8), together with the canonical coefficients.

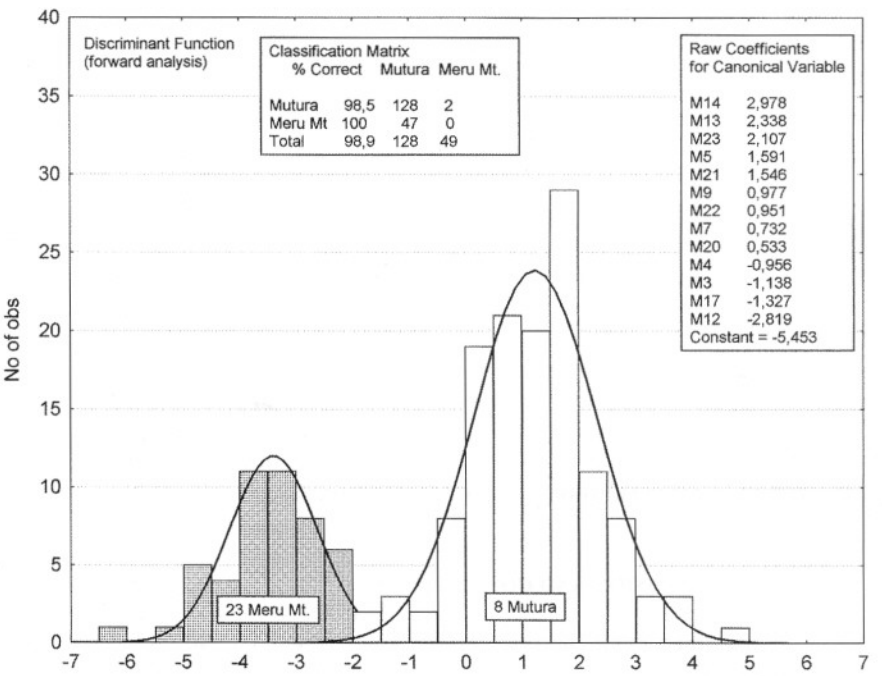


Graph. 7.
Graphic representation of the discriminant function characterizing between *L. dudui* n.sp. versus the population of "Irangi" (OTU 5).



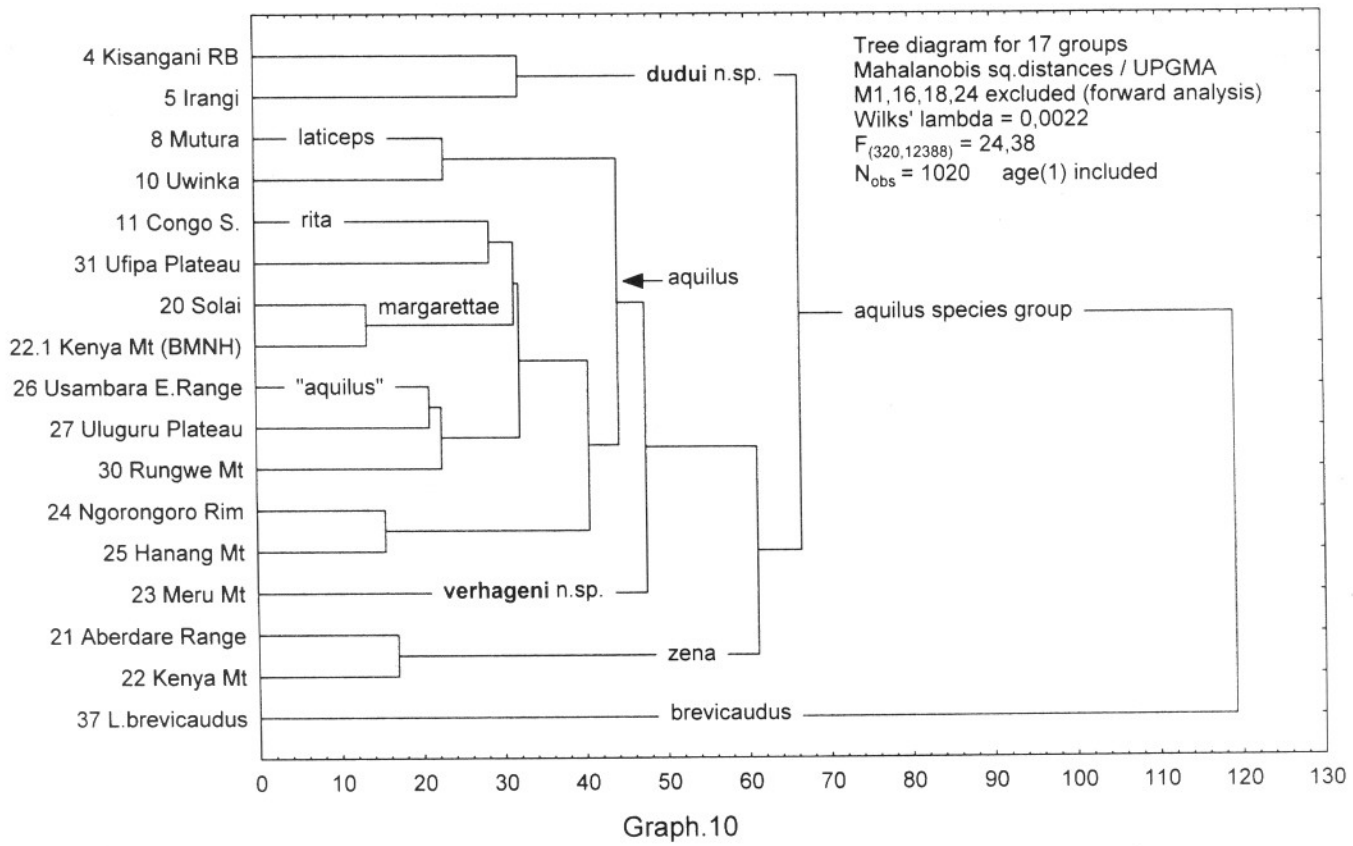
Graph.8

Graph. 8.
Graphical representation of a canonical analysis on our new species *verhageni* (OTU 23 : Mt Meru) and *dudui* (OTU 4 : Kisangani RB) and a population of the taxon *laticeps* (OTU 8 : Mutura).



Graph.9

Graph. 9.
Graphical representation of the discriminant function (forward analysis) between the Mutura population (OTU 8), representing the taxon *laticeps* and the new taxon *verhageni* of Mt Meru (OTU 23). Are also represented the coefficients for the canonical transformation, necessary for the diagnosis of the new taxon.



Graph. 10. UPGMA dendrogram based upon OTU's representing the known and our newly described east African taxa and using *brevicaudus* (OTU 37) as an outgroup.

tances are respectively = 0.078 and 0.059), even when based upon a single specimen, are likely to be reliable diagnostic differences.

Finally, the significance of the amount of genetical differentiation between *verhageni* and the nearby *aquilus* population (Mweka) is illustrated by the fact that the nucleotide substitutions observed between these two taxa result in not less than 4 inferred amino acid substitutions (p-distance = 0.031).

DISCUSSION AND CONCLUSIONS

The combination of craniometric and quantitative methods with genetical data, has proven to be a valuable approach to evaluate the current taxonomy of the *L. flavopunctatus* s.l. species complex.

One of the most important results of this study is that it illustrates the power and limitations of our methodology and approach. Indeed, concerning the use of craniometrical data in taxonomical work on rodents, our study demonstrates – at least for the studied taxa - that the differences in age and sex composition of OTU's (as long as these are sufficiently large; i.e. Nobs >20) are of no consequence for the branching of the phenetic trees based on the Mahalanobis squared distances between the centroids. This observation permitted us to screen the whole of the *L. flavopunctatus* s.l. species complex and to evaluate the validity of the already known taxa, to detect new taxa and to describe two *Lophuromys* species new to science.

Although it was not our main objective to go into the taxonomy of the Ethiopian representatives of the species complex, we had to consider a possible overlap with other east African taxa. *L. flavopunctatus* THOMAS 1888 turned out to be typical for Ethiopia and *L zaphiri* THOMAS 1906 appears to be synonymous with it. The taxa *brunneus* THOMAS 1906, *brevicaudus* OSGOOD 1936, *chrysopus* OSGOOD 1936 and *melanonyx* PETTER 1972 are craniometrically well differentiated from *flavopunctatus*. However, there is a closer craniometrical similarity between the latter and *brevicaudus*. All these Ethiopian taxa are considered to be differentiated at the species level, but the taxon *simensis* OSGOOD 1936 is possibly synonymous with *brunneus*. None of these taxa proved to belong to the *L. sikapusi* species complex; *L. major* THOMAS & WROUGHTON 1907 is not represented in the Ethiopian *Lophuromys* populations we analysed.

The craniometrical study of the type skulls from non - Ethiopian origin shows that *laticeps* THOMAS & WROUGTON 1907, *rita* DOLLMAN 1910, *margarettae* HELLER 1912 and *cinereus* DIETERLEN *et al.* 1974 are probably synonyms with *aquilus* TRUE 1892. However, the fact that each of the type specimens of *zena* DOLLMAN 1909, *rubecula* DOLLMAN 1909, *eisentrauti* DIETERLEN 1978 and *dieterleni* VERHEYEN *et al.*

1997 plots within the OTU 's 38+39 representing Ethiopian *chrysopus* suggests that this caryotypically well defined species has a much wider distribution than believed so far.
Graph.10 represents a phenetic tree incorporating the OTU's of the known and our newly described east African taxa and comparing these with a well-defined outgroup from Ethiopia (OTU 37: *brevicaudus*). This graph is an example of a multitude of different phenetic trees that can be obtained through changing or adding to the selection of east African OTU's; however, all the resulting trees are always fundamentally similar.
In the first place we note that the Ethiopian *L. brevicaudus* (OTU 37) is, as expected, different and clearly separated from all the east African taxa. Next, it is apparent that the east African populations of the "speckled" *Lophuromys* regroup in four taxonomical and geographical entities:

1° *dudui*: represents a taxon that inhabits the region of the rainforest situated between the right bank of the Congo river and the western foothills of the rift mountains (OTU's 4 and 5);
2° *zena*: groups the mountain populations of the Aberdare Range and Mt Kenya (OTU's 21 and 22);
3° *verhageni*: represents the population that lives on Mount Meru
4° *aquilus*: contains the rest of the OTU's, and covers the remainder of the known geographical range of the "speckled" *Lophuromys*.

We especially draw attention to the fact that in Kenya the "speckled" *Lophuromys* from the lower regions around Aberdare Range and Mt Kenya (OTU's 20 and 22.1) are allocated to "*aquilus*" whereas the specimens, caught higher up the flanks of both mountains (OTU's 21 and 22), are identified as belonging to *zena*. From graph 5.1 we know that *zena* is morphometrically most similar to Ethiopian *brunneus* (OTU 36) and forms, be it on a higher level of differentiation, a clade with Ethiopian *chrysopus* (OTU's 38+39).
It is obvious that we need more information, preferably chromosomal and genetical, before we can settle whether *brunneus* THOMAS 1906 has taxonomical priority over *zena* DOLLMAN 1909 and what the taxonomic position of the east African *rubecula* DOLLMAN 1909 is in connection with Ethiopian *chrysopus* OSGOOD 1936.
As to our newly described taxon, *dudui* is morphometrically and genetically sufficiently separated from the other non - Ethiopian OTU's, that it should be differentiated at the species level. This conclusion is supported by its rather important geographical range covering the rainforest from the right bank of the Congo river to the western foothills of the rift mountains and reaching to the north as far as Garamba and to the south as far as the Maniema region.
Also *L. verhageni* (OTU 23: Mt Meru) is craniometrically well defined, but is manifestly closer to *aquilus* than to *zena* and *dudui*. Moreover, it appears to have reached a genetical level of differentiation similar to levels observed among other rodent species (SMITH & PATTON, 1991). All the other formerly recognized east African taxa such as *laticeps*, *rita*, *margarettae*, *cinereus* are to be considered conspecific with *aquilus*. Consequently, we have refrained to describe and name the branch grouping the populations of Mt Hanang (OTU 25) and Ngorongoro Rim (OTU 24), because these OTU's never form a well defined higher level grouping such as we found for *dudui* and *verhageni*.
Finally, graph.5.1 shows clearly that all the east African "speckled" *Lophuromys* form together with the Ethiopian *brunneus* and *chrysopus* what we call the "*aquilus*" species group, contrasting with the rest of the Ethiopian species *melanonyx, brevicaudus* and *flavopunctatus*. We will develop this further into a forthcoming publication concerning the speckled *Lophuromys* of the Mount Ruwenzori.
Our study complements the traditional morphological approach with a genetic tool to characterize two new species and several OTU's. The partial mitochondrial cyt b sequences used here do not provide direct evidence to support our view that the described species are biologically valid. However, the fact that the mitochondrial cyt b fragment of our new species is significantly different when compared to sequences of related taxa, is suggestive to this effect.
It is documented that mitochondrial cyt b evolves at a similar rate in a wide array of vertebrates (JOHNS & AVISE, 1998). The observed amount of sequence divergence among the *Lophuromys* species and populations studied here is well within the range observed for other mammal species, including some rodents (SMITH & PATTON, 1991). Indeed, it has been reported that the cyt b sequence divergence between mammal species ranges between 0-0.32 (p distances), and for sister taxa, these values range between 0-0.2 with a mode at about 0.035 for mammals (JOHNS & AVISE, 1998). Interestingly our results appear to agree with findings suggesting that cyt b sequence divergences among South American Akodontine rodent species are in the same range or higher ($0.03 > p > 0.21$, SMITH & PATTON, 1991). However, as correctly pointed out (FERGUSON, 2002) genetic distances as such do not allow us to distinguish between species-level and population-level differences. An important problem is that it is easy to imagine how populations may have been geographically isolated for long periods of time, while they presumably constitute a single species (AVISE *et al.*, 1998). Although the evidence provided by our cyt b sequences supports that the investigated taxa and OTU's have experienced a long-term genetic isolation, the inclusion of genetic distances to the presented craniometrical evidence does not allow us to make absolute statements whether or not a population has attained the species status (FERGUSON, 2002).
Nevertheless, our observation that the investigated OTU's and taxa differ in fixed genetic characters that can be detected by their nucleotide sequences and morphological differences does provide some support about the taxonomic status of the investigated taxa. All taxa for which more than a single specimen was assayed share character states (synapomorphies), a situation that appears to be compatible with the phylogenetic species concept (CRACRAFT, 1983). The presence, and relative stability of the synapomorphies not only implies genetic differentiation, but suggests a lack of gene flow between these taxa.

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