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SHORT NOTE

THE PHYLOGENY OF SOME AFRICAN MUROIDS (RODENTIA) BASED UPON PARTIAL MITOCHONDRIAL CYTOCHROME *b* SEQUENCES

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Almost 50 % of all extant mammal species are rodents and much of the taxonomic confusion in this group concerns the Muroidea. Immunological data, DNA/DNA hybridization experiments, amino acid sequences, distribution of highly amplified Lx fragments and mitochondrial DNA sequences disagree with the morphology based systematics and suggest that the genus *Acomys* does not belong to the Murinae Illiger, 1814 (1, 2, 3, 4, 5, 6, 7, 8, 9). If the « acomyines » (*Acomys* Geoffroy, 1838, *Uranomys* Dollman, 1909 and *Lophuromys* Peters, 1874) and the Murinae are found to be separated by at least two nodes in the cladogram, the similarity between the cranial and dental morphologies of these two clades could be the result of convergent evolution.

To test recently published phylogenies based on different molecular data we analyzed the nucleic acid sequences of portions of the mitochondrial cytochrome *b* gene (cyt *b*). In order to evaluate the potential importance of biological sampling on phylogenetic inference, we studied the relations among a number of African genera instead of *Mus L.*, 1758 and *Rattus* Fisher, 1803 often used as the representatives of the Murinae. We present parsimony analyses of cyt *b* from species of six genera traditionally allocated to the Murinae (*Mastomys natalensis* (Smith, 1847) ; *Dasymys incomtus* (Sundevall, 1846) ; *Arvicanthis nairobae J.A.* Allen, 1909 ; *Acomys spinosissimus* Peters, 1852 ; *A. wilsoni* Thomas, 1892 ; *Hybomys univittatus* (Peters, 1876) ; a yet undescribed *Hybomys* species and *Lophuromys flavopunctatus* Thomas, 1888), one species of the Gerbillinae Gray, 1825 (*Tatera valida* (Bocage, 1890)), species of two genera of Cricetomyinae Roberts, 1951 (*Cricetomys gambianus* Waterhouse, 1840 and *Beamys hindei* Thomas, 1909) and sequences from the literature of species from three genera of the Sigmodontinae Wagner, 1843 (10). Throughout this contribution we use the taxonomy of Musser and Carleton (11) in which the Muroidea contain only the family Muridae Illiger, 1814 subdivided into 17 subfamilies.

DNA was isolated from frozen or ethanol-preserved muscle tissue samples from the collections of the Department of Biology of the University of Antwerp (RUCA). The primers used to amplify a 402 base pairs (bp) long cyt *b* gene segment were L13724 (5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′ and H14139 (5′-AAACTGCAGCCCCTCAGAATG ATATTTGTCCTCA-3′) (12). PCR-reactions were performed in three vials containing 25 µl of Tris buffer (10 mM, pH 9.0) containing

50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, 50-500 ng template DNA, and *Taq* polymerase (0.65 units : 30 cycles, 94°C : 60s, 49°C : 60s and 72°C : 90s). The double-stranded PCR product was cleaned on a Millipore Ultrafree-MC 30,000 spin column to remove unincorporated nucleotides and cycle sequenced on an ALF-DNA Sequencer according to the manufacturer's protocol, using 0.8 µM primer, 2.5 units of *Tth* polymerase and both primers (Pharmacia). The mtDNA sequences were analysed using the parsimony method (PAUP 3.1.1 : 13). Transversions (TV) were considered to be ten times more important than transitions (TS) and cyt *b* sequences of the marsupial *Didelphis virginiana* Kerr, 1792 and/or the Geomyidae Bonaparte, 1845 *Cratogeomys* Merriam, 1895, *Geomys* Rafinesque, 1816 and *Papageomys* Merriam, 1895 (14, 15) were used as outgroup sequences. Statistical support for the branching patterns was estimated using the bootstrap method under the same TS/TV settings (100 replications) in PAUP.

We analysed 357 bp of the mitochondrial cyt *b* fragment. In total 172 sites were variable and 152 sites were parsimony informative. A high number of amino acid replacements were observed of which 33 of the 119 sites were informative for parsimony analysis. Pairwise comparisons among the cyt *b* sequences indicated that TS/TV ratios are low, suggesting that the studied taxa have diverged long ago or have undergone unusual rapid mutation rates in comparison with other mammals (16, 17). Finally, the overall base compositions within the muroids showed a strong anti-G bias at third positions in cyt *b*. (A : 41 %, T : 21 %, C : 36 % and G : 2 %). Because of the degenerate nature of the amino acid code, many third codon positions and some first positions are less constrained and evolve at a higher rate (16). In accordance with observations on the cyt *b* sequences of arctic carnivores many third codon positions were found to be completely consistent with the obtained phylogeny; therefore we did not delete these characters (17).

Parsimony analysis yielded a single most parsimonious tree whose branching pattern was subsequently evaluated using the bootstrap method. The clades agreed with the subfamily grouping of most of the species studied (Fig. 1). Analyses that did not take the observed TS/TV ratio into account never resulted in trees in agreement with the (morphology based) classification of the studied species. The Cricetomyinae is the only subfamily in our dataset that was not supported by enough characters to yield a bootstrap value higher than 50 %. *Tatera valida*, the only representative of the Gerbillinae in our data set, did not cluster with any of the other subfamilies.

Our cyt *b* phylogeny does not exclude the possibility that the « acomyines » and the Murinae are a monophyletic group and the *Acomys-Lophuromys* clade may be a sister-clade to the African murines as has been suggested by immunological data (9). In addition, cyt *b* sequences of *Uranomys ruddi* Dollman, 1909 (not shown) confirm the existence of an *Acomys-Lophuromys-Uranomys* clade (4, 9, 18, 19). Our data did not allow us to evaluate whether or not the « acomyines » are closely related to the genus *Tatera* as suggested by recent DNA hybridization and amino acid sequence data (5, 6, 9).

Our study illustrates the limitations of partial cyt *b* sequences for phylogenetic studies within and among muroid subfamilies. The only phylogenetic statement at the « subfamily level » that is supported by our data is the inclusion of the Sigmodontinae within the Muridae. Although our cyt *b* phylogeny is consistent

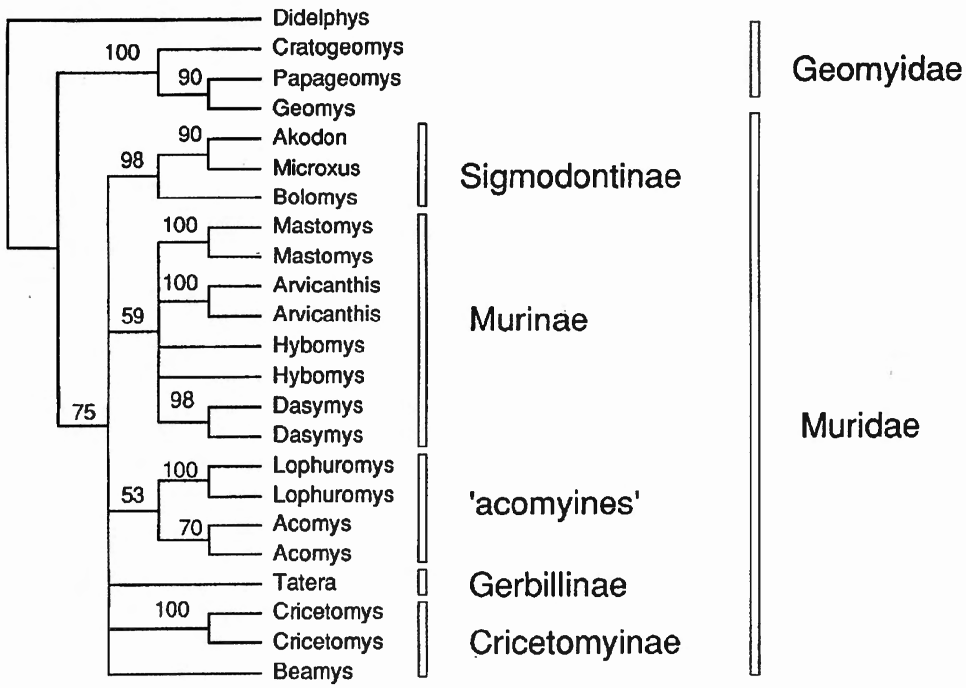

Fig. 1. — Cladogram produced by parsimony analyses of a partial mitochondrial cyt *b* gene (357 aligned sites) of representatives of twelve murid genera (see text). Numbers at nodes are bootstrap values obtained after 100 replicates (C.I. = 0.28). The Cricetomyinae is not supported by enough characters to yield a bootstrap value > 50 %.

with the subfamily groupings within the muroids, more sequence information will be required to establish the relationships between the Murinae and the « acomyines » or to determine the phylogenetic relations among muroid subfamilies. Consequently, a portion of the 16S rRNA mitochondrial gene is presently being sequenced on more taxa of various African subfamilies of the Muridae to be added to our data-base. Confirmation from our cyt *b* sequences of *Otomys lacustris* Allen and Loveridge, 1933 (subfamily Otomyinae Thomas, 1897 ; data not shown) that this genus belongs to the Murinae — as was recently suggested by immunological data (9) — illustrates the need to include taxa of all subfamilies in such analyses.

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