

Morphometric, biochemical and molecular traits in Caucasian wood mice (*Apodemus/Sylvaemus*), with remarks on species divergence

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We analysed Caucasian wood mice from Georgia ($n = 60$) and supplementary reference material of the *Apodemus/Sylvaemus* species group to evaluate the reliability of taxon identification. Traditional “expert knowledge” plus three different methodological approaches were employed and combined to perceive their discriminatory power for a reliable taxon assignment. Graphs of principal component scores derived from the analysis of 14 skull metrics displayed taxon membership of individuals. Individual multi-locus ($L = 18$) electrophoretic profiles were used to re-assess specimens to a specific genepool by an assignment test based on allele frequencies indicative of populational taxon samples of the respective sampling locations. Genotyped individuals were re-allocated to those taxa, for which they yielded the highest probability score. Genetic distances among the taxa were computed and clustered in a neighbour-joining tree. PCR-fragments of 1074bp amplified from the mitochondrial cytochrome *b* gene were cut with 2 six- and 4 four-cutter restriction enzymes, and resulting RFLP patterns were analysed phenetically to classify the specimens according to their molecular similarity. Partial cytochrome *b* sequences were used to construct a phylogenetic tree by computing neighbour-joining clusters from a matrix of percent nucleotide differences. The power of the combined classification approaches and their congruence is discussed. It is concluded that the joint application of traditional, morphometric and biochemical or genetic techniques for taxon allocation of specimens of wood mice encountered problems in species delimitation. The mtDNA topology obtained was not congruent with protein polymorphism that indicated differential historical and/or recent introgression and incomplete lineage sorting in substructured populations. Cytochrome *b* sequence DNA data analysed were not as adequate as expected to resolve phylogenetic relationships among Caucasian and European members of the *Apodemus-Sylvaemus* complex. Altogether, morphometric, biochemical and sequence data sets did not support the hypothesis of the evolutionary independence of European and Caucasian lineages of wood mice. Nonetheless, extended combined morphological and genetic analyses are considered necessary prerequisites to an in-depth study of the evolutionary lineages of the *Apodemus/Sylvaemus* group. More sequence data of a variety of genes (and plenty of nuclear markers) are needed to resolve the various levels of differentiation of the extant lineages.

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