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## Taxonomy-Genetics

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### **New rodent species described since 2000; an age of discovery, comments and prospects**

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In this contribution I present a review on the new living rodent species described since 2000. The most relevant issue to emphasize is that we are in an age of discovery. An average of ca. 14 new rodent species were described each year. Since 2000, over 250 new species have been described on the basis of specimens collected in South America, Asia, Africa, Oceania, North America, and Europe. The new rodent species belong to 21 families; most of them are allocated to the families *Muridae* and *Cricetidae*, but also to less diverse families as *Platacanthomyidae* and *Sminthidae*, as well as to the until then considered extinct *Diatomyidae*. Most discoveries were prompted by the analysis of genetic variation, although several new species have been hypothesized exclusively on the basis of morphological variation. Even when most taxonomic studies are based on one locus or few loci, we are starting a transition to an era where (sub)genomic data is analyzed with a coalescent approach. After commenting on distinct particularities of the new species, it is concluded that field collection of specimens and collections-based research needs to be intensified to characterize rodent diversity. Financial support: FONDECYT 1141055 and 1180366.

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### **Molecular phylogeny and distribution of the most widespread African rodents, the multimammate mice genus *Mastomys*: a review**

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Murid rodents of the genus *Mastomys* ("multimammate mice or rats") represent one of the most successful groups of indigenous African mammals. This genus inhabits almost the whole sub-Saharan Africa and a small population of one species lives also in Morocco. They are very important from human point of view (important agricultural pests and reservoirs of diseases), but they serve also as model taxa for fundamental biological research (speciation, phylogeography, host-parasite co-evolution, etc.). For proper use of *Mastomys* as biological models, the information about their evolutionary history is necessary. Unfortunately, most available genetic data on this genus are based on mitochondrial DNA, which provides only partial information. Further, detailed genetic data from large areas in Africa have been completely lacking. Currently, eight morphologically very similar species are recognized especially on the basis of their karyotypes and mitochondrial sequences. Some of them have large area of distribution, e.g. the most widespread species, *Mastomys natalensis*, or Sudanian savanna specialist *Mastomys erythroleucus*, while the distribution of other species is geographically very restricted (e.g. *Mastomys awashensis* in Ethiopia or *Mastomys shortridgei* in humid habitats of southwestern Africa). Here, we combine all newly produced and already published mitochondrial sequences of *Mastomys* and describe the most complete distribution of their mitochondrial variability across whole continent. For the first time we use nuclear data to reconstruct the multi-locus phylogeny of the genus. The results show that the genus is not monophyletic (*Mastomys pernanus* should not be *Mastomys*), the highest species diversity is found in Ethiopia. In addition, we also clarify other questions in species delimitations and taxonomy. The research was funded by the Czech Science Foundation project No. 18-17398S and by the Russian Foundation for Basic Research No. 18-04-00563-a.

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### **Molecular evolution, hybridization and introgression affect molecular systematics of old world mice**

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Despite the post-zygotic reproductive isolation between the house mouse (*Mus musculus domesticus*) and the Algerian mouse (*Mus spretus*) hybridization between the two species locally has resulted in introgression. We show how heterogeneity in molecular divergence, geographic variation in introgression, and incomplete lineage sorting result in a highly variable mosaic of the genome wide systematic relationships between mice. We infer how these variable patterns of molecular divergence and of introgression at protein coding genes may affect the biology of the species, and conceivably, variability in some of their unique phenotypic characteristics.

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### **Genetic structure and morphological evolution of the house mouse on the Orkney Archipelago**

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The house mouse has colonized numerous islands following human travelers. This has exposed it to a high variety of environments, prone to trigger adaptive evolution. The colonization itself, processing by successive founder events and bottlenecks, may promote evolution. Hence, both adaptive and stochastic processes could contribute to an accelerated evolution on islands. We developed an approach coupling genetic and morphometric approaches on a set of more than 250 mice from eight of the Orkney Islands. A first genetic analysis using mitochondrial D-loop sequences allowed us to determine the relationships between Orkney and western European mice. A second analysis based on 21 microsatellites provided insights into genetic structure within the archipelago, revealing a strong geographic structure at a small spatial scale. Morphometric analyses were performed on the first upper molar. Despite their phylogenetic relatedness, Orkney mice displayed a morphological diversity as high as among Western European populations. This diversification erased the phylogenetic signal in the tooth morphology, and is ascribed to an accelerated morphological evolution on Orkneys. In contrast, within Orkneys, morphological and genetic divergences are largely in agreement. This holds true at the scale of the archipelago, of the main island, and even at the scale of local genetic clusters (<5 km). This suggests that on Orkneys tooth morphology evolved in a rather neutral way in isolated populations, and does not correspond to an adaptive divergence. It further suggests that the strong genetic structure at a small spatial scale was a fuel for the important morphological diversification.

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### **Genetic differentiation and phylogeographic structure of the house mouse *Mus musculus* s.str. in the northern Palearctic**

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The systematics of the parapatric commensal taxa of house mice *Mus musculus* remains now rather controversial. We consider *Mus musculus* as a polytypic species, including subspecies *Mus musculus*, *Mus musculus wagneri*, *Mus musculus gansuensis*, *Mus musculus molossinus*. These subspecies are diagnosed by morphological, ecological, behavioral and cytogenetic features. We realized taxonomic estimation and analysis of genetic variability using nuclear (exon BRCA1) and mitochondrial (D-loop, cytb) markers. The material for genetic analysis was provided by 170 mtDNA and 46 nuclear sequences of house mice from Russia and the nearest countries of eastern Europe and central Asia. According to mtDNA variability, the phylogeographic structure turned out to be extremely heterogeneous. It was allocated 7 treasures: in Europe - 2, in Asia - 3 and 2 mixed. Two European branches divided the lines of house mice belonging to the subspecies *Mus musculus musculus* and *Mus musculus wagneri*. Basal monophyletic clade as well as control region and cytochrome b gene was lineage from Transcaucasia. Asian branches were characterized by 3 lines of subspecies of *Mus musculus* (*Mus musculus*, *Mus musculus wagneri*, *Mus musculus molossinus*). Of these, it should be noted a phylogroup from Siberia. It is assumed that mice in modern human history could be settled along major trade and transport routes. By the variability of the BRCA1 gene, *Mus musculus* was divided into two phylogroups by eight substitutions (6 transitions 2 transversions). The first group included individuals of the subspecies *Mus musculus*, in the second *Mus musculus wagneri*. Individuals of a hybrid origin formed a separate subcluster, apparently included hybrids of *Mus musculus* and *Mus domesticus*. Our data of mitochondrial and nuclear DNA analysis support the point of view that the subspecies *Mus musculus wagneri* should be considered as a separate species.

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### **Taxonomic structure and evolutionary history of mountain voles (*Alticola*, subgenus *Aschizomys*) in north-eastern Asia**

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Two species of Asian mountain voles assigned to subgenus *Aschizomys*, of the genus *Alticola*, namely, *Alticola macrotis* and *Alticola lemminus* inhabit mountain areas from Altay range at the south to Korytski Range and Chukotka peninsula in northeast Siberia. The distribution of both species is highly fragmented. The certain borders of their distribution, taxonomic status of isolated populations and phylogenetic interrelationships are obscure. We examine patterns of lineage diversification, phylogenetic and population genetic history analyzing mitochondrial *cytb* and three nuclear markers: partial *BRCA*, *GHR* and *LCAT* from specimens covering a large portion of species' ranges. We tested the hypothesis of hybridization between two species with JML software. The obtained results support the hypothesis of ancestral polymorphism and incomplete lineage sorting rather than interspecies hybridization. Genetic differentiation within both species as inferred from the *cytb* tree including museum type specimens is consistent with current species delimitations. The current intraspecies taxonomy correctly reflects evolutionary relationships. The nuclear genes species tree supports monophyly of the subgenus and included species. Further, we combine results of the phylogeographic analysis with species niche and distribution modelling with MAXENT software and use these combined results to reconstruct possible ancestral area and species distribution history from LGM to present. This study was conducted under research theme № AAAA-17-1170 424 10 167-2 and RFBR grant № 15-04-04602 and Program of Presidium RAS "Dynamics of gene pools in natural populations" and "Development of vital and biosphere processes".

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### **Multiple mitochondrial pseudogenes in the nuclear genome in two species of mole voles (*Ellobius*, *Cricetidae*)**

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Within the last decades, mitochondrial molecular markers have been widely used for phylogeographic and phylogenetic studies. They are most suitable markers when estimating genetic divergence between natural fragmented populations. However, the presence of numerous nuclear mitochondrial copies (numts) may severely complicate such studies. The mole voles, genus *Ellobius*, are specialized subterranean rodents with highly fragmented range. In our earlier study of the Northern mole vole population, *Ellobius talpinus*, we uncovered high nucleotide diversity of the mitochondrial control region fragment (Kuprina et al., 2016). Here we suggest that one of two predominating haplotypes which have been assigned to the mitochondrial control region actually represents a nuclear pseudogene (numt) of the control region. Moreover, the subsequent cloning of control region and cytochrome b fragments of the Northern mole vole and the Zaisan mole vole (*Ellobius tancrei*) showed the presence of different variants of these putative numts within the genome of each individual. To reveal an approximate time of various mitochondrial-nuclear translocations we conducted a phylogenetic analysis using the control region fragment of different taxa of subfamily *Arvicolinae* including pseudogenes of *Ellobius talpinus* and *Ellobius tancrei* obtained in this study. Our results emphasize the importance of testing for numts in every phylogenetic and phylogeographic study using mt markers. Technical and financial support: Chromas and MCT RRCs of SPbSU, RFBR (projects 16-04-00479, 18-04-00730) research theme № AAAA-17-1170 424 10 167-2 and Program of Presidium RAS "Dynamics of gene pools in natural populations" and "Development of vital and biosphere processes".

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### **Comparative study of striped field mouse *Apodemus agrarius* from continental and insular populations: the result of five microsatellite loci analysis**

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The striped field mouse inhabits a wide geographical area from central Europe to the Pacific coast of Asia including adjacent islands. The species range is subdivided into two isolated fragments (European-Kazakh-Siberian versus Russian Far East-Chinese-Korean) with disjunction in Transbaikalia. We compared the level of genetic variability and differentiation within and between these isolates using 4 regional group samples (30 animals in each group). The fragment analysis of GTTDS8, GATAE10A, CAA2A, GTTF9A and GSADT7 (selected from Makova et al 1998) was carried out. It was shown that allelic diversity in the western isolate was lower than in the eastern one. High affinity of regional groups within each of the isolates and a bit greater genetic differentiation between these isolates were found. The second aim of our investigation was to compare population structure and differentiation of mainland and island local populations of the eastern lineage. 263 mice from 6 continental localities of Russian Far East as well as 3 islands of the Peter the Great Bay (Sea of Japan) were analyzed. The allele numbers in the continental populations were higher than in the island ones. Tests for HWE indicated significant heterozygote deficiency in both types of populations. Island populations were more significantly different both from each other and in comparison with continental populations. This suggests the importance of genetic drift in the formation of their genetic structure, especially on the islands. The work was supported by grant № 18-4-031 of "Far East" Program of RAS.



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### **Phylogeography, taxonomy and diversity of montane populations of laminate-toothed rats (Muridae: Otomys) in the southern Great Escarpment, South Africa, with the description of a new species**

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Mitochondrial DNA sequences of the cytochrome b gene (cyt b, 1137 bp) were used to investigate evolutionary relationships of five putative taxa of *Otomys* (family *Muridae*, subfamily *Murinae*, tribe *Otomysini*) occurring in the Western Cape and Eastern Cape provinces of South Africa. A total of 22 specimens of three putative species (*Otomys auratus*, *Otomys sloggetti* and *Otomys cf. karoensis*) were added to data obtained from GenBank and the amalgamated data set was analysed phylogenetically. Outgroups were selected from other genera of the tribe *Arvicanthini*. To incorporate a further 28 sequences from a shorter segment of 407 bp of cyt b obtained from a recently published study we created a second dataset in which we trimmed the sequences to 407 bp and this was analysed separately. We analysed craniodental and craniometric characters of 94 adult skulls from localities from which individuals had been sequenced or karyotyped herein or previously. Phylogenetic analysis revealed the existence of a well-supported unique mitochondrial lineage of *Otomys cf. karoensis* (named herein *Otomys willani* sp. nov.) from the southern Drakensberg Range. Craniometric analysis distinguished *Otomys karoensis* from *Otomys willani* sp. nov., and added a further four localities to the range of the latter species.

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### **Computational species delimitation provides evidence for distinct evolutionary lineages of *Trinomys iheringi* (Rodentia: Echomyidae)**

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The endemic Brazilian spiny rat genus *Trinomys* comprises 13 taxa occurring in Atlantic forest, Cerrado and Caatinga biomes. This genus has a controversial systematics, and until recently six subspecies were recognized for *Trinomys iheringi* (*Trinomys iheringi iheringi*, *Trinomys iheringi graciosus*, *Trinomys iheringi bonafidei*, *Trinomys iheringi eliasi*, *Trinomys iheringi paratus*, *Trinomys iheringi panema* and *Trinomys iheringi denigratus*), all present in southeastern Brazil. Ilha Grande island populations, in Rio de Janeiro state, were considered as *Trinomys dimidiatus*, the species with geographical distribution spanning the mainland adjacent to this island. However, studies based on cytochrome b suggested that individuals from the Ilha Grande island actually belong to *Trinomys iheringi*. As a consequence, *Trinomys iheringi* now consists of a monotypic species with a disjunct distribution: in the Ilha Grande island, and in the São Paulo state, both in the São Sebastião island and in the mainland. Given this disjunct geographic distribution, we were prompted to investigate the phylogenetic relationships between populations from different localities. We sequenced the mitochondrial gene cytochrome b for 170 individuals of *Trinomys*, comprising all currently recognized species and added sequences available in GenBank to estimate within-genus divergence times. Moreover, we applied computational species delimitation methods to investigate whether *Trinomys iheringi* populations from the Ilha Grande island and São Paulo state were independent evolutionary units. Our results indicate that the Ilha Grande island and São Paulo populations separated around 0.798 Mya (0.35-1.33 Mya). Geological data poses that Ilha Grande island was completely separated from the mainland around 0.0051 Mya, which is far more recent than the estimated age of split between these two lineages. Therefore, we are suggesting that this divergence did not result from a vicariant event. Computational species delimitation methods always recovered both populations from the São Sebastião island and mainland São Paulo as unique entities. Our analysis indicates that *Trinomys iheringi* is not monotypic and possibly consists of several subspecies.

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### **Genetic analysis of type material brings logical order in geographic distribution and taxonomy. Case study of Central Asian vole genera *Neodon*, *Blanfordimus*, *Lasiopodomys***

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The systematics and nomenclature of central Asian vole species from genera *Neodon*, *Blanfordimus* and *Lasiopodomys* raised many questions. The dramatic discordance may be discovered in comparison of species distribution and taxonomy. Thus, the range of *Lasiopodomys fuscus* is very narrow and is in the very center of distribution ranges of voles from the genus *Neodon*. It is on the other hand very isolated from two other congeneric species. The range of *Neodon juldaschi* in turn lays in the center of distribution of voles from the genus *Blanfordimus* and is strongly isolated from the distribution of congeneric voles. It should be kept in mind that the proper use of species names depends entirely on the process of verifying whether additional specimens are conspecific with the specimen with which the species name is associated. Related to this the genetic studies of type material are of paramount importance in elucidating taxonomic issues. We successfully extracted DNA and obtained fragments of mitochondrial cytb from lectotype and paralectotypes of *Lasiopodomys fuscus* (collected by Przhevalskii) and the holotype of *Neodon juldaschi* (collected by Severtzov) from the collection of the Zoological Institute RAS. The results of phylogenetic analysis bring back the logic correspondence between zoogeography and systematics. Thus, *Lasiopodomys fuscus* should be without any doubt assigned to the genus *Neodon* and *Neodon juldaschi* to the genus *Blanfordimus*. Both species with a high support find their places within corresponding monophyletic clusters at the phylogenetic tree. Funding: research theme № AAAA-17-1170 424 10 167-2, RFBR grant № 15-04-04602, Program of Presidium RAS "Dynamics of gene pools in natural populations" and "Development of vital and biosphere processes".

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### **The genus *Sciurus* in Turkey: data on their distribution, morphometry, karyology and mtDNA sequence variation**

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The genus *Sciurus* is represented by two species: *Sciurus anomalus* and *Sciurus vulgaris*. The latter species is naturally distributed in European part of Turkey. *Sciurus anomalus* has a predominant distribution in Anatolia but allopatrically found with *Sciurus vulgaris* on the Northeast of Turkey. In this study, *Sciurus anomalus* and *Sciurus vulgaris* samples were collected from their natural habitats in Turkey. Morphometric characteristics of both species were studied based on standard and geometric morphometric approaches. Karyotyping was also conducted to find out the chromosomal properties of both species. Partial DNA sequences of two mitochondrial genes (Cytb and dLoop) were sequenced for genetical comparisons and phylogenetical assessment. Morphological measurements showed significant differences between two species ( $P < 0.05$ ) based on 26 cranial characters. Mandibles and the skull used in geometric morphometric analysis resulted in significant differences ( $P = 0.013$ ) in terms of shape based on mandibles but did not yield any significant differences ( $P = 0.069$ ) based on skull. Both species have the same chromosome number  $2n = 40$  but differ in the NF (*Sciurus vulgaris* 76 and *Sciurus anomalus* 80) and NFa (*Sciurus vulgaris* 72 and *Sciurus anomalus* 76) values obtained from karyological analysis. The two species were distinguishable based on both mtDNA gene regions utilized and the sequences were approved by the sequences obtained from the Genbank. Two separated *Sciurus vulgaris* populations were found in Turkey. Based on the mtDNA sequences Edirne samples (from Thrace, European part of Turkey) were clustered with the northeast squirrel population. The genetic distance between two species is found to be  $0.182 \pm 0.020$  based on mtDNA dLoop sequences. The results obtained from both mitochondrial gene regions supported each other.

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### **A new vole record from Anatolia may change the evolutionary story of voles in Anatolia**

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*Microtus* species are distributed in open meadows and agriculture areas in the Holarctic region. It is one of the most specious taxa in *Arvicolinae* by having 58 species. The speciation processes is still on going and recent studies have discovered new species for the genera. This makes the taxa a good model for the studies on sympatric issues and ecological speciation. In Turkey there are 13 vole species, and three of them are endemic to Turkey (*Microtus anatolicus*, *Microtus dogramacii*, and recently described *Microtus elbeyli*). In this study, we collected more than 400 samples from inner and eastern Anatolia and evaluate them on the basis of morphology and molecular characters. Our preliminary results showed *Microtus qazvinensis* is distributed in Elazığ Province of Turkey. This species was described from Iran and this is the first record of this species from eastern Turkey. The karyotype and morphological results support our findings. On the other hand, up to now, *Microtus socialis* was thought to be widely distribute in the east of Turkey. However, our results could not verify the presence of this species in Turkey. Additionally, our results showed that

has possibly a wider distribution in Turkey than former publication states. These results will possibly change the discussion about species distribution and evolution of voles in Anatolia. Also our preliminary results showed that *Microtus irani* and *Microtus guentheri* were separately placed in the bottom position in the phylogenetic tree of Turkish voles.