Radiation events in the subfamily Arvicolinae (Rodentia): Evidence from nuclear genes

Article *in* Doklady Biological Sciences · October 2009

DOI: 10.1134/S0012496609050196 · Source: PubMed

4 authors:

Natalia I Abramson
Russian Academy of Sciences
121 PUBLICATIONS **670** CITATIONS
SEE PROFILE

Vladimir S Lebedev
145 PUBLICATIONS **1,105** CITATIONS
SEE PROFILE

Anna Andreevna Bannikova
Lomonosov Moscow State University
198 PUBLICATIONS **1,115** CITATIONS
SEE PROFILE

Alexey Tesakov
Russian Academy of Sciences
156 PUBLICATIONS **2,414** CITATIONS
SEE PROFILE

ISSN 0012-4966, Doklady Biological Sciences, 2009, Vol. 428, pp. 458–461. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © N.I. Abramson, V.S. Lebedev, A.A. Bannikova, A.S. Tesakov, 2009, published in Doklady Akademii Nauk, 2009, Vol. 428, No. 5, pp. 713–717.

GENERAL BIOLOGY

Radiation Events in the Subfamily Arvicolinae (Rodentia): Evidence from Nuclear Genes

N. I. Abramson[a], V. S. Lebedev[b], A. A. Bannikova[c], and A. S. Tesakov[b]

Presented by Academician S.G. Inge-Vechtomov November 13, 2008

Received March 16, 2009

DOI: 10.1134/S0012496609050196

Voles and lemmings (subfamily Arvicolinae) are one of the youngest and species-rich groups of myomorphic rodents prevailing in different landscape zones of the northern hemisphere. Because of unprecedented fossil records, as well as rapid and continuing morphogenesis, they became leading forms in correlation and biostratigraphy of late Cenozoic continental deposits. For the same reasons, they are an almost ideal model group for testing various evolutionary scenarios and phylogenetic hypotheses on the one hand, and for comparison of the possibilities and limitations of different methodical approaches to phylogeny analysis and system generation, on the other hand.

This study consisted in analysis of two nuclear gene variation, which demonstrated that mole lemmings (Ellobuisini), steppe lemmings (Lagurini) and gray voles (Arvicolini) were sister groups. This divergence was the latest, third step of subfamily radiation. The new data on close sister relationships of mole lemmings, gray voles, and steppe lemmings and on the late mole lemming radiation are unexpected and contradictory to traditional views.

Originally, system generation and relationship analysis within the subfamily were based on comparatively studying the morphological traits in the modern and extinct forms; the most important results were reported in summary monographs and articles [1–4].

As new approaches (karyological, allozyme analysis, molecular methods) appeared, modified schemes were developed based on the new data [5–12]. Comprehensively studying the group revealed some distinct higher categories of the tribal rank in all summaries and systems, including the latest ones [11]. At the same time, their composition, relationships, and the time of divergence still remain obscure. Morphological approaches fail to reconstruct the evolutionary history and relationships of such a young and rapidly evolving group because of a few available traits and numerous parallelisms.

The study of Arvicolinae molecular phylogeny has been so far based on the mitochondrial cytochrome *b* gene [8, 10, 12, etc.]; note that the initial attempts, in addition, dealt with a limited set of taxa. Because of these two factors, phylogenetic relationships of supraspecific groups remained unsolved. Numerous polychotomies were observed on both the generic and suprageneric levels. This suggested explosive radiation of vole and almost simultaneous appearance of all major phyletic lineages leading to the modern genera *Ondatra, Prometheomys, Dicrostonyx, Lemmus, Ellobius, Phenacomys*, etc. [8]. However, many studies showed a high degree of mutational saturation in the *cyt b* sequences, which resulted in a loss of phylogenetic signal and could be the main cause of polychotomy. It could be expected that slower evolving nuclear genes, which were less prone to mutational saturation, would be more appropriate for determining phylogenetic relationships. However, to date, an attempt has been made to use only one gene (the 10th exon of growth hormone receptor (GHR) [9]) for the subfamily Arvicolinae. This report has brought into challenge the hypothesis on explosive radiation, which was thought to account for appearance of all suprageneric groups of this subfamily.

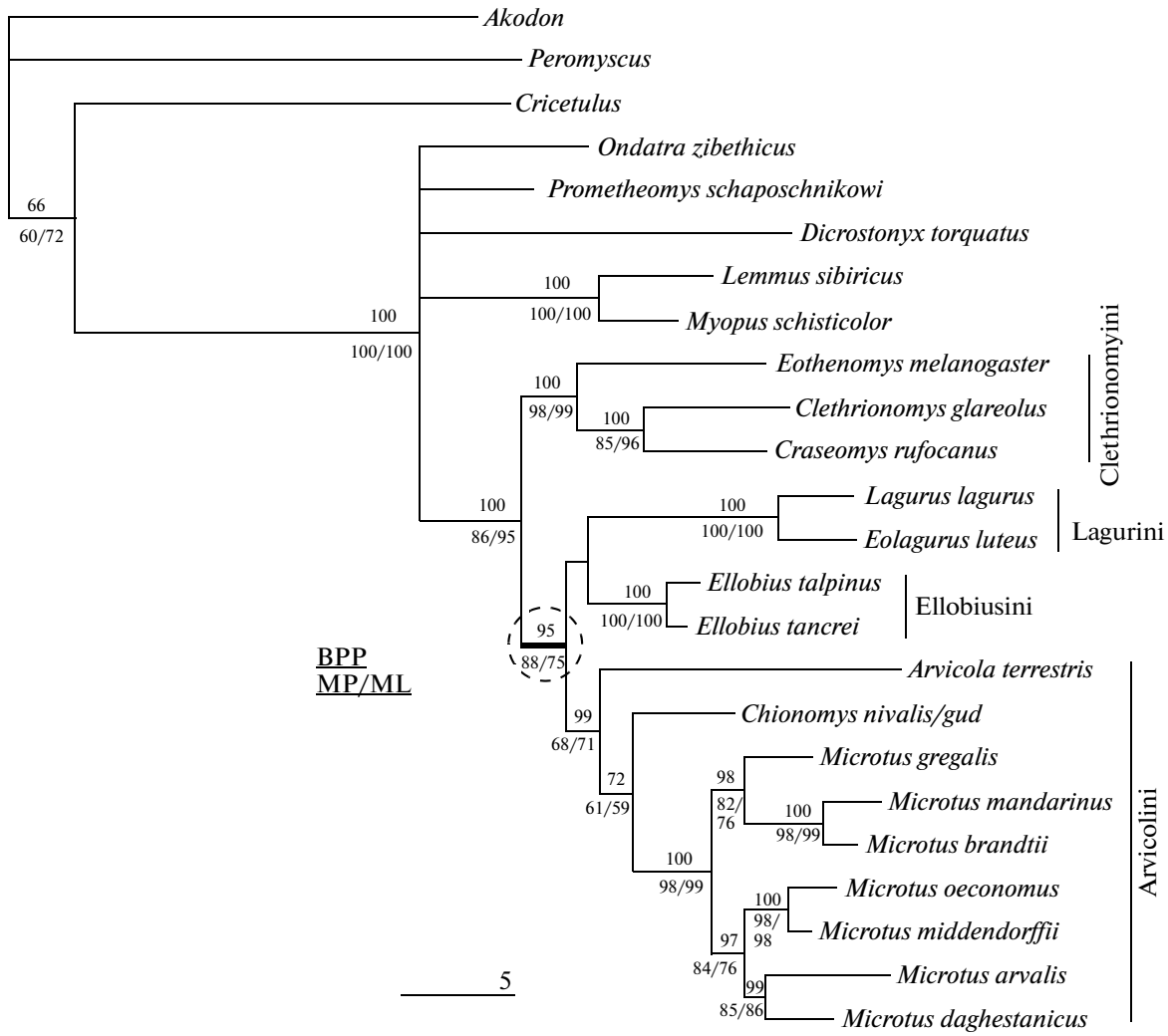
This study is the next step in the same direction; in addition to GHR, the nuclear gene encoding LCAT enzyme (lecithine–cholesterol acyltransferase) has been analyzed. Sequencing of this gene has been performed for a very limited number of the subfamily members. In addition, GHR of unstudied vole tribes, such as Ellobiusini and Lagurini, whose phyletic position is still debatable, was analyzed. The objective of this study was to identify more clearly the phyletic lineages that diverged during the basal (first) radiation from the groups that appeared at further steps of the subfamily evolution.

The material of our study was tissue samples from 25 voles whose skulls and skins are kept in collections of Zoological Institute, Russian Academy of Sciences

[a] *Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, St. Petersburg, 199034 Russia*

[b] *Geological Institute, Russian Academy of Sciences, Pyzhevskii per. 7, Moscow, 109017 Russia*

[c] *Moscow State University, Moscow, 119992 Russia*



Phylogenetic relationships between the members of Arvicolinae as determined on the basis of the MP criterion using bootstrap analysis. The bootstrap supports (MP and ML) and the posterior probabilities obtained by Bayesian analysis are shown under and above the branches, respectively.

(St. Petersburg) and in Zoological Museum of Moscow State University. In addition, 19 sequences from the GenBank database were used: those of the GHR gene (AM392378, AM392380–382, AM392384, AM392386, AM392388, AM392390, AM392395–396, AM392398–399, AY294926–927, AF332024) and those of the LCAT gene (AJ275588–AJ275592, AH005713, AH005249, AH005250). The GHR sequences were obtained using PCR with the combinations of primers Ghr_arv_F/Ghr_arvic_R or Ghr_cric_F/Ghr_arvic_R. We also specially developed primers for GHR and LCAT amplification: for GHR, Ghr _arv_F (5'-GGCGTTCATGACAACTACAAACCTGA-3'), Ghr_cric_F (5'-GGCATTCATGATAACTACAAATCTGA-3'), and Ghr_arvic_R (5'-ATAGCCACACGAGGAGAGGAACT-3'); LCATF, 5'-CACCATCTTCCTGGATCTCAA-3'; LCATR, 5'-AAGAAATACAGCACATGTAGGCA-3'. The general alignment in phylogenetic analysis 921 bp for GHR and 612 bp for LCAT. In the case of LCAT, only information on exons was used (330 bp) because of difficulty of aligning the outgroup.

Phylogenetic reconstruction was performed using the maximum parsimony (MP) and maximum likelihood (ML) algorithms of the PAUP* version 4.0b10 software. The optimum model of sequence evolution was determined using the ModelTest 3.7 software. The stability of phylogenetic reconstructions was tested using the bootstrap method (1000 and 300 replications for the MP and ML analyses, respectively). Bayesian phylogenetic analysis was performed using the MrBayes 3.04 software (figure). The age of divergence was determined for individual clades by the method of inexact molecular clock using the Multidivtime software and using the GenBank sequences for four other nuclear genes (*BRCA1, RAG1, IRBP*, and *c-myc*) known for a limited number of forms, as well as the data of fossil record.

The divergence times of superspecific groups of Arvicolinae estimated using the Multidivtime software

Sister clades		Estimated divergence time (Myr) ± SD	95% confidence interval	A priori limitation
Lagurus/Eolagurus		3.3 ± 0.3	3.0–4.0	>3.0
Lagurini/Ellobiusini		4.3 ± 0.6	3.2–5.7	
E. talpinus/E. tancrei		1.0 ± 0.6	0.1–2.5	
Lagurini + Ellobiusini/Arvicolini		4.7 ± 0.7	3.6–6.2	
Chionomys/Microtus		3.0 ± 0.3	2.3–3.4	
Arvicola/Microtus + Chionomys		3.3 ± 0.1	3.0–3.5	<3.5
Microtus s. str/Terricola		1.4 ± 0.5	0.5–2.3	>3.0
Alexandromys/Microtus		1.9 ± 0.5	1.0–2.8	
Stenocranius/Lasiopodomys		1.8 ± 0.4	1.2–2.7	>1.1
Stenocranius + Lasiopodomys/Microtus		2.4 ± 0.4	1.5–3.2	
Clethrionomys/Craseomys		3.2 ± 0.5	2.6–4.4	>2.6
Clethrionomyini/Lagurini + Ellobiusini + Arvicolini		5.8 ± 0.8	4.6–7.6	
Basal radiation Arvicolinae	Dicrostonyx/...	6.5 ± 0.9	4.9–8.6	
	Prometheomys/...	6.8 ± 1.0	5.2–9.0	
	Lemmini/...	7.2 ± 1.0	5.4–9.5	>4.0
	Ondatra/...	7.7 ± 1.1	5.9–10.3	>4.1
Arvicolinae/Cricetinae		18.1 ± 2.7	13.7–24.2	
Neotominae/Arvicolinae + Cricetinae		18.5 ± 2.8	13.9–24.8	

As in earlier reports [8–10], the monophyly of the subfamily and of all major tribes (Lemmini, Clethrionomyini, Lagurini, Ellobuisini, and Arvicolini) has been confirmed. Note that our study was the first to comprehensively analyze the Lagurini tribe (the genus *Eolaurus* was studied for the first time). Unlike the *cyt b* analysis, which failed to reveal monophyly of the tribe Arvicolini [8, 10] because of the uncertain position of the genus *Arvicola*, the data on the GHR and LCAT nuclear genes taken separately or together reliably confirmed the tribe monophyly irrespective of the method used and testified to the basic position of *Arvicola* in the tribe. The results of combined analysis of original data on both genes using various phylogenetic reconstructions (figure) indicate that *Lagurus* + *Eolagurus* and *Ellobius* constitute the same group as the members of the Arvicolini tribe (*Arvicola, Chionomys*, and *Microtus*). The supports of this clade varied from moderate to high in different analyses. A high support was obtained for the monophyletic group Lagurini (*Lagurus* + *Eolagurus*). The tribe Clethrionomyini (the second radiation) proved to be sister of the clade Ellobiusini/Lagurini/Arvicolini with a high support. The trichotomy Ellobiusini/Lagurini/Arvicolini, which in the given scheme represented the third step of vole radiation, remained unsolved. The earlier divergence (the first radiation: Ondatrini, Prometheomyini, Dicrostonychini, and Lemmini) also remains obscure so far. The divergence time of the clades can be seen in the table. The divergence time of mole lemmings (Ellobiusini), Lagurini, and Arvicolini, and of the genera within these tribes were in most cases older than the first paleontological findings of these groups.

The new data on close sister relationships between mole lemmings (viewed as the most primitive), gray voles, and yellow steppe lemmings were unexpected and contradictory to the traditional concepts. Mole lemmings proved to be more related to Arvicolini than Clethrionomyini, as it was first demonstrated on the basis of the 12s rRNA gene sequence [13]. Similar results were obtained with some trees by taxon analysis on the basis of the *cyt b* gene sequence [10]. However, the unstable *Arvicola* position and the clearly controversial position of *Prometheomys* [10] as determined by studying the nuclear GHR gene [9] leave these results in doubt.

Recall that, in [3], mole lemmings (Ellobiusini) were excluded from voles and assumed to originate independently from Miocene vole-toothed hamsters, whereas later researchers believed that the mole lemming divergence was the earliest event in the evolution of the modern voles. The assumption that mole lemmings were too ancient and independent of other voles [3, 4, 11] is based on an extremely simplified structure of their molar teeth, the presence of tooth roots, specific structure of the skull and postcranial skeleton. However, many of these traits may have developed due to specialized adaptation to underground way of life,

though they were inconclusive with respect to phylogenetic information. Fossil mole lemmings proved to have specific features, such as retaining some primitive tooth traits inherent in the so-called *Mimomys* voles of Pliocene. This group included the genera *Promimomys, Mimomys, Cromeromys*, and *Borsodia* (earlier, *Villanyia*) [14]. The *Miomys* traits in the most ancient mole lemmings suggest their origin from Early Pliocene ancestors shared by other *Miomys* voles. Our molecular data provide convincing proof for the existence of a group that includes the modern Lagurini, Arvicolini, and Ellobiusini.

The following major steps of diversification of this group were found on the basis of new molecular data.

The first radiation. Our results suggest that basal subfamily radiation occurred in the late Miocene, which is in accordance with paleontological data indicating that the most primitive undoubted members of the group appeared about 7.0 million years ago in Pontic deposits of eastern Europe [15].

The second radiation. This stage corresponds to segregation of the modern Clethrionomyini, which occurred in the late Miocene to early Pliocene, as determined from molecular data. Our results indicate that Clethrionomyini diverged from the common vole stem after the first (basal) stage of radiation but before radiation of Microtini–Lagurini–mole lemmings. Distribution of the modern members and paleontological data suggest that the group originated from an eastern Asian center, low and mountain forests being their initial habitat.

The third radiation included the Lagurini/Ellobiusini/Arvicolini divergence, which was assumed to occur in early Pliocene. Hence, the Palearctic voles of Promiomys organization represented a common ancestor of this group. Lagurini and Arvicolini followed the main line of subfamily evolution; their adaptation to feeding on the vegetative parts of herbaceous plant improved with their distribution over the meadow and steppe landscapes (Arvicolini and Lagurini, respectively). Ellobiusini demonstrated an example of rapid evolutionary and adaptive transformations caused by another, underground way of life; in particular, these changes included simplification of the root tooth structure and changes in the skull and limbs. Rapid adaptive evolution accounted for the difficulties in dwetermining the mole lemming phylogenetic position from the classical morphological data.

ACKNOWLEDGMENTS

We are grateful to A.V. Smorkacheva for the material on yellow steppe lemmings, to A.Yu. Kostygov for sequencing and invaluable comment on the study; T.V. Petrova, S.Yu. Bodrov, and E.N. Rodchenkova for technical assistance in the laboratory studies.

This study was supported by the Russian Foundation for Basic Research (project nos. 06-04-49294; 08-04-00029; 09-04-01330-a, 06-05-4049-a) and by the Programs of Basic Research of the Russian Academy of Sciences "Biodiversity and Gene Pool Dynamics of Plants, Animals, and Humans" and "The Biosphere Origin and Evolution."

REFERENCES

1. Hooper, E. and Hart, B., *A Synopsis of Recent North American Microtinae Rodents*, Michigan: Miscellaneous Publications, Museum of Zoology, Univ. Michigan, 1962, vol. 120, pp. 1–68.
2. Kretzoi, M., *Vert. Hung. Mus. Hist.-Nat. Hung*, 1969, vol. 11, nos. 1–2, pp. 155–192.
3. Gromov, I.M. and Polyakov, I.Ya., in *Fauna SSSR*, vol. 3: *Mlekopitayushchie* (The Fauna of the Soviet Union, vol. 3: Mammals), Leningrad, 1977, vol. 3, issue 8.
4. Agadzhanyan, A.K. and Yatsenko, V.N., in *Problemy izmenchivosti i filogenii mlekopitayushchikh* (Problems of Variability and Phylogeny of Mammals), Moscow: Mosk. Gos. Univ., 1984, pp. 135–190.
5. Modi, W.S., *Syst. Zool.*, 1987, vol. 36, pp. 109–136.
6. Modi, W.S., *Mol. Biol. Evol.,* 1996, vol. 13, no. 5, pp. 633–641.
7. Mezhzherin, S.V., Morozov-Leonov, S.Yu., and Kuznetsova, I.A., *Genetika,* 1995, vol. 31, no. 6, pp. 788–797.
8. Conroy, C.J. and Cook, J.A., *J. Mammal. Evol.*, 1999, vol. 6, pp. 221–245.
9. Galewski, T., Tilak, M., Sanchez, S., et al., *BMC Evol. Biol.*, 2006, vol. 6, pp. 1–17.
10. Buzan, E.V., Krystufek, B., Hanfling, B., and Hutchinson, W.F., *Biol. J. Linn. Soc.*, 2008, vol. 94, pp. 825–835.
11. Musser, G.G. and Carleton, M.D., in *Mammal Species of the World: A Taxonomic and Geographic Reference*, Baltimore: The Johns Hopkins Univ. Press, 2005, vol. 2, pp. 894–1531.
12. Jaarola, M., Martinkova, N., Gunduz, I., et al., *Mol. Phylogenet. Evol.,* 2004, vol. 33, pp. 647–663.
13. Lebedev, V.S., Pavlova, N.K., and Surov, A.V., in *Problemy filogenii, sistematiki i paleontologii melkikh mlekopitayushchikh* (Problems of Phylogeny, Taxonomy, and Paleontology of Small Mammals), St. Petersburg, 2003, p. 114.
14. Zazhigin, V.S., *Gryzuny pozdnego pliotsena i antropogena yuga Zapadnoi Sibiri* (Rodents of the Late Pliocene and Anthropogen of Southern West Siberia), Moscow: Nauka, 1980.
15. Nesin, V.A., *Vestn. Zool.*, 1996, vol. 3, pp. 74–75.