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Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences

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Abstract

Voles of the genus *Microtus* represent one of the most speciose mammalian genera in the Holarctic. We established a molecular phylogeny for *Microtus* to resolve contentious issues of systematic relationships and evolutionary history in this genus. A total of 81 specimens representing ten *Microtus* species endemic to Europe as well as eight Eurasian, six Asian and one Holarctic species were sequenced for the entire cytochrome *b* gene (1140 bp). A further 25 sequences were retrieved from GenBank, providing data on an additional 23, mainly Nearctic, *Microtus* species. Phylogenetic analysis of these 48 species generated four well-supported monophyletic lineages. The genus *Chionomys*, snow voles, formed a distinct and well-supported lineage separate from the genus *Microtus*. The subgenus *Microtus* formed the strongest supported lineage with two sublineages displaying a close relationship between the *arvalis* species group (common voles) and the *socialis* species group (social voles). Monophyly of the Palearctic pitymyid voles, subgenus *Terricola*, was supported, and this subgenus was also subdivided into two monophyletic species groups. Together, these groupings clarify long-standing taxonomic uncertainties in *Microtus*. In addition, the "Asian" and the Nearctic lineages reported previously were identified although the latter group was not supported. However, relationships among the main *Microtus* branches were not resolved, suggesting a rapid and potentially simultaneous radiation of a widespread ancestor early in the history of the genus. This and subsequent radiations discernible in the cytochrome *b* phylogeny, show the considerable potential of *Microtus* for analysis of

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historical and ecological determinants of speciation in small mammals. It is evident that speciation is an ongoing process in the genus and that the molecular data provides a vital insight into current species limits as well as cladogenic events of the past.

Keywords: Voles; *Microtus*; Arvicolinae; Mitochondrial DNA; Cytochrome *b*; Holarctic; Phylogeny; Speciation

1. Introduction

Voles of the genus *Microtus* Schrank (1798) are ecologically diverse and constitute the dominant herbivorous small mammals in many habitats in the Northern Hemisphere. Most species prefer open grasslands such as meadows and pastures but some species also occupy forests and highland habitats (Getz, 1985; Hoffmann and Koeppl, 1985; Mitchell-Jones et al., 1999; Nowak, 1999). *Microtus* represents one of the most speciose mammalian genera in the Holarctic, accounting for nearly 50 percent of the species of Arvicoline rodents (voles and lemmings) (e.g., Musser and Carleton, 1993). The genus represents one of the best known cases of rapid mammalian radiation resulting in about 65 extant species distributed throughout the Palearctic and Nearctic regions (Musser and Carleton, 1993; Chaline et al., 1999; Nowak, 1999). The shrew genus *Sorex* (Soricidae, Insectivora) is the only other mammalian genus that displays a comparable diversity across the Holarctic region, but *Sorex* is much older than *Microtus* (see Fumagalli et al., 1999).

The genus *Microtus* is apparently derived from the fossil genus *Allophaiomys*, which itself appears to have descended from *Mimomys* (Chaline and Graf, 1988; Conroy and Cook, 1999; Garapich and Nadachowski, 1996). Palaeontological data suggest that *Allophaiomys* radiated independently in northern Eurasia, central Asia-Himalayas and North America (Brunet-Lecomte and Chaline, 1991; Chaline et al., 1999). Until recently, the appearance of *Allophaiomys* was dated to approximately 2 million years ago (Mya) (Chaline and Graf, 1988), but a new finding of *Allophaiomys* from China traces the origin of the lineage back to 2.3–2.4 Mya (Zheng and Zhang, 2000). The majority of extant *Microtus* species, however, do not appear in the fossil record until Middle Pleistocene about 0.7–0.5 Mya (Chaline et al., 1999; Rabeder, 1986; Richmond, 1996) and it has even been suggested that some species trace their origin to the last glaciation (e.g., Brunet-Lecomte and Chaline, 1990; Chaline and Graf, 1988).

The genus *Microtus* displays a number of features that makes it ideal for evolutionary studies of speciation and the role of Quaternary glacial cycles on diversification. However, the phylogenetic relationships within *Microtus* and its closest relatives are uncertain and difficulties remain both in delimiting species and defining subgenera (e.g., Musser and Carleton, 1993; Nadachowski and Zagorodnyuk, 1996; Zagorodnyuk, 1990).

Current species' boundaries and phylogenetic relationships in *Microtus* rely mainly on morphology and karyotypes but these taxonomic characters have not been sufficient for solving all systematic questions in *Microtus*. The treatment of this genus is consequently characterized by inconsistency and lack of consensus (Musser and Carleton, 1993). For example, studies of dental and skull characters in *Microtus* demonstrate great intra-specific variability, a high incidence of adaptive convergence and many pairs of sibling species (Chaline et al., 1999; Chaline and Graf, 1988; Nadachowski and Zagorodnyuk, 1996; Zakrzewski, 1985). Karyotype evolution on the other hand appears largely uncoupled from morphological evolution (Baskevich, 1996; Suchentrunk et al., 1998). The *Microtus* karyotype varies between $2n = 17$–62 (Zagorodnyuk, 1990; Zima and Král, 1984) and exhibits one of the highest rates of karyotypic change in mammals (Maryama and Imai, 1981; Modi, 1987). Although some phylogenetic relationships can be deduced, especially from G-banded karyotypes (Mazurok et al., 2001; Meier et al., 1985, 1996; Modi, 1987; Orlov et al., 1983; Zagorodnyuk, 1990), the overall picture is that of extensive karyotypic variation among closely related species with no apparent phylogenetic trends (e.g., Akhverdyan et al., 1999; Chaline et al., 1999; Macholán et al., 2001; Modi, 1987). The systematic uncertainties in *Microtus* are perhaps best illustrated by the fact that the number of recognized species varies widely in different accounts (e.g., Gromov and Polyakov, 1992; Musser and Carleton, 1993; Nowak, 1999; Panteleyev, 1998; Zagorodnyuk, 1990) and that several new species have been proposed over recent years (Golenishchev et al., 2003; Kefelioğlu and Kryštufek, 1999; Kryštufek and Kefelioğlu, 2001; Yigit and Colak, 2002). Other unresolved issues concern the delineation and validity of higher-order relationships and species groups such as *Chionomys*, *Volemys*, *Lasiopodomys*, *Blanfordimys*, and *Terricola* that are alternately given either generic or subgeneric rank (e.g., Gromov and Polyakov, 1992; Musser and Carleton, 1993; Nowak, 1999).

Attempts to reconstruct the *Microtus* phylogeny using molecular approaches have been made using allozymes (e.g., Chaline and Graf, 1988; Gill et al., 1987; Graf, 1982; Mezhzherin et al., 1993, 1995; Suchentrunk et al., 1998), restriction enzyme analysis of mitochondrial DNA (DeBry, 1992) and RAPD analysis (Potapov et al., 1999) based on a limited number of *Microtus* species. However, the most comprehensive molecular

phylogenetic studies to date have been carried out by Conroy and Cook (1999, 2000a) and Conroy et al. (2001) using mitochondrial cytochrome *b* gene sequences. These studies comprised the North American endemics as well as some Asian and Eurasian *Microtus* species. In addition, Mazurok et al. (2001) analyzed cytochrome *b* sequences of four species of the *M. arvalis* group and Haring et al. (2001) inferred a phylogeny for species of the *M. multiplex* species group by analysis of mitochondrial D-loop sequences. Phylogeographic surveys of North American and Eurasian *Microtus* have demonstrated extensive cytochrome *b* variation within species (Brunhoff et al., 2003; submitted; Conroy and Cook, 2000b; Haynes et al., 2003; Jaarola and Searle, 2002, 2004). The results even suggest the existence of hitherto unidentified, cryptic species in *Microtus* (Jaarola and Searle, 2002, 2004; Hellborg, 2004), thereby pointing out the importance of within-species sampling for phylogeny reconstruction in *Microtus*.

The aim of this study is to further the understanding of phylogenetic relationships and evolutionary history in *Microtus* voles. We establish a molecular phylogeny for Palearctic *Microtus* including all the currently recognized European species (Mitchell-Jones et al., 1999) except *M. bavaricus*, as well as eight Eurasian and six Asian species. For this purpose we use DNA sequence analysis of the entire cytochrome *b* gene (1140 bp) since it evolves rapidly over the expected divergence times and because it enables us to incorporate previously published data on Nearctic species (Conroy and Cook, 1999, 2000a; Conroy et al., 2001). The combined phylogeny includes 48 out of 65 species of *Microtus*, with multiple representatives of many of the species. This near-comprehensive phylogeny of the genus allows us not only to resolve long-standing taxonomic controversies in *Microtus* but also to provide a phylogenetic tool to help understand speciation and species' radiations in small mammals.

2. Materials and methods

2.1. Samples

A total of 81 specimens representing 25 *Microtus* species were analyzed for variation in the mitochondrial cytochrome *b* gene (Table 1). All 25 species except for the Holarctic *M. oeconomus* are Palearctic; 10 are endemic to Europe, eight occur in Eurasia and six are restricted to Asia (cf. Gromov and Polyakov, 1992; Mitchell-Jones et al., 1999; Musser and Carleton, 1993). The only European species not analyzed is *M. bavaricus* that was considered extinct (Mitchell-Jones et al., 1999), but recently rediscovered in the northern Tyrol (Hutterer, 2001). The samples contain two species from the genus *Chionomys*, until recently often classified as *Microtus*, as well as six subgenera of *Microtus* (Table 1). Up to five individuals per taxon were surveyed to account for intra-specific variation. When possible, conspecific individuals were chosen from geographically distant localities. A total of 17 sequences representing *M. agrestis*, *M. oeconomus*, and *M. arvalis* have been published previously (Brunhoff et al., 2003; Haynes et al., 2003; Jaarola and Searle, 2002); their GenBank accession numbers are given in Table 1. In addition, 24 sequences representing 19 North American endemics and five Asian/Palearctic *Microtus* species (including one species that we sequenced, *M. gregalis*) were retrieved from GenBank (Conroy and Cook, 1999, 2000a; Conroy et al., 2001); for accession numbers see Table 2. A second *M.* (*Volemys*) *kikuchii* sequence was obtained from the whole mtDNA sequence in GenBank (AF348082, Lin et al., 2002).

2.2. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from frozen or ethanol preserved tail tips, ears, kidneys or liver. A few samples consisted of skulls collected from owl and raptor pellets. The Qiagen Dneasy Tissue kit was used for all types of samples. Pure mtDNA was isolated from two *M. rossiaemeridionalis* samples according to Jaarola and Tegelström (1995).

The primers used in this study are given in Table 3. For most specimens, the complete mitochondrial cytochrome *b* gene (1140 bp) was amplified in a single PCR reaction using the L14727-SP and H-15195-SP/H-ISO-SP primers complementary to glutamate and threonine/proline tRNA sequences, respectively. Alternatively, two to four separate amplifications that produced overlapping fragments were carried out. Due to the presence of nuclear copies of the cytochrome *b* gene in several *Microtus* species (DeWoody et al., 1999; Jaarola and Searle, 2004; Jaarola et al., in prep.), the majority of amplifications involved only *Microtus*- or species-specific primers designed by us (Table 3).

PCR amplification was carried out using Ampli*Taq* Gold DNA polymerase (Applied Biosystems). The PCR protocol for tissue samples consisted of an initial 7 min denaturation step at 95 °C, 30–35 cycles of denaturation at 94 °C for 1 min, annealing at 49 or 50 °C for 1 min and extension at 72 °C for 1–2 min, and a final 10-min extension step at 72 °C. PCR products were purified using the Qiagen QIAquick kit. The PCR protocol for skull samples is described in Jaarola and Searle (2004).

We sequenced each DNA fragment in both directions using a combination of PCR primers and internal primers (Table 3). Cycle sequencing reactions were carried out using the BigDye Terminator cycle sequencing kit (Applied Biosystems). Amplifications and sequencing reactions were performed in a PTC-200 thermal cycler (MJ Research). Sequencing products were purified using

Table 1
Microtus species, specimens, locations, and GenBank accession numbers of cytochrome *b* sequences

Subgenus (species group)	Species	Common name	No.	Location	Country	Accession Nos.
Agricola (*agrestis*)	*Microtus agrestis*	Field vole	1	Novosibirsk	Russia	AY167149
			2	Bonn	Germany	AY167210
			3	Sion, Valais	Switzerland	AY167160
			4	Pyrenees	Spain	AY167187
Microtus (*arvalis*)	*M. arvalis arvalis*	Common vole	1	Mantet, Pyrenees	Spain	AY220789
			2	Trento	Italy	AY220766
			3	Nuijamaa	Finland	AY220770
			4	Lauwersee	Netherlands	AY220778
Microtus (*arvalis*)	*M. arvalis obscurus*	(Altai vole)	1	Crimea	Ukraine	AY220762
			2	Kavka River, Serov	Russia	AY220764
			3	Neiva River	Russia	AY220765
			4	Sisian	Armenia	AY220761
			5	Ninotsminda	Georgia	AY220760
Agricola (*agrestis*)	*M. cabrerae*	Cabrera's vole	1	Alandron	Portugal	AY513788
			2	Idanha-a-Velha	Portugal	AY513789
Terricola (*subterraneus/majori*)	*M. daghestanicus*	Daghestan pine vole	1	Beniani	Georgia	AY513790
			2	Bağdaşan	Turkey	AY513791
			3	Handere	Turkey	AY513792
Microtus (*socialis*)	*M. dogramacii*		1	Ortaköy-Aksaray	Turkey	AY513793
			2	Amasya	Turkey	AY513794
			3	Boyalı Köyü-Amasya*	Turkey	AY513795
Terricola (*duodecimcostatus*)	*M. duodecimcostatus*	Mediterranean pine vole	1	Setùbal	Portugal	AY513796
			2	Algarve	Portugal	AY513797
Terricola (*savii*)	*M. felteni*	Balkan pine vole		Mt. Pelister, Begova Češma	Macedonia	AY513798
Terricola (*savii*)	*M. gerbei*	Pyrenean pine vole	1	Arrós, Vall d'Aran	Spain	AY513799
			2	Riba	Spain	AY513800
			3	Hecho	Spain	AY513801
			4	Hecho	Spain	AY513802
Stenocranius	*M. gregalis*	Narrow-headed vole	1	Yamal Peninsula	Russia	AY513803
Microtus (*socialis*)	*M. guentheri*	Guenther's vole	1	Gravia	Greece	AY513804
			2	Aqrabat	Syria	AY513805
			3	Locality unknown	Israel	AY513806
			4	Locality unknown	Israel	AY513807
Neodon	*M. juldaschi*	Juniper vole		Mazarsay	Kyrgyzstan	AY513808
Microtus (*arvalis*)	*M. kirgisorum*	Tien Shan vole	1	Balkash	Kyrgyzstan	AY513809
			2	Balkash	Kyrgyzstan	AY513810
Terricola (*multiplex*)	*M. liechtensteini*	Liechtenstein's pine vole		Anhovo	Slovenia	AY513811
Terricola (*duodecimcostatus*)	*M. lusitanicus*	Lusitanian pine vole	1	Burgos	Spain	AY513812
			2	Melgar de Fernamental	Spain	AY513813
Terricola (*subterraneus/majori*)	*M. majori*	Major's pine vole		Damar	Turkey	AY513814
Terricola (*multiplex*)	*M. multiplex*	Alpine pine vole	1	Staffarda, Piedmont	Italy	AY513815
			2	Trento	Italy	AY513816
			3	Lillaz	Italy	AY513817
			4	Méribel	France	AY513818
Pallasiinus (*oeconomus*)	*M. oeconomus*	Root vole (Tundra vole)	1	Ivvavik Nat. Park	Canada	AY220028
			2	Krasnoyarsk	Russia	AY220018
			3	Hamningberg	Norway	AY219988
			4	Texel	Netherlands	AY220006
Microtus (*arvalis*)	*M. rossiaemeridionalis*	Sibling vole	1	Kauhava	Finland	AY513819
			2	Svalbard	Norway	AY513820
			3	Gerede, Istanbul	Turkey	AY513821

Table 1 (*continued*)

Subgenus (species group)	Species	Common name	No.	Location	Country	Accession Nos.
			4	Erciyes Mt., Kayseri	Turkey	AY513822
			5	Kangal-Sivas	Turkey	AY513823
Terricola (*savii*)	*M. savii*	Savi's pine vole	1	Viterbo	Italy	AY513824
			2	Torino, Piedmont	Italy	AY513825
			3	Cerano, Piedmont	Italy	AY513826
			4	Fiume Freddo	Italy	AY513827
			5	Fiume Freddo	Italy	AY513828
Microtus (*socialis*)	*M. socialis*	Social vole	1	Iori River valley	Georgia	AY513829
			2	Iori River valley	Georgia	AY513830
			3	Reine	Iran	AY513831
Terricola (*subterraneus/majori*)	*M. subterraneus*	Common pine vole	1	Seli	Greece	AY513832
			2	Glocknerhaus	Austria	AY513833
			3	Çiğlikara	Turkey	AY513834
			4	Çiğlikara	Turkey	AY513835
			5	Güzyurdu	Turkey	AY513836
Terricola (*multiplex*)	*M. tatricus*	Tatra vole	1	Tretie Roháčske pleso lake	Slovakia	AY513837
			2	Smutná dolina valley	Slovakia	AY513838
			3	Veĺká studená dolina valley*	Slovakia	AY513839
Terricola (*duodecimcostatus*)	*M. thomasi*	Thomas's vole	1	Agios Stefanos	Greece	AY513840
			2	Ano Kastritsi	Greece	AY513841
			3	Kyparissia	Greece	AY513842
			4	Itea	Greece	AY513843
			5	Trebinje, Herzegovina	Bosnia	AY513844
	Chionomys nivalis	Snow vole	1	Trento	Italy	AY513845
			2	Trento	Italy	AY513846
			3	Prvé Roháčske pleso lake	Slovakia	AY513847
			4	Queralbs, Girona	Spain	AY513848
			5	Saleh, As Suwayda	Syria	AY513849
	C. roberti	Robert's vole	1	Altındere Vadisi	Turkey	AY513850
			2	Datvisi	Georgia	AY513851

Subgenus and species group designation follows Musser and Carleton (1993) whose classification is largely based on the reclassification of Zagorodnyuk (1990).

* Type localities.

standard protocols and run in an ABI 310 or 3100 automated DNA sequencer (Applied Biosystems).

2.3. Phylogenetic analysis

Sequences were aligned and ambiguous bases resolved by eye using Sequencher v. 3.1.1 (Gene Codes Corp.). Nucleotide and amino acid composition was analyzed using MacClade v. 4.05 (Maddison and Maddison, 2000). Frequencies of transitions and transversions were estimated from maximum parsimony trees using MacClade.

The phylogenetic relationships among haplotypes were reconstructed using neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) algorithms implemented in PAUP* v. 4.0b10 (Swofford, 2002) as well as the Bayesian approach (Huelsenbeck et al., 2001) using the program MrBayes 3 (Ronquist and Huelsenbeck, 2003). The parsimony analyses were carried out ten times with the heuristic search approach using the TBR swapping algorithm, steepest descent option, random addition and 100–1000 replicates. Strict and 50% majority consensus trees were constructed from multiple equally parsimonious MP trees. The hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) implemented in the computer program MODELTEST v. 3.06 (Posada and Crandall, 1998), were used to identify the most appropriate model of DNA substitution for our data. The model selected was the general time reversible model, GTR (Yang, 1994) with a gamma distributed shape parameter (α) of 0.8722 and the proportion of invariable sites (I) equaling 0.5320. This model, with parameters determined by MODELTEST, as well as several simpler substitution models, was implemented in the NJ and ML analyses. The ML tree search was conducted as described for MP but with the "as is" addition replicate (i.e. alphabetically by species). Relative stability of NJ and MP trees was assessed with bootstrap analysis using 10 000 and 1000 replicates, respectively. Bootstrapping of the ML tree could not be carried out due to the excessive computer capacity required.

Table 2
Microtus cytochrome *b* sequences retrieved from GenBank (Conroy and Cook, 1999, 2000a; Conroy et al., 2001; Lin et al., 2002)

Species	Accession Nos.
Microtus abbreviatus	AF163890
M. californicus	AF163891
M. canicaudus	AF163892
M. chrotorrhinus	AF163893
M. fortis	AF163894
M. gregalis	AF163895
M. guatemalensis	AF410262
M. kikuchii	AF163896
M. kikuchii	AF348082
M. longicaudus	AF187230
M. mexicanus	AF163897
M. middendorffi	AF163898
M. miurus	AF163899
M. montanus	AF119280
M. montebelli	AF163900
M. oaxacensis	AF410260
M. ochrogaster	AF163901
M. oregoni	AF163903
M. pennsylvanicus	AF119279
M. pinetorum	AF163904
M. quasiater	AF410259
M. richardsoni	AF163905
M. townsendii	AF163906
M. umbrosus	AF410261
M. xanthognathus	AF163907

We conducted Bayesian phylogenetic analyses using the GTR + I + G model with unequal base frequencies. Model parameters were estimated as part of the analysis. Altogether four independent runs, each with four Markov chain Monte Carlo (MCMC), were performed. All Bayesian analyses were initiated with random starting trees and run for two million generations. Trees were sampled every 10 generations. Log-likelihood scores of trees were plotted against generation time to determine the "burn-in" period and ensure that equilibrium log-likelihood values for different runs approached similar mean values (Huelsenbeck et al., 2002; Ronquist and Huelsenbeck, 2003). After discarding burn-in trees, we generated 50% majority rule consensus trees in PAUP for each single run and compared posterior probabilities for convergence among runs.

Since the correlation between and significance of tree support values as estimated by standard, nonparametric bootstrap values as opposed to posterior probabilities is not well understood and intensely debated (e.g., Huelsenbeck et al., 2002; Suzuki et al., 2002; Erixon et al., 2003), we follow the conservative approach of Leaché and Reeder (2002). Thus, only bootstrap values of $\geqslant 70\%$ (corresponding to 95% CI) and posterior probabilities of $\geqslant 95\%$ were considered significant.

Table 3
Primers used for PCR amplification and sequencing of the cytochrome *b* gene in *Microtus*

Primer	Sequence (5′–3′)	Reference
L14724B	CGAGATCTGAAAAACCATCGTTG	Kocher et al. (1989)
L14727-SP	GACAGGAAAAATCATCGTTG	Jaarola and Searle (2002)
L14841M	CCATCAAATATTTCATCATGATGAAA	Jaarola and Searle (2002)
L15162M2	GCTACGTACTTCCATGAGGACAAATATC	Jaarola and Searle (2002)
L15162Marv	G(CT)TACGT(CT)CTTCCATGAGGCCAAATATC	Haynes et al. (2003)
L15162MO	CTTCCATGAGGCCAAATATC	Brunhoff et al. (2003)
L15408M	GCAGACAAAATCCCGTTCCA	Jaarola and Searle (2002)
L15408Marv	GCAGACAAAATCCCATTCCA	Haynes et al. (2003)
L15408-SP	GCAGACAAA AT(TC)CC(AG)TT(TC)CA	Present study
H15177-SP2	AGGAGGTTTGT(AG)ATGACTG	Present study
H15177-SP3	A(AG)GAGGTTTGT(AG) ATNACTG	Present study
H15177Marv	AAGAGATTTGTAAT(CT)ACTG	Present study
H15177MO	AGGAGGTTTGTGATTACTG	Brunhoff et al. (2003)
H 15319Marv	AAAGGTGGACTAATACGAGG	Haynes et al. (2003)
H15348A-SP	GTTGGA(CT)CCTGTTTCGTG	Jaarola and Searle (2002)
H15408M	TGGAACGGGATTTTGTCTGC	Jaarola and Searle (2002)
H15408MO	TGGAATGGGATTTTGTCTGT	Brunhoff et al. (2003)
H15497-SP	T(AG)TAATT(AG)TCNGGGTCTCC	Present study
H15497-SP2	TGTAATT(AG)TCGGGGTCTCC	Present study
H15549M	AAGAGGAAATACCATTCTGGTTTAA	Jaarola and Searle (2002)
H15576M	GACCGTAAAATGGCGTAGG	Jaarola and Searle (2002)
H15576MO	GATCGTAGGATGGCGTAGG	Brunhoff et al. (2003)
H15915	AACTGCAGTCATCTCCGGTTTACAAGAC	Irwin et al. (1991)
H15915-SP	TTCATTACTGGTTTACAAGAC	Jaarola and Searle (2002)
H-ISO-M	AAGTAGTTTAATTAGAATGTCAG	Haynes et al. (2003)
H-PRO	AAGTAGTTTAATTAGAATATCAG	Brunhoff et al. (2003)
H-ISO-SP	AGTAGTTTAATTAGAATGTCAGC	Jaarola and Searle (2002)

SP: *Microtus* spp.; M: *M. agrestis*; Marv: *M. arvalis*; and MO: *M. oeconomus*.

Various combinations of lemmings (*Dicrostonyx*, *Lemmus*), *Clethrionomys rutilus* and *C. glareolus* and *Arvicola terrestris* were tested as outgroups. Since the position of *Arvicola* proved unstable and often tended to occur within *Microtus*, we discarded this option. The two *Chionomys* species analyzed, *C. nivalis* and *C. roberti*, were also tested but were too closely related to *Microtus* to function as outgroups. Our findings corroborate the conclusion of Conroy and Cook (1999, 2000a) that *Clethrionomys* is a sister taxon to *Microtus*, and we therefore used *C. glareolus* and/or *C. rutilus* as outgroups.

2.4. Tests of sequence saturation and a cytochrome b clock

To diagnose sequence saturation and homoplasy at the third position, we constructed a scatter plot of uncorrected pairwise transitions and transversion frequencies versus corrected pairwise divergences. Sequence divergence was corrected with the Jukes–Cantor (JC, Jukes and Cantor, 1969) model as well as a maximum likelihood model based on the GTR + G + I model of substitution. Total and net divergence (Dxy and Da) between species was estimated according to Nei (1987). We also tested for a molecular clock by comparing log likelihood scores of ML trees constructed with and without a molecular clock constraint (Felsenstein, 1988) in PAUP.

3. Results

GenBank accession numbers for the 64 new 1140 bp cytochrome *b* sequences representing 22 species are given in Table 1 (AY513788–AY513851) together with accession numbers of 17 sequences, representing three additional species, that we have published previously. We are confident that the new sequences represent the mitochondrial cytochrome *b* gene and not nuclear pseudogenes since they closely match previously reported *Microtus* sequences (Brunhoff et al., 2003; Conroy and Cook, 2000a,b; Conroy et al., 2001; Haynes et al., 2003; Jaarola and Searle, 2002) and because they did not display any of the anomalies typical for nuclear copies (cf. Bensasson et al., 2001; Mirol et al., 2000). We did, however, also obtain pseudogenes for the cytochrome *b* gene in a number of samples and taxa despite using only *Microtus*-specific primers. These findings will be reported elsewhere (Jaarola et al., unpublished).

3.1. Sequence composition and variation

The total, aligned data matrix included 100 cytochrome *b* haplotypes derived from 106 sequences representing 48 currently recognized species. A total of 504 (44%) variable sites were observed and 456 of these were informative for the parsimony analyses. More than one type of nucleotide substitution was observed in 199 (17%) sites and 83 (7%) of these displayed all four nucleotides. The majority of polymorphic sites were at third positions (364, 72%), followed by first positions (115, 23%) and second positions (25, 5%). Most substitutions were transitions (78%). The light strand nucleotide composition was characterized by a deficit of guanines (13%) similar to that described in North American *Microtus* species (Conroy and Cook, 2000a,b) as well as other mammals (e.g., Irwin et al., 1991). All but eight of the 86 variable amino acid residues and ten of the 136 amino acid replacements were located within the variable matrix and transmembrane region of cytochrome *b* (cf. Irwin et al., 1991; McClellan and McCracken, 2001).

Inter-specific distances varied between 4.2% and 18.0% using the JC model, whereas maximum likelihood distances estimated under the GTR + G + I model ranged from 4.5% to 51.6%. Corresponding intra-specific distances ranged up to 6.2% and 7.2% (*M. agrestis*). *M. arvalis*, *M. agrestis*, *M. daghestanicus*, *M. guentheri*, *M. oeconomus*, *M. savii*, *M. subterraneus*, and *C. nivalis* showed intra-specific divergences of 4–7%, values similar to net distance estimates between closely related species such as *M. duodecimcostatus–M. lusitanicus*, *M. dogramacii–M. guentheri*, and *M. liechtensteini–M. multiplex*. Some saturation occurred at third position transitions for divergences at the subgenus and genus levels (not shown), similar to that described in other studies of rodent cytochrome *b* (e.g., Yang and Yoder, 1999).

3.2. Phylogenetic results

The four phylogenetic methods used (MP, NJ, ML, and Bayesian) displayed trees with very similar topologies (Figs. 1–3). All methods discriminated two, well-supported major lineages corresponding to the two subgenera *Microtus* and *Terricola*. Each of the two subgenera was further divided into two well-supported sublineages. The two *Microtus* lineages previously described by Conroy and Cook (2000a) and Conroy et al. (2001), the "Asian" and the Nearctic, were also observed although the latter group was not supported. The two species of the genus *Chionomys* formed a highly supported branch outside the genus *Microtus*. Monophyly of the genus *Microtus* was supported although the bootstrap support depended much on the position of *M. gregalis*.

We obtained 48 MP trees (3744 steps, CI = 0.214) when using *C. rutilus* as outgroup, and 156 trees (3792 steps, CI = 0.212) when *C. glareolus* and *C. rutilus* were used as outgroups. The strict consensus tree of the 48 trees is given in Fig. 1. The same tree topology was recovered in the two consensus trees except for the position of *M. gregalis*. The 48 MP trees rooted with *C. rutilus* only

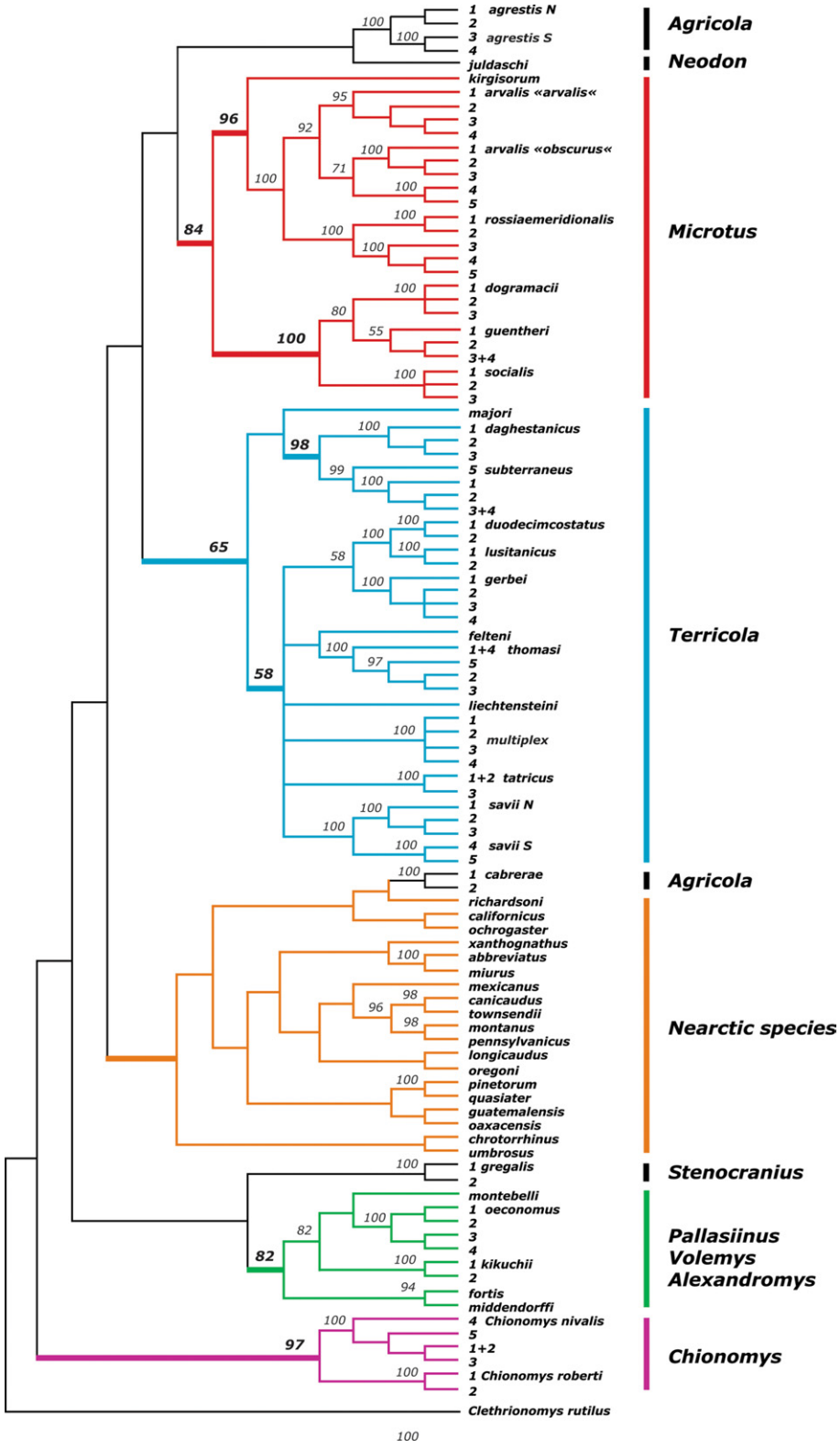

Fig. 1. Strict consensus tree of 48 maximum parsimony (MP) trees from cytochrome *b* haplotypes for 46 *Microtus* and two *Chionomys* species rooted with *Clethrionomys rutilus*. Numbers above branches denote percentage bootstrap resampling support (>50%) from 1000 replications.

differed because of unresolved intra-specific branches of *M. multiplex*, *M. gerbei*, *M. socialis*, and *M. dogramacii*. However, the importance of intra-specific sampling was clearly illustrated by the fact that removal of this intra-specific variation for one or several of these taxa resulted in fewer MP trees but also a decreased resolution of some

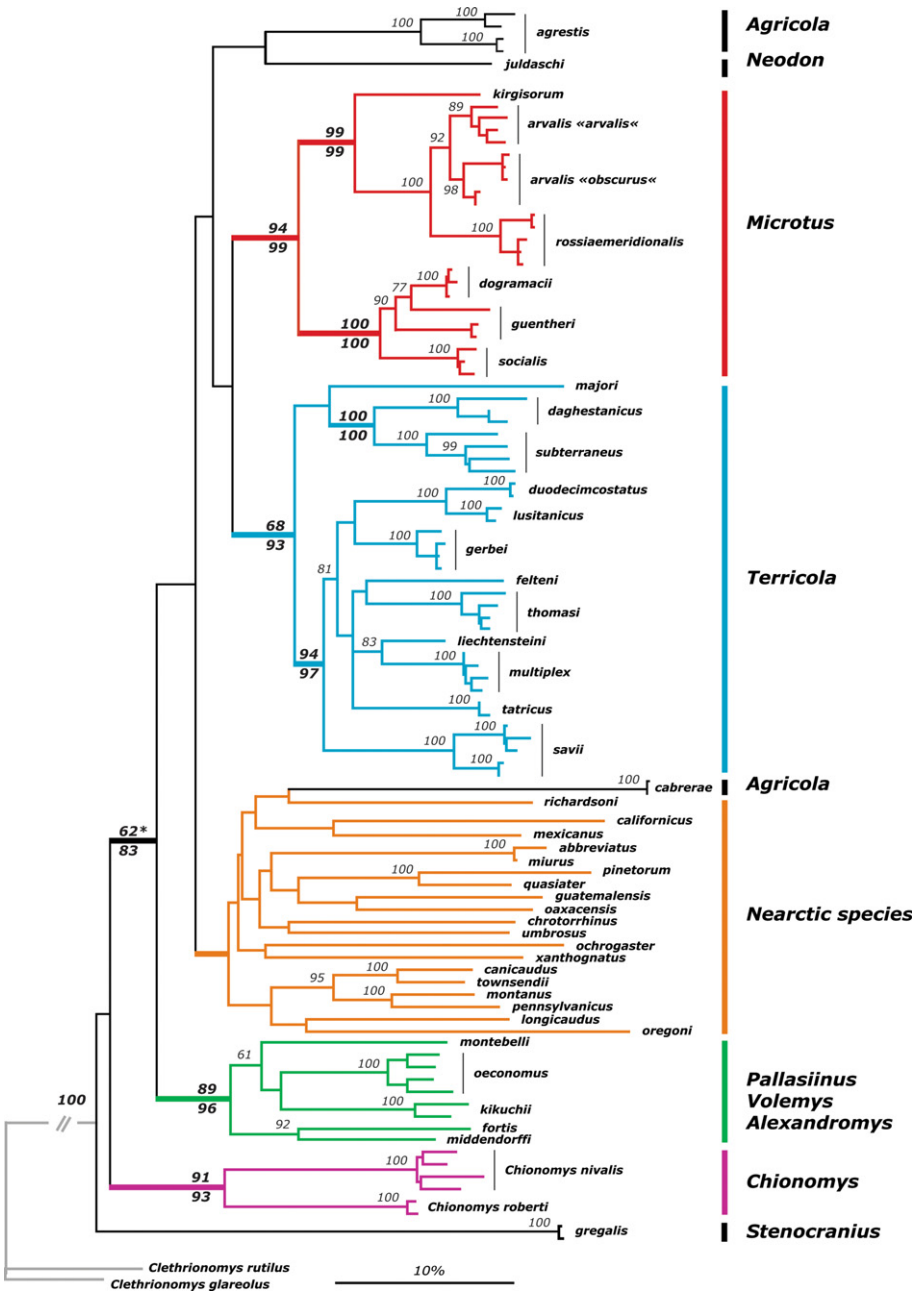

Fig. 2. Maximum likelihood (ML) tree based on GTR + G + I distances and rooted with *Clethrionomys glareolus* and *C. rutilus*. The tree shows the inferred phylogenetic relationships among 100 cytochrome *b* haplotypes representing 46 *Microtus* and two *Chionomys* species. Bootstrap resampling support from 10,000 iterations for a neighbor-joining (NJ) tree based on the JC model is listed above main branches. Estimates below branches show corresponding bootstrap support values when *M. gregalis*, *M. majori* and *M. cabrerae* are removed from the phylogenetic analyses. Only values greater than 50 percent are shown. *Percentage bootstrap support for *Microtus* monophyly in a NJ tree with *M. gregalis* included in *Microtus*. Subgenus designation follows Musser and Carleton (1993) (see Table 1).

of the deeper branches in the tree. The MP trees clearly contained an excessive degree of homoplasy, but all major lineages, except for the Nearctic, as well as many sublineages exhibited high bootstrap values, and relatively few MP trees were generated. Phylogenetic analyses using only first and second positions did not provide enough resolution (data not shown).

The NJ algorithm recovered the same topology independent of substitution model used, except for statistically unsupported differences in the positions of Nearctic species and basal taxa such as *M. agrestis*, *M. cabrerae*, *M. gregalis*, and *M. juldaschi*. The bootstrap values increased with the simplicity of the substitution model, the JC model generating the highest estimates.

The ML tree (Fig. 2) based on the GTR + G + I model showed the same topology as the MP and NJ trees. The ML tree constructed under a molecular clock constraint differed significantly from the unconstrained ML tree

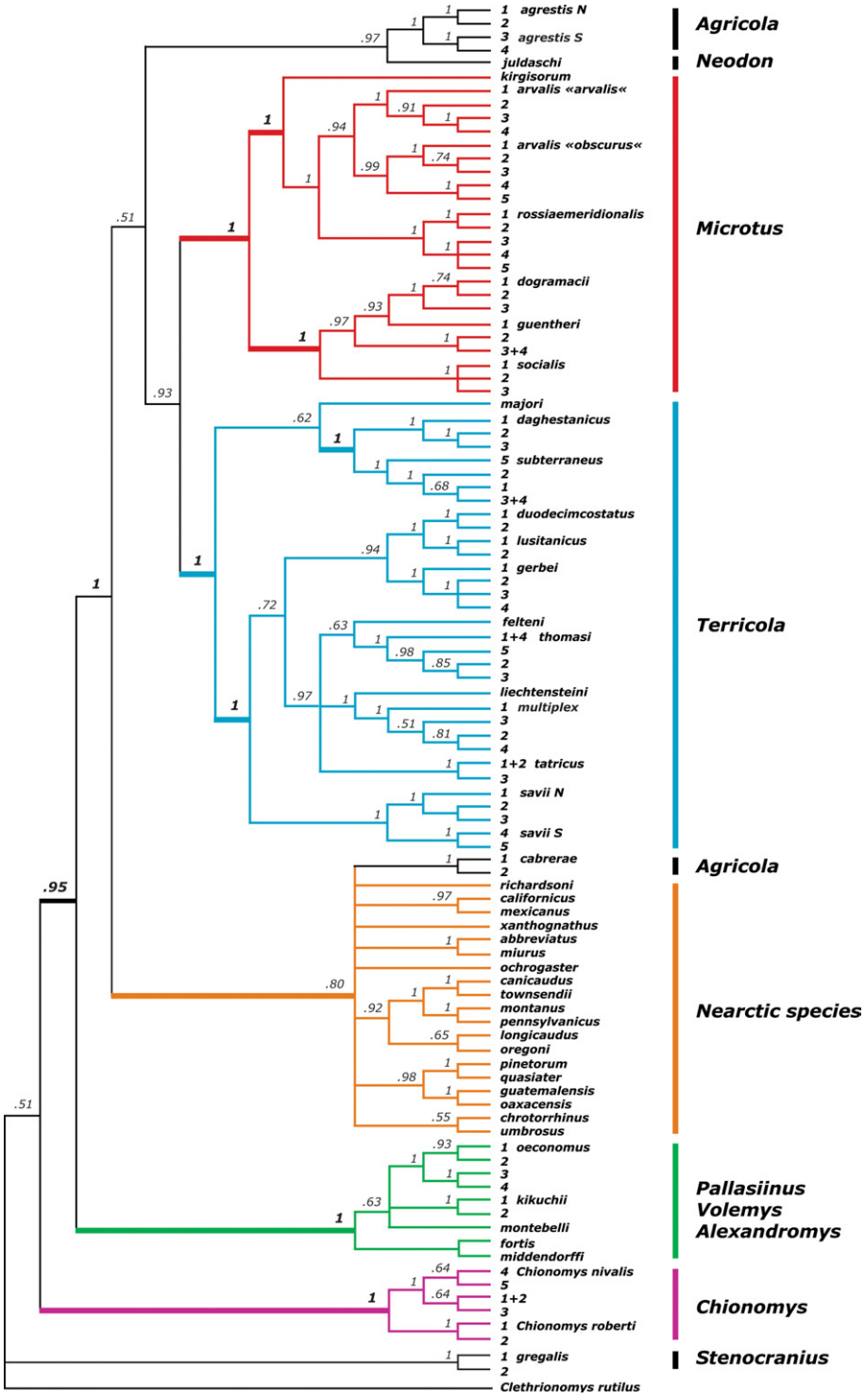

Fig. 3. Fifty percent majority rule consensus tree of 190, 000 trees from a Bayesian analysis of cytochrome *b* haplotypes for 46 *Microtus* and two *Chionomys* species rooted with *Clethrionomys rutilus*. Numbers above branches represent posterior probability values (>0.50).

when outgroups were included ($\chi^2 = 140.4$, df = 100, $P < 0.001$), but the difference was not significant when outgroups were excluded ($\chi^2 = 120.5$, df = 98, $P > 0.05$).

The Bayesian analyses reached stationarity around 100,000 generations (equaling 10,000 saved trees), so that the last 190,000 trees were used to compute a majority rule consensus tree for each run. The four independent analyses converged on similar log-likelihood values and the mean ln L score for the posterior distribution of trees was −17674. Topology of consensus trees, values of posterior probabilities and parameter estimates were highly similar in all four analyses. The

marginal probabilities of the nucleotide frequencies were similar to those obtained with MODELTEST. The average gamma distribution shape parameter (α) was 0.80 and the proportion of invariable sites (I) averaged 0.48. The lineages and sublineages also supported by standard, nonparametric bootstrapping values $\geqslant 70\%$, calculated using MP and/or NJ (Figs. 1 and 2), all showed posterior probabilities of 100% (Fig. 3). In addition, significance levels around 95% were obtained for a few branches not supported by bootstrapping (Fig. 3).

The long branches of *M. agrestis*, *M. juldaschi* and, especially, *M. gregalis* and *M. cabrerae* were basal and only formed non-significant associations that were dependent on the taxa present and the substitution model used. The apparent relationship of the Iberian endemic *M. cabrerae* with the Nearctic species represents an example of this type of clustering (Figs. 1 and 3). The Bayesian analyses supported the grouping of *M. agrestis* with *M. juldaschi* with a significance level of 97% (Fig. 3), but this was not supported by bootstrapping (Figs. 1 and 2). The position of *M. gregalis* was especially unstable. Within the *Terricola* lineage, the position of the single *M. majori* sequence could not be determined. Removal of the unstable taxa *M. gregalis*, *M. cabrerae*, and *M. majori* increased the bootstrap support for most major lineages in the NJ tree so that all except the Nearctic branch generated bootstrap values over 90% as well as high (83%) support for *Microtus* monophyly (Fig. 1).

4. Discussion

4.1. The cytochrome b tree and Microtus taxonomy

Although there are many issues relating to details of particular relationships, overall there is much concordance between the *Microtus* cytochrome *b* tree and current taxonomy based on morphology and karyotype. Four monophyletic lineages with good bootstrap support and 100% posterior probabilities were identified (Figs. 1–3). First, the two species of snow voles, genus *Chionomys*, formed a lineage separate from *Microtus*. Second, species of the subgenus *Microtus* formed a well-supported lineage with two highly supported sublineages representing the *arvalis* species group (common voles) and the *socialis* species group (social voles). Third, monophyly of the Palearctic pitymyid voles, i.e. subgenus *Terricola* was supported. Fourth, the "Asian" lineage previously reported by Conroy and Cook (2000a) and Conroy et al. (2001) was identified. All the Nearctic species clustered as previously described by Conroy and Cook (2000a) and Conroy et al. (2001) but this grouping was not well supported. The following discussion will deal with phylogenetic relationships, biogeographic scenarios and species delimitations in *Microtus*. Data on molecular evolution involving estimation of a molecular clock and datings will be reported elsewhere (Jaarola et al., unpublished). Our data support the analyses of Conroy and Cook (2000b) suggesting rapid cytochrome *b* evolution in *Microtus*.

4.2. Genus Chionomys (snow voles)

Chionomys nivalis and *C. roberti* form a distinct and well-supported clade separate from *Microtus* (Figs. 1–3). Thus, our cytochrome *b* results corroborate the ranking of *Chionomys* as a genus separate from *Microtus*. The genus *Chionomys* was previously included in *Microtus* but recent analyses have suggested separation (reviewed in Musser and Carleton, 1993; Nadachowski, 1991). According to palaeontological data, *Chionomys* represents an early split, 1.3–1.5 Mya, from *Allophaiomys* or, possibly, *Mimomys*, the ancestor of *Allophaiomys* (Horáček, pers. comm.). The distant relationship in cytochrome *b* between *C. nivalis* and *C. roberti* is also in accord with fossil data (cf. Nadachowski, 1991).

4.3. Genus Microtus

The relationship among the main *Microtus* branches was not resolved and the data indicate that Eurasian species are not basal to North American endemics as suggested by Conroy and Cook (2000a). This "hard" polytomy is likely to indicate an early burst of rapid diversification of a widespread ancestor resulting in the nearly simultaneous appearance and radiation of major *Microtus* lineages in Eurasia, central Asia-Himalayas and North America as advocated by palaeontologists (Brunet-Lecomte and Chaline, 1991; Chaline et al., 1999). We cannot, however, entirely dismiss the added effects of a high degree of homoplasy at third positions in the cytochrome *b* gene confounding the resolution of basal relationships (cf. Conroy and Cook, 1999; Reed and Sperling, 1999). Furthermore, some relationships among subgenera were indicated by Bayesian inference (Fig. 3) but not supported by bootstrapping of NJ or MP trees (Figs. 1 and 2).

4.4. Subgenus Microtus

The *Microtus* subgenus represents the strongest supported lineage in the cytochrome *b* tree, displaying a close relationship between the *arvalis* species group (common voles) and the *socialis* species group (social voles) *sensu* Zagorodnyuk (1990). The close phylogenetic relationships among the morphologically cryptic species in the *arvalis* group are concordant with the cytochrome *b* studies of Haynes et al. (2003) and Mazurok et al. (2001) involving members of this group. Mazurok et al. (2001) also included *M. transcaspicus* but since the sequence was not submitted to GenBank, we could not include it in our analyses.

The systematics of social voles has proven much more complex than previously thought (see Kefelioğlu and Kryštufek, 1999; Kryštufek and Kefelioğlu, 2001). Only recently a number of new species have been suggested (Golenishchev et al., 2003; Kefelioğlu and Kryštufek, 1999; Kryštufek and Kefelioğlu, 2001; Yigit and Colak, 2002). Besides the established species, *M. socialis* and *M. guentheri*, we also included sequences of the newly described *M. dogramacii* (Kefelioğlu and Kryštufek, 1999) in our analyses. The cytochrome *b* data demonstrate a recent divergence of *M. dogramacii* ($2n = 48$) from *M. guentheri* ($2n = 54$). The NJ, ML and Bayesian trees indicated a paraphyletic relationship (Figs. 2 and 3) but the MP analyses supported reciprocal monophyly (Fig. 1). However, the situation is more complex in that our *M. guentheri* specimens from Syria and Israel may be more appropriately considered *M. irani* (cf. Kefelioğlu and Kryštufek, 1999; Kryštufek and Kefelioğlu, 2001). The distribution of *M. irani* remains, however, uncertain (Kryštufek and Kefelioğlu, 2001; Mitchell-Jones et al., 1999). Overall, it is clear that a much more detailed molecular investigation of the phylogenetic relationships among social voles is warranted.

4.5. Subgenus Terricola (ground voles)

The cytochrome *b* tree supports Brunet-Lecomte and Chaline (1992), Chaline and Graf (1988), and Zagorodnyuk (1989) in the separation of pitymyine forms into Nearctic (*Pitymys*) and Palearctic (*Terricola*) components (Figs. 1–3). The monophyly of the subgenus *Terricola* was recently questioned by Kryštufek et al. (1996) who claimed that it constituted an artificial group of unrelated convergently evolved species with no shared apomorphies. However, our molecular data demonstrate that *Terricola* species do share a common ancestor. The Bayesian analyses yielded strong supported for *Terricola* (Fig. 3), but the bootstrap support was relatively low (Figs. 1 and 2). Removal of *M. majori* from the analyses, however, increased the bootstrap values drastically for the whole group as well as the two subgroups within *Terricola* (Fig. 2). Analysis of additional *M. majori* sequences might stabilize the group, but the results may also indicate that the Asian endemic *M. majori* represents a separate evolutionary lineage (see below).

The cytochrome *b* data strongly support two monophyletic species groups within *Terricola*: one subgroup with *M. subterraneus* and *M. daghestanicus*, species with ranges extending to Asia Minor, and the other subgroup consisting of the European endemics. The cytochrome *b* phylogeny does not agree fully with the prevailing species groups within *Terricola* (cf. Table 1). However, extensive karyotypic and morphological polymorphism in *Terricola* has spawned many alternative classifications (e.g., Chaline et al., 1999; Kratochvíl and Král, 1974) and our data imply that yet another systematic revision is necessary.

Microtus majori, *M. subterraneus*, and *M. daghestanicus* are believed to have diverged recently (reviewed in Macholán et al., 2001). Our data fully support a sister relationship between *M. subterraneus* and *M. daghestanicus* but the position of *M. majori* remains uncertain (see above)—even in the Bayesian analyses. This result is somewhat surprising since Macholán et al. (2001) found a close allozymic relationship between *M. majori* and *M. subterraneus*. Our results are, however, in accordance with Zagorodnyuk (1990) who considered *M. majori* the sole member of its own species group.

Species' relationships in the European subgroup of *Terricola* are poorly resolved, although, again, the Bayesian analyses support some groupings not recognized by MP or NJ bootstrap analysis (Fig. 3). Since these species are so closely related, the hard polytomy observed cannot be ascribed to saturation in cytochrome *b*. Instead, strong bootstrap support above and below unresolved polytomies indicate a rapid radiation involving nearly simultaneous diversification of many lineages (cf. Conroy and Cook, 1999; Lessa and Cook, 1998). Such a surge of speciation could have occurred during a single or, more likely, a few consecutive glacial periods by geographic isolation of small and genetically differentiated populations in different glacial refugia as suggested by Chaline (1987). The relative importance of Mediterranean peninsulas as opposed to more northern mountain areas as "speciation traps" deserves further attention (cf. Chaline, 1987; Bilton et al., 1998; Martínková and Dudich, 2003). In this context it is noteworthy that all taxa in this subgroup except *M. savii* (see below) seem to harbor little intra-specific variation.

4.6. Subgenera Pallasiinus, Alexandromys, and Volemys (the "Asian" lineage)

The taxonomic validity of the subgenera *Pallasiinus*, *Alexandromys*, and *Volemys* was not supported by our cytochrome *b* analysis since their representatives formed a strongly supported monophyletic group. This "Asian" group was previously described by Conroy and Cook (2000a). Our addition of within-species sequences and more species to the *Microtus* tree has significantly increased the support for this lineage. The Japanese *M. montebelli* and Taiwanese *M. kikuchii* are sister species to the Holarctic *M. oeconomus* and constitute examples of allopatric speciation on islands. The results are supported by chromosome data. Thus, pairing of the X and Y chromosome in *Microtus* meiosis seems to be a rare lineage-specific phenomenon that can be used in reconstructing systematic relationships in the genus (Mekada et al., 2002; Megías-Nogales et al., 2002). To

date, X–Y pairing is only reported for the three species forming the Asian lineage (see Mekada et al., 2002) as well as *Chionomys nivalis* (Megías-Nogales et al., 2002) and two species representing the subgenera *Lasiopodomys* and *Neodon* not included here (Gu et al., 1999; Mekada et al., 2002).

4.7. Subgenera Agricola, Neodon, and Stenocranius

Microtus agrestis, *M. cabrerae*, *M. juldaschi*, and *M. gregalis* were placed basal in the phylogenetic analyses and did not form significant associations with other species. For example, the association of *M. cabrerae* with the Nearctic species was not supported by either bootstrapping or posterior probabilities (Figs. 1–3) but most probably due to long-branch attraction (e.g., Hendy and Penny, 1989). These four, basal species represent three subgenera—*Agricola*, *Neodon*, and *Stenocranius*—characterized by few and ancestral species. Our data suggest that *M. agrestis* and *M. cabrerae* should not both be placed in the subgenus *Agricola* since they do not show any sister relationship. Especially the *M. cabrerae* lineage seems to be either very old or has undergone accelerated evolution in cytochrome *b*. Actually, *M. cabrerae* displays morphological characters that are archaic (Gromov and Polyakov, 1992), and Chaline (1972) described a new subgenus, *Iberomys*, for the fossil vole *Microtus* (*Iberomys*) *brecciensis*, a direct ancestor of *M. cabrerae*. Altogether, our data strongly support the classification of *M. cabrerae* in the separate subgenus *Iberomys*.

Microtus juldaschi belongs to the subgenus *Neodon* also containing *M. irene* and *M. sikimensis* (Musser and Carleton, 1993; Zagorodnyuk, 1990). *Neodon* as well as the subgenera *Blanfordimys* and *Phaiomys* (not analyzed) are considered old Pleistocene relicts that probably descended directly from the *Allophaiomys* stock (Nadachowski and Garapich, 1998; Nadachowski and Zagorodnyuk, 1996). Consequently, the position of *M. juldaschi* in the phylogenetic tree is expected to be basal, in line with our result.

Both our data and those of Conroy and Cook (2000a) indicate that the subgenus *Stenocranius* is a polyphyletic and artificial group as the Asian *M. gregalis* does not cluster with the North American *M. miurus* and *M. abbreviatus*. Nor does *M. gregalis* cluster with *M. middendorffi* as suggested by the alternative classification of Zagorodnyuk (1990). Thus, the morphological similarity of these species is most probably due to adaptive convergence as suggested by Chaline et al. (1999) and Conroy and Cook (2000a). *M. gregalis* is by far the most divergent *Microtus* species in our cytochrome *b* data set and its position is unclear even in relation to *Chionomys* (cf. Figs. 1–3). According to palaeontological data, *M. gregalis* represents an early split from the *Allophaiomys* stock (Rekovets and Nadachowski, 1995). The support for *Microtus* as a bona fide taxonomic group is mainly influenced by the instability of *M. gregalis*. Thus, in order to fully evaluate monophyly of the genus *Microtus*, the position of *M. gregalis* needs to be determined. In addition, the position of the genus *Arvicola* needs to be evaluated.

4.8. Species limits in Microtus?

Our data confirm that the genus *Microtus* contains many closely related species as well as many species that are characterized by extensive intra-specific variation (Fig. 2). Consequently, there is an overlap between inter- and intra-specific cytochrome *b* distances around 4–8%, similar to what has been described in other mammals (e.g., Avise, 2000; Bradley and Baker, 2001). These data corroborate the notion that genetic distances and/or reciprocal monophyly in mtDNA are highly uncertain criteria for delimiting closely related species (Bradley and Baker, 2001; Hudson and Coyne, 2002; Hudson and Turelli, 2003; Nichols, 2001; Rosenberg and Nordborg, 2002). Moreover, the overlap in cytochrome *b* divergence between and within currently recognized species demonstrates that speciation is an ongoing process in *Microtus* and that many taxa and species groups offer a huge potential for molecular, evolutionary research, particularly with regards to phylogeography and speciation (cf. Barraclough and Nee, 2001).

Examples of sister species that show low cytochrome *b* divergence, but do represent widely accepted species include *M. duodecimcostatus—lusitanicus* and *M. arvalis—rossiaemeridionalis* (Fig. 1). The cytochrome *b* distance of 4–5% between *M. duodecimcostatus* and *M. lusitanicus* is among the lowest recorded for *Microtus* species, the exception being *M. miurus—M. abbreviatus* that differ by only 1.5%. However, the latter two taxa are probably conspecific (Conroy and Cook, 2000a). The taxonomic rank of *M. lusitanicus* has varied in recent years but it is now considered a species (reviewed in Spitzenberger et al., 2000), its ranking validated by sterility of male F_1 hybrids between *M. duodecimcostatus* and *M. lusitanicus*.

The sibling species *M. arvalis* ($2n = 46$) and *M. rossiaemeridionalis* ($2n = 54$) are closely related, demonstrating a divergence of only 6–8% in cytochrome *b*. However, hybrids between the two taxa are sterile (Meier et al., 1985). The status of the eastern '*obscurus*' taxon in the *arvalis* group is unclear. While some authors regard this taxon as a species, *M. obscurus*, separate from the western *M. arvalis*, other authors describe the taxa as two karyotypic forms, *M. arvalis* '*obscurus*' and *M. arvalis* '*arvalis*'. We have used the latter classification since hybridization between the two taxa appears to occur in the wild (Bulatova, unpublished) and hybridization studies have shown that F_1, F_2 and subsequent backcrosses are fertile, albeit with lowered fertility (Malygin and Panteleichuk, 2003). The cytochrome *b* data imply a re-

cent split between '*arvalis*' and '*obscurus*'; the divergence is only 2–4%. For references and a recent discussion based on cytochrome *b* data see Haynes et al. (2003).

Other species with unclear status accompanied by low cytochrome *b* divergence include *M. liechtensteini* and *M. atticus*. *M. liechtensteini* seems to represent a cytochrome *b* lineage distinct from *M. multiplex* (Figs. 1–3). Our observation is consistent with the results obtained by Haring et al. (2001) who conducted a more extensive study of the Alpine voles of the *M. multiplex* complex using mitochondrial D-loop sequences. However, additional data on hybridization and fertility of offspring in the *M. multiplex* complex are needed to fully evaluate the taxonomic ranking of *M. liechtensteini*. The *M. thomasi* samples 1–3 represent the karyotype form '*atticus*' previously ascribed to a separate species, *M. atticus*. Our data corroborate the present ranking of '*atticus*' as a mere karyotype form of *M. thomasi* (references in Tsekoura et al., 2002).

Species with high intra-specific cytochrome *b* variation include *M. savii*, *M. agrestis*, *M. daghestanicus*, *M. oeconomus*, *M. subterraneus*, and *C. nivalis* (Fig. 1). The subspecies *M. savii savii* in northern and central Italy and *M. s. brachycercus* in southern Italy differ in karyotype (Galleni et al., 1992) and because the male F_1 hybrids are sterile, Galleni et al. (1994) suggested that the two subspecies should be elevated to species rank. Our data set is limited, but indicates a recent mtDNA split between southern and central-northern *M. savii*, with cytochrome *b* divergences of 4–5%. Recent molecular data on *M. agrestis* demonstrate a south-north split in Europe, with a net divergence of 5.2% in cytochrome *b* and 0.7% in the X and Y chromosome, all three genealogies exhibiting reciprocal monophyly. These data suggest that the southern *M. agrestis* represent a new, morphologically and karyotypically cryptic species (Jaarola and Searle, 2002, 2004; Hellborg, 2004).

Another species with high intra-specific variation is *M. daghestanicus* that exhibits a highly divergent haplotype from Georgia differing by 4% from the two Turkish haplotypes. Georgian specimens are sometimes ascribed to *M. nasarovi*, but *M. daghestanicus* and *M. nasarovi* differ in karyotype ($2n = 52/54$ and 38, respectively), habitat preference and distribution range (Bukhnikashvili and Kandaurov, 2002). Since the Georgian *M. daghestanicus* originated from the eastern part of the country, i.e. outside the distribution range of *M. nasarovi*, and the Turkish specimens both carried a $2n = 54$ karyotype (Macholán, pers. comm.), the large cytochrome *b* divergence observed between Georgian and Turkish *M. daghestanicus* is likely to reflect intra-specific variation.

Microtus oeconomus is divided into four divergent cytochrome *b* lineages differing by net distances up to 3.5% (Brunhoff et al., 2003). The largely allopatric distribution of these lineages and inferences on their late Quaternary history is presented in Brunhoff et al. (2003, submitted). Yet another species that is characterized by very high cytochrome *b* distances between haplotypes is *M. subterraneus*; one of the haplotypes from Turkey differed from the other by 6–7%. Finally, *C. nivalis*, a species with fragmented distribution over Europe and Middle East mountain ranges, exhibited intra-specific distances up to 4%. Studies of morphological characters (Kryštufek, 1999; Nadachowski, 1991) and allozymes (Filippucci et al., 1991) have also demonstrated much diversity in this species.

5. Conclusions

'Small mammals' are far more speciose than their larger relatives, and it is interesting to speculate on the reasons for this (Searle, 1996). However, before the present study, there have been few detailed attempts to obtain molecular phylogenies for particularly species-rich genera of small mammals. Our study, through its coverage of almost all European and North American species, as well as many from Asia, provides a fascinating insight into *Microtus*, the most speciose genus of Arvicoline rodents and one of the most speciose genera of all mammals (Nowak, 1999). The cytochrome *b* phylogeny demonstrates species' radiations at a variety of temporal and spatial scales. In a temporal sense, an early rapid radiation about 2 Mya appears to have generated the major subgenera, which then subsequently radiated further to generate the variety of extant species. Moreover, the currently recognized species are not static forms, but are clearly differentiating and contain cryptic entities that may in many cases best be considered species (e.g., within *M. agrestis*: Jaarola and Searle, 2004). In a spatial sense, the radiations of subgenera and equivalent groupings of *Microtus* have occurred in different geographic areas as evidenced by the current geographic ranges of major cytochrome *b* lineages.

Now that a molecular phylogeny of *Microtus* is available, there is an opportunity to use it to understand the ecological and historical circumstances that lead to speciation of small mammals (cf. Searle, 1996). The phylogeny can also be used for a range of future comparative studies in ecology, behaviour, physiology, parasitology etc. It will, for instance, be possible to use the phylogeny for coevolutionary studies (e.g., *Microtus* and their parasites; Wickström, 2004) and for analyses of trait evolution (e.g., mating systems).

A further, practical achievement of this study is to resolve a variety of long-standing taxonomic uncertainties in the genus *Microtus* since the molecular results are clear-cut and provide an objective basis for taxonomic revision. Our data show that there is a justification for subdividing the genus into subgenera, as proposed by previous workers, but there are also groupings of species within subgenera (Figs. 1–3) and an appropriate nomen-

clature will need to be developed, building on that previously established.

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References

Akhverdyan, M.R., Lyapunova, E.A., Vorontsov, N.N., Teslenko, S.V., 1999. Intrapopulation autosomal polymorphism in the common vole *Microtus arvalis* of the Transcaucasian region. Russ. J. Genet. 35, 1452–1463.

Avise, J.C., 2000. Phylogeography: the History and Formation of Species. Harvard University Press, Cambridge, MA.

Barraclough, T.G., Nee, S., 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16, 391–399.

Baskevich, M.I., 1996. On the karyological differentiation in Caucasian populations of common vole (Rodentia, Cricetidae, *Microtus*). Zool. Zh. 75, 297–308 (in Russian with English summary).

Bensasson, D., Zhang, D.-X., Hartl, D.L., Hewitt, G.M., 2001. Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends Ecol. Evol. 16, 314–321.

Bilton, D.T., Mirol, P.M., Mascheretti, S., Fredga, K., Zima, J., Searle, J.B., 1998. Mediterranean Europe as an area of endemism for small mammals rather than a source for northwards postglacial colonization. Proc. R. Soc. Lond. Ser. B 265, 1219–1226.

Bradley, R.D., Baker, R.J., 2001. A test of the genetic species concept: cytochrome *b* sequences and mammals. J. Mammal. 82, 960–973.

Brunet-Lecomte, P., Chaline, J., 1990. Relations phylogénétiques et évolution des campagnols souterrains d'Europe (*Terricola*, Arvicolidae, Rodentia). C.R. Acad. Sci. Paris, Série II 311, 745–750.

Brunet-Lecomte, P., Chaline, J., 1991. Morphological evolution and phylogenetic relationships of the European ground voles (Arvicolinae, Rodentia). Lethaia 24, 45–53.

Brunet-Lecomte, P., Chaline, J., 1992. Morphological convergences versus biochemical divergences in the holarctic ground voles: *Terricola* and *Pitymys* (Arvicolidae, Rodentia). N. Jb. Geol. Paläont. Mh. 12, 721–734.

Brunhoff, C., Galbreath, K.E., Fedorov, V.B., Cook, J.A., Jaarola, M., 2003. Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. Mol. Ecol. 12, 957–968.

Bukhnikashvili, A., Kandaurov, A., 2002. The annotated list of mammals of Georgia. Proc. Inst. Zool. Acad. Sci. Georgia, Metsniereba, Tbilisi 21, 319–340.

Chaline, J., 1972. Les rongeurs du Pléistocène moyen et supérieur de France. Cahiers Paléont., CNRS, Paris, pp. 1–410.

Chaline, J., 1987. Arvicolid data (Arvicolidae, Rodentia) and evolutionary concepts. Evol. Biol. 21, 237–310.

Chaline, J., Brunet-Lecomte, P., Montuire, S., Viriot, L., Courant, F., 1999. Anatomy of the arvicoline radiation (Rodentia): palaeogeographical, palaeoecological history and evolutionary data. Ann. Zool. Fenn. 36, 239–267.

Chaline, J., Graf, J.-D., 1988. Phylogeny of the Arvicolidae (Rodentia): biochemical and paleontological evidence. J. Mammal. 69, 22–33.

Conroy, C.J., Cook, J.A., 1999. MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. J. Mamm. Evol. 6, 221–245.

Conroy, C.J., Cook, J.A., 2000a. Molecular systematics of a holarctic rodent (*Microtus*: Muridae). J. Mammal. 81, 344–359.

Conroy, C.J., Cook, J.A., 2000b. Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Rodentia: Muridae). Mol. Ecol. 9, 165–175.

Conroy, C.J., Hortelano, Y., Cervantes, F.A., Cook, J.A., 2001. The phylogenetic position of southern relictual species of *Microtus* (Muridae: Rodentia) in North America. Mamm. Biol. 66, 332–344.

DeBry, R.W., 1992. Biogeography of New World taiga-dwelling *Microtus* (Mammalia: Arvicolidae): a hypothesis test that accounts for phylogenetic uncertainty. Evolution 46, 1347–1357.

DeWoody, A.J., Chesser, R.K., Baker, R.J., 1999. A translocated mitochondrial cytochrome *b* pseudogene in voles (Rodentia: *Microtus*). J. Mol. Evol. 48, 380–382.

Erixon, P., Svennblad, B., Britton, T., Oxelman, B., 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst. Biol. 52, 665–673.

Felsenstein, J., 1988. Phylogenies from molecular sequences—inference and reliability. Annu. Rev. Genet. 22, 521–565.

Filippucci, M.G., Fadda, V., Kryštufek, B., Simson, S., Amori, G., 1991. Allozyme variation and differentiation in *Chionomys nivalis* (Martins, 1842). Acta Theriol. 36, 47–62.

Fumagalli, L., Taberlet, P., Stewart, D.T., Gielly, L., Hausser, J., Vogel, P., 1999. Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. Mol. Phylogenet. Evol. 11, 222–235.

Galleni, L., Stanyon, R., Tellini, A., Giordano, G., Santini, L., 1992. Karyology of the Savi pine vole, *Microtus savii* (De Sélys-Longchamps, 1838) (Rodentia, Arvicolidae): G-, C-, DA/DAPI-, and *Alu*I-bands. Cytogenet. Cell Genet. 59, 290–292.

Galleni, L., Tellini, A., Stanyon, R., Cicalò, A., Santini, L., 1994. Taxonomy of *Microtus savii* (Rodentia, Arvicolidae) in Italy: cytogenetic and hybridization data. J. Mammal. 75, 1040–1044.

Garapich, A., Nadachowski, A., 1996. A contribution to the origin of *Allophaiomys* (Arvicolidae, Rodentia) in Central Europe: the relationships between *Mimomys* and *Allophaiomys* from Kamyk (Poland). Acta Zool. Cracov. 39, 179–184.

Getz, L.L., 1985. Habitats. In: Tamarin, R.H.(Ed.), Biology of New World *Microtus*. American Society of Mammalogists. Special Publication No. 8, pp. 286–309.

Gill, A., Petrov, B., Zivkovic, S., Rimsa, D., 1987. Biochemical comparison in Yugoslavian rodents of the families Arvicolidae and Muridae. Z. Säugetierk. 52, 247–256.

Golenishchev, F.N., Malikov, V.G., Nazari, F., Vaziri, A.S., Sablina, O.V., Polyakov, A.V., 2003. New species of *guentheri* group (Rodentia, Arvicolinae, *Microtus*) from Iran. Russ. J. Theriol. 1, 117–123.

Graf, J.-D., 1982. Génétique biochimique, zoogéographie et taxonomie des Arvicolidae (Mammalia, Rodentia). Rev. Suisse Zool. 89, 749–787.

Gromov, I.M., Polyakov, I.Y., 1992. Fauna of the USSR, vol. 3, Voles (*Microtinae*). Brill Publishing Company, Leiden.

Gu, W., Wang, T., Zhu, B., 1999. Study on the morphology of sex chromosomes pairing of the synaptonemal complex in Mandarin vole (*Microtus mandarinus*). Acta Theriol. Sin. 19, 150–154 (in Chinese).

Haring, E., Herzig-Straschil, B., Spitzenberger, F., 2001. Phylogenetic analysis of Alpine voles of the *Microtus multiplex* complex using the mitochondrial control region. J. Zool. Syst. Evol. Res. 38, 231–238.

Haynes, S., Jaarola, M., Searle, J.B., 2003. Phylogeography of the common vole (*Microtus arvalis*) with particular emphasis on the colonization of the Orkney archipelago. Mol. Ecol. 12, 951–956.

Hellborg, L., 2004. Evolutionary studies of the mammalian Y chromosome. Ph.D, thesis, Uppsala University.

Hendy, M.D., Penny, D., 1989. A framework for the quantitative study of evolutionary trees. Syst. Zool. 38, 297–309.

Hoffmann, R.S., Koeppl, J.W., 1985. Zoogeography. In: Tamarin, R.H. (Ed.), Biology of New World *Microtus*. American Society of Mammalogists. Special Publication No. 8, pp. 84–0115.

Hudson, R.R., Coyne, J.A., 2002. Mathematical consequences of the genealogical species concept. Evolution 56, 1557–1565.

Hudson, R.R., Turelli, M., 2003. Stochasticity overrules the three-times rule: genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution 57, 182–190.

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.

Hutterer, R., 2001. Bavarian pine vole still alive. Oryx 35, 185.

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. 32, 128–144.

Jaarola, M., Searle, J.B., 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. Mol. Ecol. 11, 2613–2621.

Jaarola, M., Searle, J.B., 2004. A highly divergent mitochondrial DNA lineage of *Microtus agrestis* in southern Europe. Heredity 92, 229–234.

Jaarola, M., Tegelström, H., 1995. Colonization history of north European field voles (*Microtus agrestis*) revealed by mitochondrial DNA. Mol. Ecol. 4, 299–310.

Jukes, T.H., Cantor, C.R., 1969. Evolution of protein molecules. In: Munro, H.N. (Ed.), Mammalian Protein Metabolism. Academic Press, New York, pp. 21–132.

Kefelioğlu, H., Kryštufek, B., 1999. The taxonomy of *Microtus socialis* group (Rodentia: Microtinae) in Turkey, with the description of a new species. J. Nat. Hist. 33, 289–303.

Kocher, T.D., Thomas, W.K., Meyer, S.A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Kratochvíl, J., Král, B., 1974. Karyotypes and relationships of Palaearctic 54-chromosome *Pitymys* species (Microtidae, Rodentia). Zool. Listy 23, 289–302.

Kryštufek, B., 1999. Snow voles, genus *Chionomys*, of Turkey. Mammalia 63, 323–339.

Kryštufek, B., Griffiths, H.I., Vohralík, V., 1996. The status and use of *Terricola* Fatio, 1867 in the taxonomy of Palaearctic pine voles' (*Pitymys*) (Rodentia, Arvicolinae). Bull. Inst. R. Sci. Nat. Belg. Biol. 66, 237–240.

Kryštufek, B., Kefelioğlu, H., 2001. Redescription of *Microtus irani*, the species limits and a new social vole from Turkey. Bonn. Zool. Beitr. 50, 1–14.

Leaché, A.D., Reeder, T.W., 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): A comparison of parsimony, likelihood and Bayesian approaches. Syst. Biol. 51, 44–68.

Lessa, E.P., Cook, J.A., 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Mol. Phylogenet. Evol. 9, 88–99.

Lin, Y.-H., Waddell, P.J., Penny, D., 2002. Pika and vole mitochondrial genomes increase support for both rodent monophyly and Glires. Gene 294, 119–129.

Macholán, M., Filippucci, M.G., Zima, J., 2001. Genetic variation and zoogeography of pine voles of the *Microtus subterraneus/majori* group in Europe and Asia Minor. J. Zool. 255, 31–42.

Maddison, D.R., Maddison, W.P., 2000. MacClade 4: Analysis of phylogeny and character evolution, Version 4.0. Sinauer Associates, Sunderland, MA.

Malygin, V.M., Panteleichuk, T.M.S.L., 2003. Efficiency of reproductive isolation mechanisms in six species of common voles (*Microtus*, Rodentia). In: Kryokov, A.P., Yakimenko, L.V. (Eds.), Problems of Evolution, vol. V. Russian Academy of Sciences, Far East Division, Vladivostok, Dalnauka, pp. 198–206 (in Russian with English summary).

Martínková, N., Dudich, A., 2003. The fragmented distribution range of *Microtus tatricus* and its evolutionary implications. Folia Zool. 52, 11–22.

Maryama, T., Imai, H.T., 1981. Evolutionary rate of the mammalian karyotype. J. Theor. Biol. 90, 111–121.

Mazurok, N.A., Rubtsova, N.V., Isaenko, A.A., Pavlova, M.E., Slobodyanyuk, S.Y., Nesterova, T.B., Zakian, S.M., 2001. Comparative chromosome and mitochondrial DNA analyses and phylogenetic relationships within common voles (*Microtus*, Arvicolidae). Chrom. Res. 9, 107–120.

Megías-Nogales, B., Marchal, J.A., Acosta, M.J., Bullejos, M., Díaz de la Guardia, R., Sánchez, A., 2002. Sex chromosome pairing in two Arvicolidae species: *Microtus nivalis* and *Arvicola sapidus*. Hereditas 138, 114–121.

McClellan, D.A., McCracken, K.G., 2001. Estimating the influence of selection on the variable amino acid sites of the cytochrome *b* protein functional domains. Mol. Biol. Evol. 18, 917–925.

Meier, M.N., Golenishchev, F.N., Radjabli, S.I., Sablina, O.V., 1996. Voles (subgenus *Microtus* Schrank) of Russia and adjacent territories. Proc. Zool. Inst. Russ. Acad. Sci. 232, 1–320 (in Russian).

Meier, M.N., Radjabli, S.I., Bulatova, N. Sh., Golenishchev, F.N., 1985. Karyological peculiarities and probable relations of common voles of the group '*arvalis*' (Rodentia, Cricetidae, *Microtus*). Zool. Zh. 64, 417–428 (in Russian with English summary).

Mekada, K., Koyasu, K., Harada, M., Narita, Y., Shrestha, K.C., Oda, S.-I., 2002. Karyotype and X–Y chromosome pairing in the Sikkim vole (*Microtus* (*Neodon*) *sikimensis*). J. Zool. 257, 417–423.

Mezhzherin, S.V., Morozov-Leonov, S.Yu., Kuznetsova, I.A., 1995. Biochemical variation and genetic divergence in Palearctic voles (Arvicolidae): subgenus *Terricola*, true lemmings *Lemmus* Link 1795, pied lemmings *Dicrostonyx* Gloger 1841, steppe lemmings *Lagurus* Gloger 1842, mole voles *Ellobius* Fischer von Waldheim 1814. Genetika 31, 788–797 (in Russian with English summary).

Mezhzherin, S.V., Zykov, A.E., Morozov-Leonov, S.Y.u., 1993. Biochemical variation and genetic divergence in Palearctic voles (Arvicolidae): meadow voles *Microtus* Schrank 1798, snow voles *Chionomys* Miller 1908, water voles *Arvicola* Lacepede 1799. Genetika 29, 28–41 (in Russian with English summary).

Mirol, P.M., Mascheretti, S., Searle, J.B., 2000. Multiple nuclear pseudogenes of mitochondrial cytochrome *b* in *Ctenomys* (Caviomorpha, Rodentia) with either great similarity to or high divergence from the true mitochondrial sequence. Heredity 84, 538–547.

Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J., 1999. The Atlas of European Mammals. Poyser, London.

Modi, W.S., 1987. Phylogenetic analyses of chromosomal banding patterns among the Nearctic Arvicolidae (Mammalia: Rodentia). Syst. Zool. 36, 109–136.

Musser, G.G., Carleton, M.D., 1993. Family Muridae. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference, second ed. Smithsonian Institution Press, Washington, pp. 510–756.

Nadachowski, A., 1991. Systematics, geographic variation, and evolution of snow voles (*Chionomys*) based on dental characters. Acta Theriol. 36, 1–45.

Nadachowski, A., Garapich, A., 1998. *Allophaiomys* evolutionary stage in extant *Microtus*. Paludicola 2, 91–94.

Nadachowski, A., Zagorodnyuk, I., 1996. Recent *Allophaiomys*-like species in the Palaearctic: Pleistocene relicts or a return to an initial type? Acta Zool. Cracov. 39, 387–394.

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nichols, R., 2001. Gene trees and species trees are not the same. Trends Ecol. Evol. 16, 358–364.

Nowak, R.M., 1999. Walker's Mammals of the World, sixth ed. The Johns Hopkins University Press, Baltimore, ML.

Orlov, V.N., Yatsenko, V.N., Malygin, V.M., 1983. Karyotype homology and species phylogeny in a group of field mice (Cricetidae, Rodentia). Doklady Biol. Sci. 269, 217–219.

Panteleyev, P.A., 1998. The Rodents of the Palaearctic Fauna: Composition and Areas. Russian Academy of Sciences, Moscow.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Potapov, S.G., Orlov, V.N., Kovalskaya, Yu.M., Malygin, V., M, Ryskov, A.P., 1999. Genetic differentiation in the voles of the tribe Arvicolini (Cricetidae, Rodentia) using DNA taxonprint and RAPD-PCR. Russ. J. Genetics 35, 403–410.

Rabeder, G., 1986. Herkunft und frühe Evolution der Gattung *Microtus* (Arvicolidae, Rodentia). Z. Säugetierk. 51, 350–367.

Reed, R.D., Sperling, F.A.H., 1999. Interaction of process partitions in phylogenetic analysis: an example from the swallowtail butterfly genus *Papilio*. Mol. Biol. Evol. 16, 286–297.

Rekovets, L., Nadachowski, A., 1995. Pleistocene voles (*Arvicolidae*) of the Ukraine. Paleont. Evolutió 28-29, 145–245.

Richmond, G.M., 1996. The INQUA-approved provisional Lower-Middle Pleistocene boundary. In: Turner, C. (Ed.), The Early Middle Pleistocene in Europe. Balkema, Rotterdam.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rosenberg, N.A., Nordborg, M., 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. Nat. Rev. Genet. 3, 380–390.

Searle, J.B., 1996. Speciation in small mammals. Symp. Zool. Soc. Lond. 69, 143–156.

Spitzenberger, F., Brunet-Lecomte, P., Nadachowski, A., Bauer, K., 2000. Comparative morphometrics of the first lower molar in *Microtus* (*Terricola*) cf. *liechtensteini* of the Eastern Alps. Acta Theriol. 45, 471–483.

Suchentrunk, F., Markov, G., Haiden, A., 1998. On gene pool divergence of the two karyotypically distinct sibling vole species *Microtus arvalis* and *M. rossiaemeridionalis* (Arvicolidae, Rodentia). Folia Zool. 47, 103–114.

Suzuki, Y., Glazko, G.V., Nei, M., 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proc. Natl. Acad. Sci. USA 99, 16138–16143.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Tsekoura, N., Fraguedakis-Tsolis, S., Chondropoulos, B., Markakis, G., 2002. Morphometric and allozyme variation in central and southern Greek populations of *Microtus* (*Terricola*) *thomasi*. Acta Theriol. 47, 137–149.

Wickström, L.M., 2004. Phylogeny, phyletic coevolution and phylogeography of anoplocephaline cestodes in mammals. Ph.D. thesis, University of Helsinki, Finnish Forest Research Institute, Research Papers 918.

Yang, Z., 1994. Estimating the pattern of nucleotide substitutions. J. Mol. Evol. 39, 105–111.

Yang, Z., Yoder, A.D., 1999. Estimation of the transition/transversion rate bias and species sampling. J. Mol. Evol. 48, 274–283.

Yigit, N., Colak, E., 2002. On the distribution and taxonomic status of *Microtus guentheri* (Danford and Alston, 1880) and *Microtus lydius* Blackler, 1916 (Mammalia: Rodentia) in Turkey. Turk. J. Zool. 26, 197–204.

Zagorodnyuk, I.V., 1989. Taksonomiya, rasprostranenie i morfologicheskaya izmenchivost'polevok roda *Terricola* vostochnoi Evropy. [Taxonomy, distribution and morphological variation of the *Terricola* voles in East Europe]. Vestnik Zool. 5, 3–14 (in Russian with English summary).

Zagorodnyuk, I.V., 1990. Kariotipicheskaya izmenchivost'i sistematika serykh polevok (Rodentia, Arvicolini). Soobshchenie1. Vidovoi sostav i khromosomnye chisla. [Karyotypic variability and systematics of the gray voles (Rodentia, Arvicolini). Communication 1. Species composition and chromosomal numbers]. Vestnik Zool. 2, 26–37 (in Russian).

Zakrzewski, R.J., 1985. The fossil record. In: Tamarin, R.H.(Ed.), Biology of New World *Microtus*. American Society of Mammalogists. Special Publication No. 8, pp. 1–51.

Zheng, S.-H., Zhang, Z.-Q., 2000. Late Miocene–Early Pleistocene micromammals from Wenwanggou of Lingtai, Gansu, China. Vertebrata PalAsiatica 38, 58–71.

Zima, J., Král, B., 1984. Karyotypes of European mammals II. Acta Sc. Nat. Brno 18, 1–62.