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Systematics of snow voles (*Chionomys*, Arvicolinae) revisited

Glenn Yannic [a,b,*], Reto Burri [a,c], Vladimir G. Malikov [d], Peter Vogel [a]

[a] Department of Ecology and Evolution, Biophore Building, University of Lausanne, 1015 Lausanne, Switzerland
[b] Département de Biologie and Centre d'Études Nordiques, 1045 Avenue de la Médecine, University Laval, Québec, QC, Canada G1V 0A6
[c] Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-75236 Uppsala, Sweden
[d] Laboratory of Theriology, Zoological Institute, Russian Academy of Sciences, 199034 St. Petersburg, Russia

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ABSTRACT

To elucidate the evolutionary history of snow voles, genus *Chionomys*, we studied the phylogeography of *Chionomys nivalis* across its range and investigated its relationships with two congeneric species, *Chionomys gud* and *Chionomys roberti*, using independent molecular markers. Analyses were based on mitochondrial (~940 bp cyt *b*) and Y-chromosomal (~2020 bp from three introns) genetic variation. Our data provide conclusive evidence for a Caucasian and Middle Eastern origin for the three species and a subsequent westward expansion of *C. nivalis*. In addition, we discuss the taxonomic status of the genus *Chionomys* in relation to the genus *Microtus*.

1. Introduction

First placed in the genus *Arvicola* (Lacepede, 1799) then included in the genus *Microtus* (Schrank, 1798), snow voles were later elevated to their own genus, *Chionomys*, by Miller (1908) only to be subsequently demoted to subgenus (Miller, 1912). This status was maintained over decades in the literature and all major syntheses of vole systematics (*e.g.*, Andera and Leffler, 1981; Corbet, 1978; Ellerman and Morrison-Scott, 1966; Krapp, 1982). More recently, snow voles were re-elevated to full genus level based on multiple criteria (reviewed in Musser and Carleton, 1993). The three currently recognized species inhabit the mountainous regions of Europe, Asia Minor, and Western Asia. All three species occur in the Caucasus. Two of them, the Gudaur Snow Vole, *Chionomys gud* (Satunin, 1909), and the Robert's Snow Vole, *Chionomys roberti* (Thomas, 1906), are endemic to the Caucasus and Asia Minor. In contrast, the European Snow Vole, *Chionomys nivalis* (Martins, 1842), occupies a much larger distribution, ranging from the Kopet-Dag (South Turkmenistan) and the Binaloud Mountains (Northeastern Iran) in the east to the Sierra Nevada (Spain) in the west.

Due to its rock-dwelling lifestyle in alpine habitats, the distribution of the European Snow Vole is highly patchy and the species is mostly restricted to altitudes from 1500 to 3000 m a.s.l., although it can also be found in rocky habitats close to sea level (Amori, 1999). Within this discontinuous distribution, populations are highly isolated, and considerable morphological diversity is found among populations (Amori, 1999). Consequently, a large number of subspecies have been described based on morphology. Corbet (1978) recognized four subspecies, while 13 were distinguished by Krapp (1982), 16 by Ellerman and Morrison-Scott (1966) and Kratochvil (1981), and up to 18 subspecies have been listed by Nadachowski (1991) in the most comprehensive revision. Allozyme variation in European populations revealed an alpine clade and a clade including populations from Italy, France and Spain (Graf, 1982). Analyses employing extended sampling from the Alps suggested a double colonization from the west and the east, with the Middle Eastern population from Mount Hermon occupying a basal position (Filippucci et al., 1991).

The reconstruction of the genus' evolutionary history has proved challenging in the past, and the phylogeographic origin of *Chionomys* remains unclear. Nadachowski (1991) reconstructed the phylogeography of all three species based on tooth morphology. Like other subsequent authors (*e.g.*, Chaline et al., 1999), he postulated a split between *Chionomys* and *Microtus* from an ancestral *Allophaiomys* sp. during the Lower Pleistocene. According to Nadachowski (1991) fossils of *C. nivalis* appeared simultaneously in Europe and Asia Minor in the Middle Pleistocene, with *C. n. leucurus* and *C. n. lebrunii* from France being the most primitive morphotypes. An eastward expansion of this species from Europe to Turkey (*C. n. spitzenbergerae*), the Caucasus (*C. n. trialeticus*)

* Corresponding author at: Département de Biologie and Centre d'Études Nordiques, 1045 Avenue de la Médecine, University Laval, Québec, QC, Canada G1V 0A6.
E-mail address: Glenn.Yannic@gmail.com (G. Yannic).

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and Kopet-Dag (*C. n. dementievi*) followed. In contrast, fossils of *C. gud* and *C. roberti* appeared in the Caucasus only in the Upper Pleistocene, and the two species are currently restricted to this region (*i.e.*, the Caucasus and Minor Asia). The mitochondrial phylogeography of *C. nivalis* recently published by Castiglia et al. (2009), including samples from Spain to Syria and Turkey, supports Nadachowski's (1991) hypothesis of a European origin of *C. nivalis* and a subsequent eastward expansion during the Middle Pleistocene. Based on morphological criteria, Kryštufek (1999) also hypothesized a European origin for *C. nivalis* and an eastern origin of *C. gud* and *C. roberti*. According to these studies, speciation would have taken place in the area of the Bosphorus land bridge, where the land connection between Europe and Asia was frequently disrupted by Pleistocene sea level oscillations (Kerey et al., 2004). However, paleontological data do not provide conclusive evidence supporting the European origin of *C. nivalis*. Rather, they show that during the Middle Pleistocene, *C. nivalis* was already widespread in Europe (Kowalski, 2001) and in Asia Minor (Kryštufek and Vohralik, 2005), contradicting the molecular dating of the species' eastward expansion (Castiglia et al., 2009).

In order to elucidate the evolutionary history of the genus *Chionomys*, we studied the phylogeography of *C. nivalis* over its entire range and investigated the relationships among all three species of *Chionomys* based on variation in mitochondrial and Y-chromosome DNA. Our analyses support a Caucasian origin of the genus and a subsequent westward expansion of *C. nivalis*. In addition, we discuss the ambiguous taxonomic status of *Chionomys* in relation to the genus *Microtus*.

2. Material and methods

2.1. Specimens

We obtained tissue samples as (1) ethanol-preserved tissues taken from voucher specimens in the IZEA collection of Musée de Zoologie (Lausanne, Switzerland), and from the Zoological Institute of Saint Petersburg (Russia), and (2) eight DNA extracts kindly provided by Peter Wandeler, Zoologisches Museum, Universität Zürich (Switzerland). A total of 34 specimens representing 7 *Chionomys* species or subspecies were analyzed for variation in the mitochondrial cytochrome *b* gene (cyt *b*) and in three Y-chromosome introns (see Fig. 1 and Table 1 for details). Due to its male-only strict paternal inheritance and a slow mutation rate relative to mtDNA, information from Y-chromosomal variation is expected to shed light on the evolutionary history at a more ancient timescale. Mitochondrial DNA only provides information about the female germ line and the rapid evolution of mtDNA makes it prone to mutational saturation (homoplasy) over long evolutionary timescales, unlike the mammalian Y-chromosome. Therefore, studying both mtDNA and the Y chromosome should enable comparative analysis of genes with different patterns of inheritance and also of recent and ancient evolutionary history.

Our sample included three *C. nivalis* subspecies (*nivalis, trialeticus* and *dementievi*) out of the 18 listed by Nadachowski (1991), and each two subspecies of *C. gud* (*gud* and *lghesicus*) and *C. roberti* (*personnatus* and *occidentalis*). Additional cyt *b* sequences representing seven *C. nivalis* subspecies, and one *C. gud* and two *C. roberti* sequences published previously were included in the data set; their GenBank accession numbers are given in Table 2. *Microtus agrestis* and *M. arvalis* were used as outgroups, and *Arvicola terrestris* was used to root all trees based on the results of Fink et al. (2006) and Galewski et al. (2006).

Insufficient taxon sampling is often cited as a major source of error in phylogenetic analysis (see for example Hillis et al., 2003 and references therein). Therefore, to infer the phylogenetic relationships between *Chionomys* and closely related genera and other arvicoline species, 77 sequences representing 17 arvicoline genera (10 out of the 11 recognized arvicoline tribes; sensu Musser and Carleton, 1993) were retrieved from GenBank (for origin and accession numbers see Supplementary Table S1). This second dataset included 51 *Microtus* species and notably *Microtus gregalis* (*Stenocranius*), the phylogenetic position of which in relation to *Chionomys* and other *Microtus* was ambiguous in previous analyses of the cyt *b* data (*i.e.*, Buzan and Kryštufek, 2008; Jaarola et al., 2004). *Cricetulus barabensis* from the subfamily Cricetinae and *Peromyscus truei* from the subfamily Neotominae, thought to be two sister clades to Arvicolinae (Michaux et al., 2001) were used as outgroups.

Mitochondrial fragments that have been integrated into the nuclear genome (*numt* pseudogenes) are not rare in Arvicolinae and can cause problems in phylogenetic and phylogeographic inference if *numts* are inadvertently included among mitochondrial sequences (Triant and DeWoody, 2007, 2008). We evaluated the mitochondrial origin of cyt *b* sequences by checking for the presence of indels, frame-shift mutations, or premature stop codons that would suggest a nuclear origin (Triant and DeWoody, 2009).

In addition, we used all published data to reconstruct concatenated sequence trees based on nuclear information available on GenBank for *C. nivalis* and nine *Microtus* species (*M. oeconomus, M. arvalis, M. kikuchii, M. ochrogaster, M. richardsoni, M. chrotorrhinus, M. longicaudus, M. thomasi and M. agrestis*). This analysis included

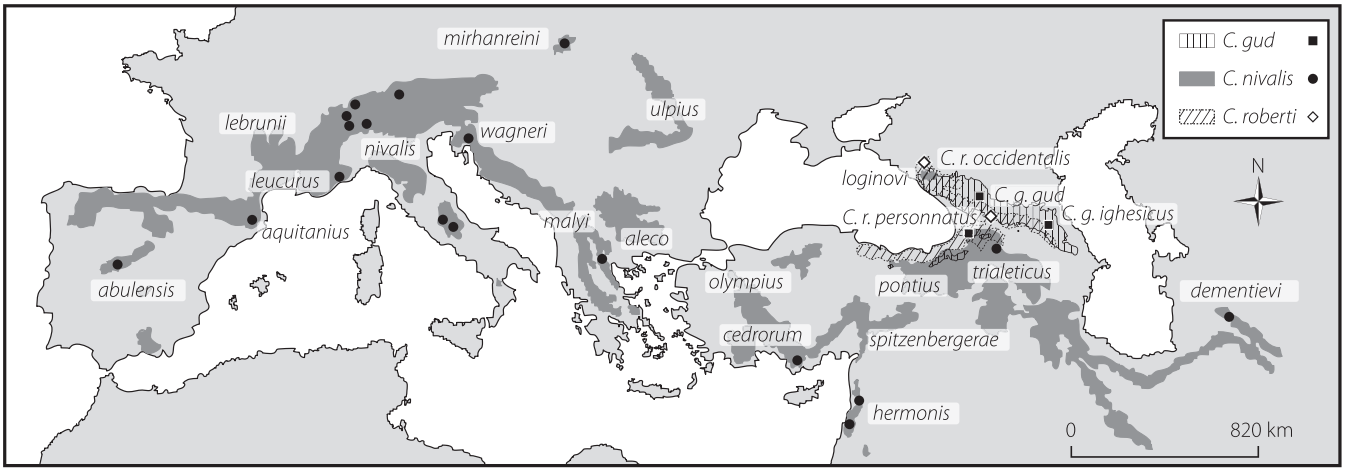

Fig. 1. Distribution of *Chionomys* species and subspecies and sampling localities. Distribution modified from Nadachowski (1991) and Kryštufek and Amori (2008).

Table 1
Species and specimens used in the present study, specimen identification code for each species (ID), and geographic origin of the samples. Type locality of a taxon is indicated by *. GenBank Accession No. are provided. NA, Failure to amplify the target sequence.

Species	ID	Country	Locality	Sex	DBY7	DBY14	UTY11	Cyt *b*	Hap. Cyt *b*
C. g. gud	IZEA.2175	Georgia	Gudaury, Krestovy Pereval*	Male	HQ901938	HQ901911	NA	HQ901797	H16
C. g. gud	IZEA.2176	Georgia	Gudaury, Krestovy Pereval*	Female				HQ901797	H16
C. g. gud	IZEA.2177	Georgia	Gudaury, Krestovy Pereval*	Female				HQ901797	H16
C. g. gud	IZEA.2178	Georgia	Gudaury, Krestovy Pereval*	Female				HQ901797	H16
C. g. lghesicus	IZEA.2180	Russian Federation, Republic of Dagestan	Andyiskoye Koisu*	Male	HQ901939	HQ901912	HQ901973	HQ901798	H17
C. g. lghesicus	IZEA.2181	Russian Federation, Republic of Dagestan	Andyiskoye Koisu*	Male	HQ901940	HQ901913	HQ901974	HQ901796	H12
C. g. lghesicus	IZEA.2179	Russian Federation, Republic of Dagestan	Andyiskoye Koisu*	Female				HQ901798	H17
C. n. dementievi	IZEA.4189	Turkmenistan	Ashabad, Kopet-Dag	Male	**NA**	HQ901914	HQ901958	HQ901806	H5
C. n. dementievi	IZEA.4191	Turkmenistan	Ashabad, Kopet-Dag	Male	HQ901934	HQ901915	HQ901959	HQ901805	H4
C. n. dementievi	IZEA.4193	Turkmenistan	Ashabad, Kopet-Dag	Male	HQ901935	HQ901916	HQ901960	HQ901807	H6
C. n. dementievi	IZEA.4188	Turkmenistan	Ashabad, Kopet-Dag	Female				HQ901804	H3
C. n. trialeticus	IZEA.4195	Georgia	Pass of Tskhra-tskharo, Transcaucasia*	Male	HQ901936	HQ901917	HQ901969	HQ901803	H7
C. n. trialeticus	IZEA.4197	Georgia	Pass of Tskhra-tskharo, Transcaucasia*	Male	HQ901937	HQ901918	HQ901970	HQ901803	H7
C. n. trialeticus	IZEA.2183	Georgia	Pass of Tskhra-tskharo, Transcaucasia*	Male	HQ901941	HQ901919	HQ901971	HQ901799	H21
C. n. trialeticus	IZEA.2184	Georgia	Pass of Tskhra-tskharo, Transcaucasia*	Male	HQ901942	HQ901920	HQ901972	HQ901801	H22
C. n. trialeticus	IZEA.4196	Georgia	Pass of Tskhra-tskharo, Transcaucasia*	Female				HQ901802	H18
C. n. trialeticus	IZEA.2182	Georgia	Pass of Tskhra-tskharo, Transcaucasia*	Female				HQ901800	H23
C. n. trialeticus	IZEA.2187	Georgia	Bacuriany, Caucasus	Female				HQ901802	H18
C. n. nivalis	FL101	Liechtenstein	Trisen, Lawena	Male	HQ901949	HQ901921	HQ901967		
C. n. nivalis	FL108	Liechtenstein	Trisen, Lawena	Male	HQ901950	HQ901922	HQ901966		
C. n. nivalis	FL133	Liechtenstein	Trisen, Lawena	Male	HQ901951	HQ901923	HQ901965		
C. n. nivalis	FL134	Liechtenstein	Trisen, Lawena	Male	HQ901952	HQ901924	HQ901968		
C. n. nivalis	CN00_05	Switzerland	Churwalden, Grison	Male	HQ901953	**NA**	HQ901964		
C. n. nivalis	CN00_07	Switzerland	Churwalden, Grison	Male	HQ901954	HQ901925	HQ901963		
C. n. nivalis	CN06_002	Switzerland	Churwalden, Grison	Male	HQ901955	HQ901927	HQ901961		
C. n. nivalis	CN06_005	Switzerland	Churwalden, Grison	Male	HQ901956	HQ901926	HQ901962		
C. r. occidentalis	IZEA.3454	Russian Federation, Republic of Adygea	Caucasian Biosphere Nature Reserve	Male	HQ901945	HQ901930	HQ901978	HQ901793	H2
C. r. occidentalis	IZEA.3456	Russian Federation, Republic of Adygea	Caucasian Biosphere Nature Reserve	Male	HQ901946	HQ901931	HQ901980	HQ901792	H11
C. r. occidentalis	IZEA.3458	Russian Federation, Republic of Adygea	Caucasian Biosphere Nature Reserve	Male	HQ901947	HQ901932	HQ901979	HQ901791	H1
C. r. occidentalis	IZEA.3455	Russian Federation, Republic of Adygea	Caucasian Biosphere Nature Reserve	Female				HQ901791	H1
C. r. personnatus	IZEA.3452	Russian Federation, Republic of North Ossetia-Alania	Tarskoe, Vladikavkaz*	Male	HQ901943	HQ901928	HQ901975	HQ901794	H8
C. r. personnatus	IZEA.3453	Russian Federation, Republic of North Ossetia-Alania	Tarskoe, Vladikavkaz*	Male	HQ901944	HQ901929	HQ901976	HQ901795	H9
C. r. personnatus	IZEA.3459	Russian Federation, Republic of North Ossetia-Alania	Tarskoe, Vladikavkaz*	Male	HQ901949	**NA**	HQ901977	HQ901794	H8
C. r. personnatus	IZEA.3466	Russian Federation, Republic of North Ossetia-Alania	Tarskoe, Vladikavkaz*	Female				HQ901794	H8
Microtus arvalis	IZEA.MB09	Switzerland	Vallée de Joux, Vaud	Male	HQ901957	HQ901933	HQ901981		

Table 2
Chionomys specimen information of sequences retrieved from GenBank and used in the mtDNA analyses; Geographic origin of the samples and GenBank Accession No. are provided.

Species	Country	Locality	GenBank Acc. No.	Hap. cyt *b*
C. nivalis	Israel	Mt. Hermon	GQ150789[a]	H13
C. nivalis	Israel	Mt. Hermon	GQ150790[a]	H34
C. nivalis	Italy	Marta Alpi Liguri	GQ150794[a]	H24
C. nivalis	Italy	Val Masino	GQ150795[a]	H25
C. nivalis	Italy	Trento	AY513845[b]	H27
C. nivalis	Italy	Val Masino	GQ150796[a]	H15
C. nivalis	Italy	Valle d'Aosta	GQ150797[a]	H26
C. nivalis	Italy	Valle d'Aosta	GQ150798[a]	H29
C. nivalis	Italy	Gran Sasso	GQ150799[a]	H32
C. nivalis	Italy	Marta Alpi Liguri	GQ150800[a]	H39
C. nivalis	Italy	Valle d'Aosta	GQ150801[a]	H36
C. nivalis	Italy	Duchessa	GQ150802[a]	H38
C. nivalis	Italy	Trento	AY513846[b]	H27
C. nivalis	Macedonia	Mt. Pelister	GQ150791[a]	H10
C. nivalis	Slovakia	West Tatra Mts	AY513847[b]	H30
C. nivalis	Slovenia	Mt. Sneznik	GQ150792[a]	H35
C. nivalis	Slovenia	Mt. Sneznik	GQ150793[a]	H35
C. nivalis	Spain	Girona	AY513848[b]	H31
C. nivalis	Spain	Sierra de Gredos	AM392367[c]	H37
C. nivalis	Syria	Saleh	AY513849[b]	H14
C. nivalis	Turkey	Ciglikara	GQ150786[a]	H19
C. nivalis	Turkey	Ciglikara	GQ150787[a]	H20
C. nivalis	Turkey	Ciglikara	GQ150788[a]	H33
C. nivalis	Switzerland	Unknown	DQ663668[d]	
C. nivalis	Switzerland	Derborence	GU954316[e]	
C. nivalis	Switzerland	Derborence	GU954317[e]	
C. gud	Turkey	Ardahan	EU700087[f]	
C. roberti	Georgia	Datvisi	AY513851[b]	
C. roberti	Turkey	Altındere Vadisi	AY513850[b]	

References:
[a] Castiglia et al. (2009).
[b] Jaarola et al. (2004).
[c] Galewski et al. (2006).
[d] Fink et al. (2006).
[e] Fink et al. (2010).
[f] Buzan and Kryštufek (2008).

data from the growth hormone receptor gene (GHR) (Galewski et al., 2006), from the interphotoreceptor retinol-binding protein, exon 1 (IRPB) (Galewski, T., Tilak, M.K., Coskun, Y., Paradis, E., Douzery, E.J.P., data available on Genbank), from the first exon (EXON1) of the arginine vasopressin 1a receptor (avpr1a) gene, and the flanking non-coding upstream region (UPSTREAM) of the avpr1a EXON1 (Fink et al., 2007, 2010) (for origin and accession numbers see Supplementary Table S2). *A. terrestris* was used as the outgroup.

Supplementary tables and the alignments generated in this study have been deposited in the Dryad Repository: doi:10.5061/dryad.n5k77dd4.

2.2. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted using the QIAgen DNeasy Blood and Tissue kit (QIAgen, Germantown, MD, USA). Double-stranded DNA amplifications of partial cyt *b* were performed with primers L14841 and H15915 (Irwin et al., 1991; Kocher et al., 1989). PCR amplification was performed in a final volume of 25 µl generally containing 50–100 ng DNA. Cyt *b* amplification reaction contained 1× PCR buffer, 0.4 µM each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$ and 0.5 U *Taq* polymerase (QIAgen, Germantown, MD, USA), with cycling conditions as follow: 95 °C for 4 min, 40 cycles at 94 °C for 30 s, 58 °C for 1 min and 72 °C for 2 min, and a final elongation step at 72 °C for 10 min. Y-chromosome intron sequences (DBY7, DBY14 and UTY11) were obtained using Y-CATS primer pairs developed by Hellborg and Ellegren (2003). Amplification of the Y-chromosome introns carried out in a final volume of 25 µl containing 1× PCR buffer, 0.2 µM of each primer, 200 µM dNTPs, 2.5 mM $MgCl_2$, and 1 U *Taq* polymerase (QIAgen). PCR conditions included an initial denaturation step at 95 °C for 5 min, followed by a touchdown program including 40 cycles at 95 °C for 45 s, $T°C_{annealing}$ for 1 min and 72 °C for 1 min 30 s, where annealing temperature was decreased from 55 to 45 °C (UTY11) or from 60° to 50 °C (DBY7 and DBY14) by 0.5 °C/cycle in the first 20 cycles and followed by 20 cycles at the lower annealing temperature (*i.e.*, 45 °C or 50 °C, respectively) and a final extension of 72 °C for 10 min (see Yannic et al. (2008), for details). The specificity of Y-chromosome primers was determined by the absence of amplification products in females. PCRs were performed on a GeneAmp PCR Systems 2700 or 9700 (Applied Biosystems, Foster City, CA).

PCR products were checked on a 1% agarose electrophoresis gel and visualized with ethidium bromide staining to verify PCR quality. Purification of PCR products was conducted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Direct sequencing was performed using the Big Dye 3.1 Terminator cycle-sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions and nucleotide sequences were determined using an ABI PRISM 3130XL genetic analyzer (Applied Biosystems Foster City, CA, USA).

2.3. Phylogenetic analysis

Nucleotide sequences were edited in mega 4.0 (Tamura et al., 2007) and aligned using clustalx 2.0.12 (Thompson et al., 1997) using default parameters and then visually inspected, manually corrected and collapsed into haplotypes using DnaSP 5.10.01 (Librado and Rozas, 2009). The models of DNA substitution were selected using jModeltest 0.1.1 (Posada, 2008), based on the Akaike Information Criterion (AIC). The GTR + G + I model and HKY substitution models best fitted the cyt *b* dataset and the three Y-chromosome introns, respectively. The best-fitting nucleotide substitution model for non-coding nuclear gene and each codon position per coding nuclear gene was also evaluated using jmodeltest according to the AIC. Based on these selected substitution models, phylogenetic trees were constructed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML heuristic searches and bootstrap analyses (1000 replicates) were performed using PhyML 3.0 (Guindon et al., 2010; Guindon and Gascuel, 2003), optimizing the topology with both simultaneous NNI and SPR, using a BioNJ starting tree and adding 5 SPR tree searches using random starting trees. For cyt *b* and nuclear genes, BI was conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), using a full partition strategy (*i.e.*, each codon position for each coding gene was entered in a separate partition; Y-chromosome introns and concatenated alignments). The analyses were performed on concatenated sequences for the Y-chromosome, with an additional binary character matrix representing the presence/absence of indels and non-sequenced positions were treated as missing data in subsequent analyses (see Table 1). For all BI, two independent runs were performed, each consisting of four parallel MCMC chains of ten million generations. Trees were sampled every 1000 generations. To assess convergence among MCMC runs, the trends and distributions of log-likelihoods and parameter values were examined in Tracer 1.4 (Rambaut and Drummond, 2007), and the correlations of split frequencies among runs were examined in awty (Nylander et al., 2008). Samples showed patterns consistent with stationarity and convergence after at most one million generations for all runs and data sets; hence the first 10% of samples were discarded as burn-in for all analyses. The remaining trees were used to construct a 50% majority rule consensus tree. Node support was estimated using non-parametric bootstrap values

(BVs) (1000 replicates) in PhyML and posterior probabilities (PPs) in MrBayes. Median-joining (MJ) networks (Bandelt et al., 1999) depicting the evolutionary relationships among the cyt *b* haplotypes were inferred with Network 4.2.0.1 (http://www.fluxus-technology.com). The sequences were deposited in GenBank (see Table 1).

2.4. Molecular dating

We estimated divergence times with Beast 1.5.4 (Drummond and Rambaut, 2007) using a coalescent tree prior, which is adequate to study intraspecific diversification (Drummond et al., 2007). Many systematic uncertainties remain in the genus *Microtus*. Whereas the fossil record seems to indicate that separation of *Chionomys* from *Microtus* occurred less than 1.0×10^6 years ago (Myr), biochemical data suggest that isolation of *Chionomys* took place more than 2.4 Myr (Chaline and Graf, 1988). A fossil record is missing for most extant *Microtus* species, or it appears relatively late (Tamarin, 1985). Clock calibration was therefore based on the assumption of a Late Pliocene radiation of the basal lineages of *Microtus* (Chaline and Graf, 1988). To account for uncertainty of the calibration date, we used 0.2 Myr as its standard error. Preliminary analyses were performed with an uncorrelated lognormal relaxed clock to test if a strict molecular clock can be rejected (ucld.stdev parameter >1 with a frequency histogram not abutting 0). Because in our simulation the mean of the "ucld.stdev" parameter was 0.1 with a frequency histogram abutting 0, we chose a strict molecular clock for the final analyses (Drummond et al., 2007). Analyses were performed with two independent chains and 10 million generations; chains were sampled every 1000 generations with a burn-in of 2 million generations. We selected an appropriate burn-in based on examination of the trends and distributions of log-likelihoods and parameter values using TRACER 1.4 (Rambaut and Drummond, 2007).

3. Results

3.1. Chionomys and other Arvicolinae: cytochrome b gene and nuclear genes

The 941 bp analyzed for cyt *b* among arvicoline species showed 450 (48%) variable sites, of which 403 (43%) were parsimony-informative and 47 (5%) were singletons. No insertions or deletions were observed. The two phylogenetic methods yielded an identical topology of the main branches; only the topology from BI is shown (Fig. 2). The phylogenetic reconstruction revealed strong support for the monophyly of *Microtus* (and its allies *Blanfordimys* and *Neodon*)–*Chionomys*–*Stenocranius* (0.98/80), in a trichotomous relationship. The monophyly of *Chionomys* is evident (1.00/96) and *Microtus* emerged as paraphyletic with respect to *Blanfordimys* and *Neodon*. *Chionomys* is definitively related to *Microtus* and more phylogenetically distant to *Arvicola*, which was expected to be a sister genus to the clade of *Microtus* and its relatives. The phylogenetic position of *Arvicola* was, however, actually poorly resolved and *Lagurus lagurus* emerged as the closest sister genus of the clade with *Microtus*–*Chionomys*–*Stenocranius* (0.92/82), as previously shown by others (*e.g.*, Buzan et al., 2008).

A tree based on the combined nuclear sequences from IRBP (653 pb), GHR (860 bp), UPSTREAM (698 bp) and EXON1 (783 bp), showed *C. nivalis* as an offshoot of the *Microtus* species, irrespective of their region of origin (Nearctic: *M. ochrogaster, M. richardsoni, M. chrotorrhinus,* and *M. longicaudus*; Europe: *M. arvalis, M. agrestis* and *M. thomasi*; Asia: *M. kikuchii*; Holarctic: *M. oeconomus*). Phylogenies based on BI and ML methods revealed the same tree topologies (Fig. 3).

3.2. Chionomys: cytochrome b

The 41 *Chionomys* sequences of 941 bp used in this study showed 348 (37%) variable sites, of which 298 (32%) were parsimony-informative. No insertions or deletions were observed. The two phylogenetic methods yielded identical topologies of the main branches; only the topology from BI is shown (Fig. 4A). Each of the three species within *Chionomys* had strong support (Fig. 4A). Their respective origin is polytomic, although *C. gud* and *C. roberti* seem to be more closely related (but supported only by BI: 0.98/n.s.). Within *C. gud*, the two subspecies *gud* and *lghesicus* from Georgia and Dagestan, respectively, group together. The genetic differentiation between the two subspecies is not supported at all, while the specimen from Çam Geçidi (Ardahan, Turkey) is more phylogenetically distant. Within *C. roberti*, the two subspecies (*C. r. occidentalis* from Adygeya and *C. r. personnatus* from Northern Ossetia, Georgia and Turkey) have strong support (1.00/93).

Chionomys nivalis shows a strongly-supported basal dichotomy between *C. n. dementievi* (1.00/99) from Turkmenistan and all other specimens. The monophyly of the remaining *C. nivalis* subspecies is well supported (0.98/84) and several geographical groupings can be recognized. The first well-supported clade subspecies from the Caucasus (*C. n. trialeticus*), Turkey (*C. n. cedrorum*) and Israel (*C. n. hermonis*) (1.00/81). The second, poorly-supported group contains specimens from western European subspecies, *i.e.*, subspecies from Slovenia and Macedonia (1.00/95), Slovakia, Spain (1.00/98), and from the Alps and the Apennine. The samples from the Alps and the Apennine are divided into two clades (0.99/88 and 1.00/93, respectively).

3.3. Chionomys: Y-chromosome

The concatenation of the three Y-chromosome introns (DBY7, DBY14 and UTY11) produced a 2027 bp alignment comprising 24 different haplotypes. These exhibit 1493 informative sites, of which 54 are parsimony informative, and numerous insertions and deletions. The two methods (BI and ML) yielded similar results and only the results from BI are presented (Fig. 4B). The phylogeny obtained from the Y-chromosome introns is in agreement with results from cyt *b*, though the relationships between clades are partially unresolved (presumably due to the lower polymorphism inherent to Y-chromosomes). According to the Y-chromosome phylogeny, *C. roberti* and *C. gud* formed a strongly -supported clade (1.0/91), which is clearly differentiated from *C. nivalis*. Subspecies differentiations within these taxa are, however, poorly supported. No differentiation between *C. g. gud* and *C. g. lghesicus* haplotypes was observed. A polytomy within *C. roberti* does not allow differentiating *C. r. personnatus* from *C. r. occidentalis* with confidence. Importantly, these results further support the basal position of the haplotypes of the most eastern subspecies, *C. n. dementievi*, within *C. nivalis*. The sampling within other *C. nivalis* taxa is scarce and does not allow for further inference of phylogenetic relationships within *C. nivalis*.

3.4. Molecular dating

The dating analyses suggested an initial divergence between *Microtus* and *Chionomys* about 2.35 Myr (95% HPD: 1.94-2.73; with a calibration point from Chaline and Graf, 1988). The subsequent basal radiation of *Chionomys* was dated to 1.77 Myr (95% HPD: 1.36–2.19). *C. roberti* and *C. gud* diverged 1.48 Myr ago (95% HPD: 1.10–1.85). The basal radiation of *C. roberti* took place 0.299 Myr ago (95% HPD: 0.186–0.417) and the diversification of *C. gud* was dated to about 1.067 Myr (95% HPD: 0.766–1.397). The basal radiation of *C. nivalis* occurred 0.597 Myr ago (95% HPD: 0.417–0.794), when *C. nivalis dementievi* and the other *C. nivalis* subspecies

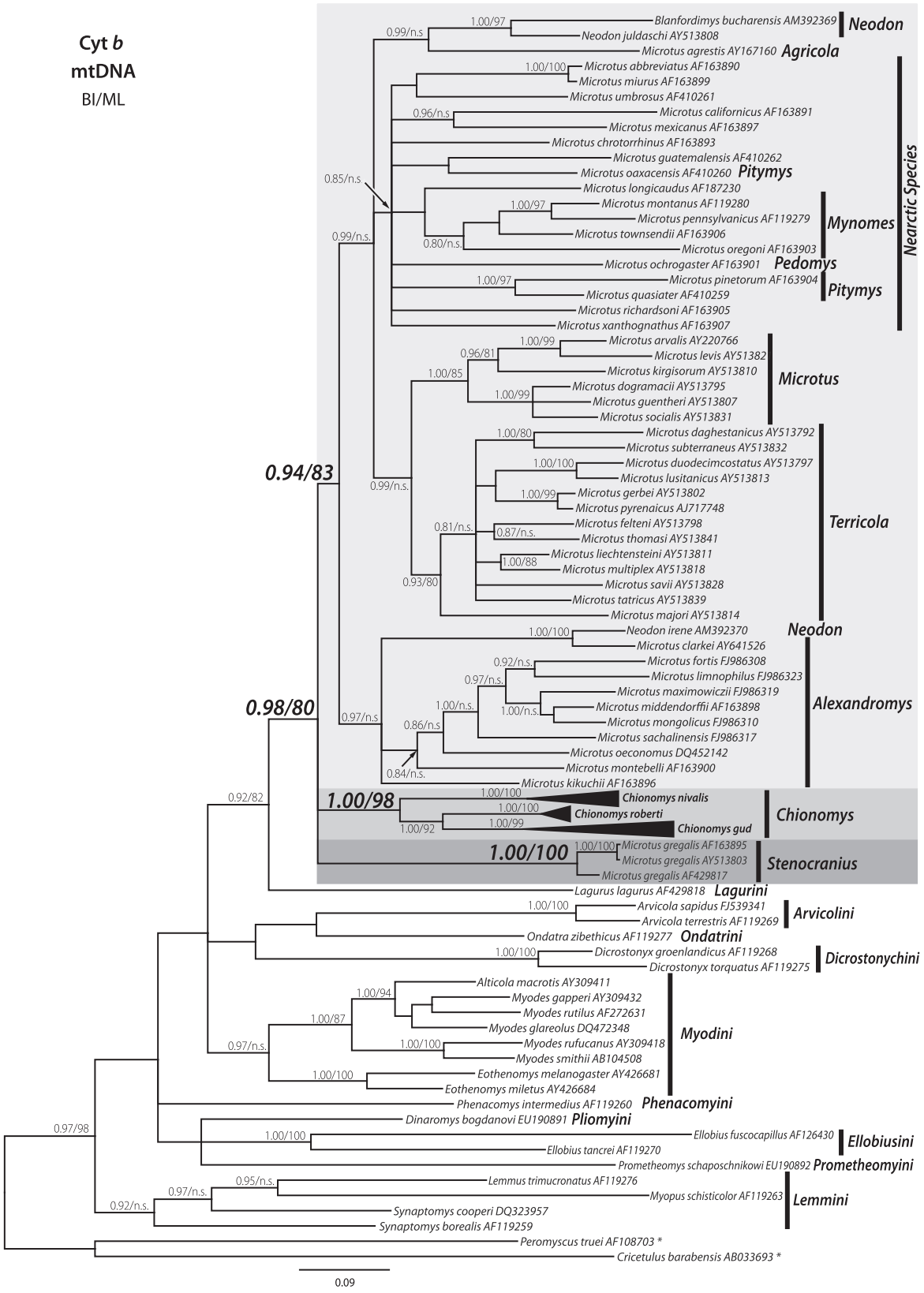

Fig. 2. Consensus Bayesian trees (50% majority rule) of the mitochondrial cyt *b* gene for 77 sequences representing 17 arvicoline genera generated using separate models for the three codon positions. Only PP values ⩾0.80 and BS values ⩾80% are given on branches. n.s. indicates that a method does not show a support value ⩾0.80/80%. Arvicoline tribe assigned according to Musser and Carleton (1993) and *Microtus* subgenus (Arvicolini) assigned according to Wilson and Reeder (2005). For unclear taxonomic status, only subgenus geographic region of origin is given. Arvicoline tribes assigned according to Musser and Carleton (1993). *Designated the outgroups.

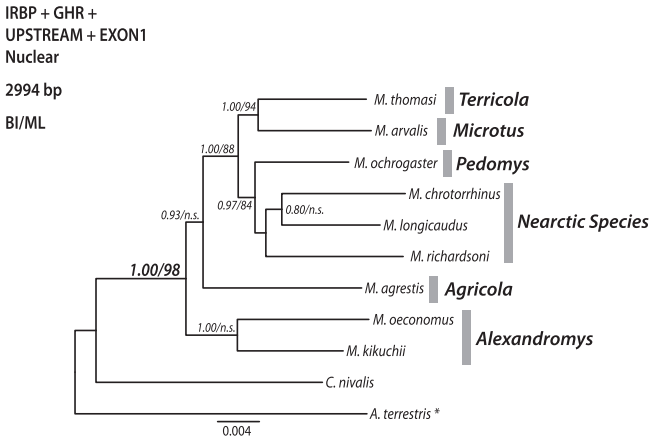

Fig. 3. Consensus Bayesian trees (50% majority rule) of the combined dataset of four nuclear markers (IRBP gene, part of the exon11 of the GHR gene, UPSTREAM and EXON1 of the avpr1a gene) obtained for *C. nivalis* and nine *Microtus* species, with *A. terrestris* used as the outgroup. Only PP values ⩾0.80 and BS values ⩾80% are given on branches. n.s. indicates that a method does not show a support value ⩾0.80/80%. *Microtus* subgenus assigned according to Wilson and Reeder (2005). For unclear taxonomic status, only subgenus geographic region of origin is given. *Designated the outgroup.

diverged. The western European subspecies of *C. nivalis* appeared about 0.271 Myr ago (95% HPD: 0.204–0.387).

4. Discussion

4.1. Phylogenetic origin of the genus Chionomys

There is a great deal of controversy regarding the systematics of the taxon *Chionomys*. The genus was originally treated as subgenus of *Microtus* for over 60 years (since Miller, 1912). *Chionomys* was later recognized as an independent genus based on isozymes (Graf and Scholl, 1975). Graf (1982) further supported this taxonomic position using isozymes again and showed an earlier divergence among *Chionomys* and the sister taxa *Arvicola–Microtus*, in agreement with paleontological data (Chaline and Graf, 1988). Taxonomic studies based on morphological traits later lead to the same conclusion (Gromov and Polyakov, 1992). Since then, the generic rank has not been debated (reviewed in Musser and Carleton, 1993; Nadachowski, 1991). Thereafter, several studies attempted to use molecular markers to resolve the phylogenetic position of *Chionomys* with regard to other *Microtus* species. Based on the cyt *b* gene, Jaarola et al. (2004) corroborated the ranking of *Chionomys* as a genus separate from *Microtus*. However, according to this study,

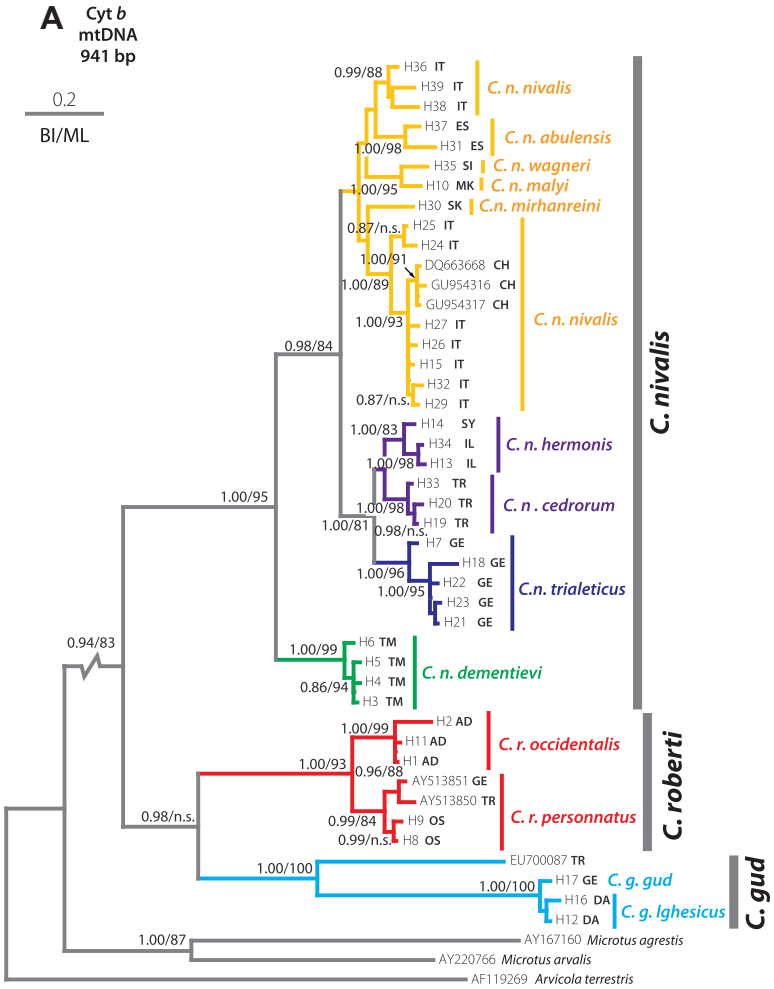

Fig. 4. Consensus Bayesian trees (50% majority rule) resulting from analyses of (A) the mitochondrial cyt *b* gene, generated using separate models for the three codon positions and (B) the combined dataset of the three Y-chromosome markers, DBY7, DBY14 and UTY11 (see Table 1 for specimen designations). Only PP values ⩾0.80 and BS values ⩾80% are given on branches. n.s. indicates that a method does not show a support value ⩾0.80/80%. List of the 2-letters country codes: Adygea (AD), Dagestan (DA), Georgia (GE), Liechtenstein (LI), Israel (IL), Italy (IT), North Ossetia-Alania (OS), Macedonia (MK), Slovakia (SK), Slovenia (SI), Spain (ES), Syria (SY), Switzerland (CH), Turkey (TR), Turkmenistan (TM).

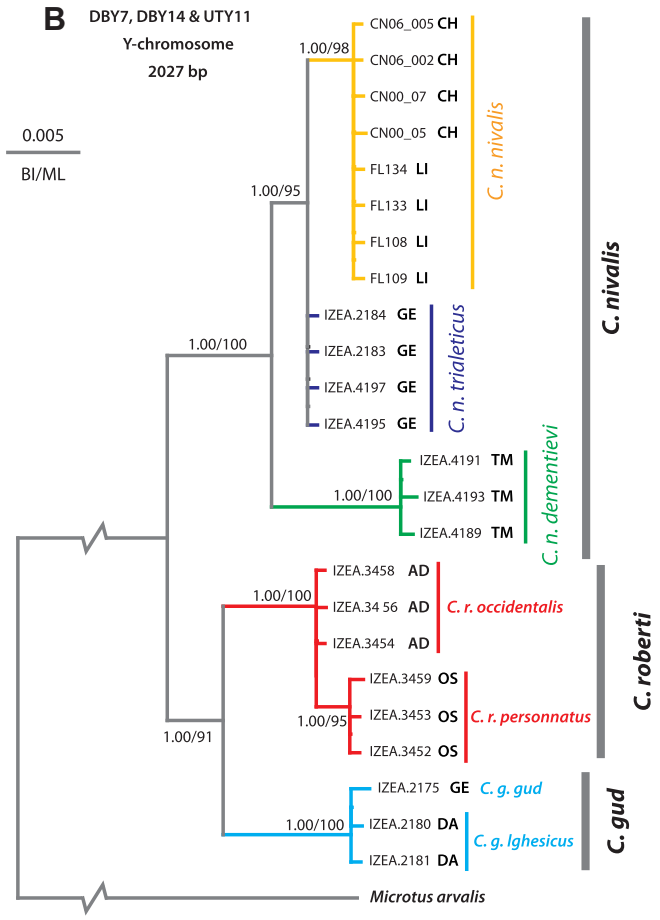

Fig. 4 (*continued*)

M. gregalis (subgenus *Stenocranius*) split earlier than *Chionomys*, supporting a closer relationship between other *Microtus* species and *Chionomys* (but with no or weak support according to the reconstruction method). In contrast, Buzan and Kryštufek (2008) suggested a *Chionomys* + *M. gregalis* clade, resulting in a problematic paraphyly of the genus *Microtus*. Combined data from the mitochondrial cyt *b* gene and the nuclear GHR gene revealed a basal position of *Chionomys* in the *Microtus* phylogeny with *Arvicola* placed at the base of Arvicolini (Galewski et al., 2006). Robovsky et al. (2008) reached the same conclusion by adding morphological characters to cyt *b* and GHR data sets. They found *Chionomys* consistently placed as a sister group of the rest of *Microtus* (Robovsky et al., 2008). The divergence between *Chionomys* and *Microtus* after the split leading to *Arvicola* was also shown by the combined analyses of the nuclear genes GHR and LCAT (Abramson et al., 2009). A recent genome-wide approach based on amplified fragment length polymorphisms (AFLP) and several DNA sequence markers showed *C. nivalis* as a basal offshoot of the other *Microtus* species for all but one marker, for which *C. nivalis* grouped within other *Microtus* species (Fink et al., 2010). The comprehensive species data set used in the present cyt *b* study includes all three recognized *Chionomys* species, in addition to 51 *Microtus* species (including *M. gregalis*), and 23 additional sequences, representing 10 out the 11 recognized arvicoline tribes. The results obtained suggest a closer phylogenetic position of *Chionomys* to *Microtus* and more distantly related to *Arvicola*. Our results thereby contradict the earlier conclusion of Graf and Scholl (1975) on a ancestral position of *Chionomys* to the clade including *Microtus* and *Arvivola*. The suggested sister relationship between *M. gregalis* (*Stenocranius*) and *Chionomys* (Buzan and Kryštufek, 2008) was not observed; it appeared with *Chionomys* and the main lineages of *Microtus* as a trichotomy. Our total evidence analyses based on four nuclear gene markers suggest a basal position of *C. nivalis* with respect to *Microtus*. At this stage, no objective criteria allow us to conclude on the generic or subgeneric status of *Chionomys*. Recent publications included *Chionomys* as a subgenus of *Microtus* (Chaline et al., 1999; Fink et al., 2010). The particular petricolous lifestyle of most *Chionomys* species (*C.* roberti is found in forest habitat; Kryštufek and Vohralik, 2005) distinguishes this taxon from *Microtus* and may be an argument in favor of generic ranking. However, considering the subjective nature of Linnean categories, no objective criterion can be used to assign rank of taxa (*e.g.*, see Dubois, 2007; Laurin, 2010). A solution is to include *Chionomys* and *Stenocranius* as subgenera of the genus *Microtus*. This is not only justified by the close phylogenetic relationships, but also by the avoidance of a paraphyletic taxon.

4.2. Phylogenetic relationships among Chionomys species

MtDNA and Y-chromosome molecular evidence suggest a division of *Chionomys* into two monophyletic lineages, the *nivalis* and *roberti/gud* groups. This division is also supported by dental morphological data (Nadachowski, 1991) and differences in the fundamental number of chromosomal arms (Zima and Král, 1984). Our molecular clock reconstruction estimates this split to have occurred in the Lower Pleistocene (1.77 Myr, 95% HPD: 1.36-2.19), while fossil data estimate it to have taken place later in the Early Pleistocene (Nadachowski, 1991). It is commonly accepted that *C. gud* and *C. roberti* probably appeared and evolved in the Near East or Caucasus (Buzan and Kryštufek, 2008), with subsequent divergence during the Middle Pleistocene, whereas *C. nivalis* would have evolved from a western mountain reclusion in the Alps, Carpathians or Pyrenees (*e.g.*, Castiglia et al., 2009; Nadachowski, 1991), consistent with paleontological data from the Holsteinian (420–375 ka) (Kowalski, 2001). Such a hypothesis, however, did not fit the Middle Pleistocene record of *C. nivalis* in Emirkaya-2 (Montuire et al., 1994) and on the island of Chios (connected to the mainland at that time; Storch, 1975), in better agreement with the estimated radiation of *C. nivalis* 0.597 Myr ago (95% HPD: 0.417–0.794). While the eastern origin of *C. gud* and *C. roberti* has never been questioned, the western origin of *C. nivalis* remained uncertain. The inclusion of the eastern *C. nivalis* subspecies was essential to obtain a complete picture of the phylogeographic origin of *C. nivalis*. The basal phylogenetic position of the eastern species *C. gud* and *C. roberti*, and of the eastern *C. nivalis* subspecies unambiguously establishes the Caucasus and Middle East as the region of origin of all *Chionomys* species, including *C. nivalis*.

4.3. Intraspecific relationships in C. nivalis

The phylogenetic reconstructions based on mtDNA and Y-chromosome data are congruent and reveal that the easternmost subspecies of *Chionomys nivalis* (*C. n. dementievi*) represents the oldest lineage within *C. nivalis*. This basal position clearly supports an eastern origin of the species. The question of specific or subspecific rank of this taxon is pertinent. The level of divergence between *C. n. dementievi* and other *C. nivalis* subspecies (3.78 ± 0.53% for the cyt *b*; see Supplementary Table S3) lies below the pragmatic >5% limit of interspecific differentiation as suggested by Baker and Bradley (2006); hence, mitochondrial data provide no evidence supporting the recognition of *C. n. dementievi* as a full species. Furthermore, crossing experiments between European Snow Voles from the Swiss Alps and Kopet-Dag showed no hampered reproduction (V. Malikov and P. Vogel, unpublished data) and may rather indicate subspecies level. In contrast to conclusions based on morphology

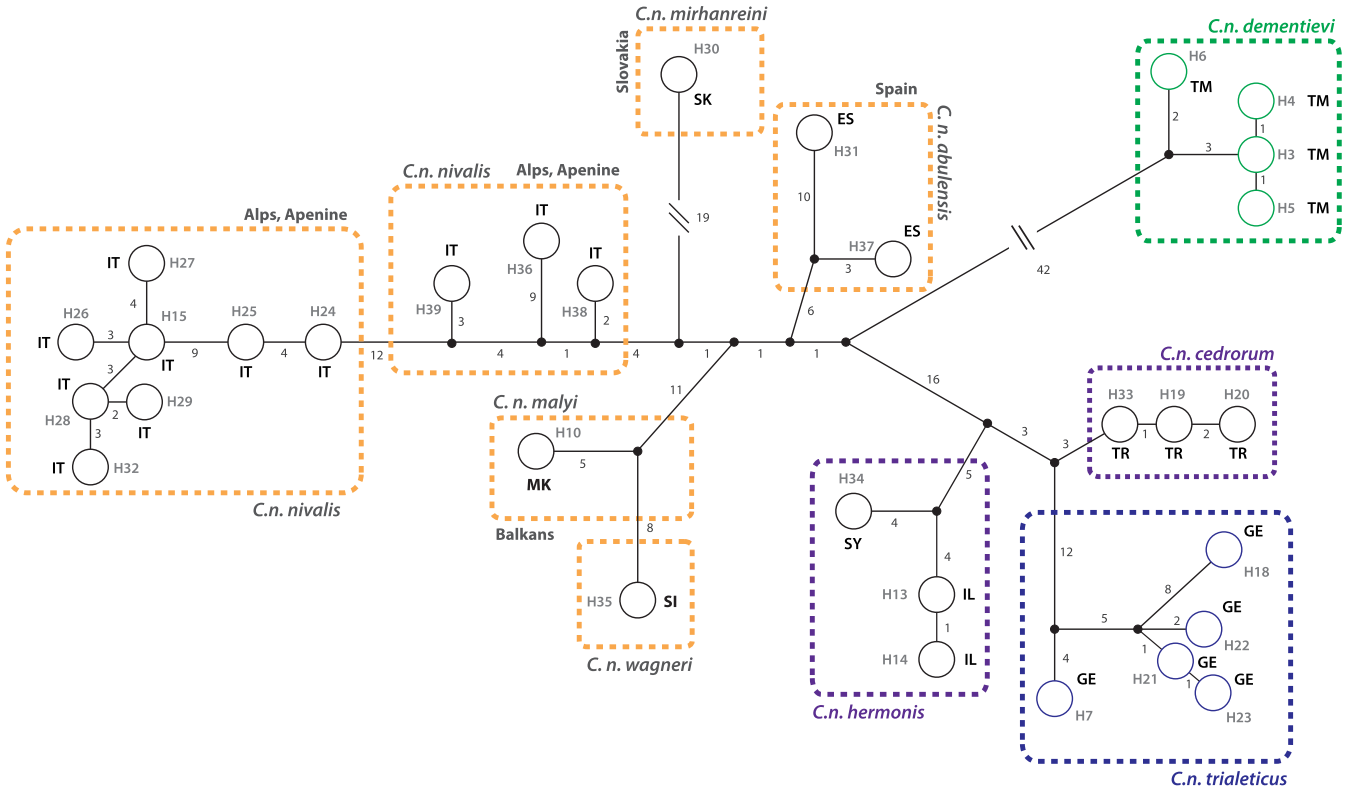

Fig. 5. Median-joining network depicting the evolutionary relationships among *C. nivalis* cyt *b* haplotypes inferred using Network 4.2.0.1. The haplotypes corresponding to the lineages and subspecies identified by the phylogenetic analyses are also indicated. List of the 2-letters country codes: Adygea (AD), Dagestan (DA), Georgia (GE), Liechtenstein (LI), Israel (IL), Italy (IT), North Ossetia-Alania (OS), Macedonia (MK), Slovakia (SK), Slovenia (SI), Spain (ES), Syria (SY), Switzerland (CH), Turkey (TR), Turkmenistan (TM).

(Nadachowski, 1991), our molecular data show that the subspecies *C. n. dementievi* (Kopet-Dag) and *C. n. trialeticus* (Caucasus) are not closely related. *C. n. trialeticus* is rather closely linked to the clade from the Near East, namely *C. n. hermonis* (Israel and Syria) and *C. n. cedrorum* (Turkey). The remaining haplotypes from Western Europe are closely related to each other. This is consistent with previous phylogeographic conclusions, but better explained by our median-joining network (Fig. 5) than by the minimum spanning network by Castiglia et al. (2009) that did not include the eastern clades (and see also Cassens et al. (2005) or; Woolley et al. (2008), for a discussion on the use of minimum spanning network method for phylogenetic reconstruction). It suggests that during the glaciations' cycles, *C. nivalis* persisted in several refugia, from which the species recolonized the mountain chains. However, larger sample sizes are needed for a sound reconstruction and corroboration of the phylogenetic relationships between the patchily distributed populations and subspecies of *C. nivalis* in order to disentangle effects of past isolation during the Last Glacial Maximum from current discontinuity due to strong geographical barriers on population structure.

4.4. Conclusion

The more comprehensive sampling of *Chionomys* snow voles, including the most eastern populations of *C. nivalis*, corroborates Caucasus and Middle East as the phylogeoprahic origin of the species *C. nivalis* and the genus *Chionomys.*

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References

Abramson, N., Lebedev, V., Bannikova, A., Tesakov, A., 2009. Radiation events in the subfamily Arvicolinae (Rodentia): evidence from nuclear genes. Doklady Biol. Sci. 428, 458–461.

Amori, G., 1999. *Chionomys nivalis* (Martins 1842). In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P., Spitzenberger, F., Stubbe, M., Thissen, J., Vohralik, V., Zima, J. (Eds.), Atlas of European Mammals. Academic Press, London, pp. 256–257.

Andera, M., Leffler, S., 1981. Microtus Schrank, 1798. In: Honacki, J., Kinman, K., Koeppl, J. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference. Allen Press, Inc. and The Association of Systematic Collections, Lawrence, Kansas, USA.

Baker, R.J., Bradley, R.D., 2006. Speciation in mammals and the genetic species concept. J. Mammal. 87, 643–662.

Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Buzan, E.V., Kryštufek, B., 2008. Phylogenetic position of *Chionomys gud* assessed from a complete cytochrome b gene. Folia Zool. 57, 274–282.

Buzan, E.V., Kryštufek, B., Hänfling, B., Hutchinson, W.F., 2008. Mitochondrial phylogeny of Arvicolinae using comprehensive taxonomic sampling yields new insights. Biol. J. Linn. Soc. 94, 825–835.

Cassens, I., Mardulyn, P., Milinkovitch, M., 2005. Evaluating intraspecific "network" construction methods using simulated sequence data: do existing algorithms outperform the global maximum parsimony approach? Syst. Biol. 54, 363–372.
Castiglia, R., Annesi, F., Kryštufek, B., Filippucci, M.G., Amori, G., 2009. The evolutionary history of a mammal species with a highly fragmented range: the phylogeography of the European Snow Vole. J. Zool. 279, 243–250.
Chaline, J., Graf, J.-D., 1988. Phylogeny of the Arvicolidae (Rodentia): biochemical and paleontological evidence. J. Mammal. 69, 22–33.
Chaline, J., Brunet-Lecomte, P., Montuire, S., Viriot, L., Curant, F., 1999. Anatomy of the arvicoline radiation (Rodentia): palaeogeo-graphical, palaeoecological history and evolutionary data. Ann. Zool. Fenn. 36, 239–267.
Corbet, G.B., 1978. The Mammals of the Palaearctic Region. A Taxonomic Review. British Museum of Natural History, London.
Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Drummond, A.J., Ho, S.Y.W., Rawlence, N., Rambaut, A., 2007. A Rough Guide to BEAST 1.4. <http://beast.bio.ed.ac.uk>.
Dubois, A., 2007. Phylogeny, taxonomy and nomenclature: the problem of taxonomic categories and of nomenclatural ranks. Zootaxa 1519, 27–68.
Ellerman, J., Morrison-Scott, T., 1966. Checklist of Palaearctic and Indian Mammals 1758–1946. British Museum (Natural History), London, United Kingdom.
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.
Fink, S., Excoffier, L., Heckel, G., 2006. Mammalian monogamy is not controlled by a single gene. Proc. Natl. Acad. Sci. USA 103, 10956–10960.
Fink, S., Excoffier, L., Heckel, G., 2007. High variability and non-neutral evolution of the mammalian avpr1a gene. BMC Evol. Biol. 7, e176.
Fink, S., Fischer, M.C., Excoffier, L., Heckel, G., 2010. Genomic scans support repetitive continental colonization events during the rapid radiation of voles (Rodentia: *Microtus*): the utility of AFLPs versus mitochondrial and nuclear sequence markers. Syst. Biol. 59, 548–572.
Galewski, T., Tilak, M., Sanchez, S., Chevret, P., Paradis, E., Douzery, E.J.P., 2006. The evolutionary radiation of Arvicolinae rodents (voles and lemmings): relative contribution of nuclear and mitochondrial DNA phylogenies. BMC Evol. Biol. 6, 80.
Graf, J.-D., 1982. Génétique biochimique, zoogéographie et taxonomie des Arvicolidae (Mammalia, Rodentia). Rev. Suisse Zool. 89, 749–787.
Graf, J.-D., Scholl, A., 1975. Variations enzymatiques et relations phylétiques entre neuf espèces de Microtinae (Mammalia, Rodentia). Rev. Suisse Zool. 82, 681–687.
Gromov, I.M., Polyakov, I.Y., 1992. Fauna of the USSR. Mammals, vol. III, No. 8. Voles (Microtinae). Nauka, Moscow-Leningrad.
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 692–704.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.
Hellborg, L., Ellegren, H., 2003. Y chromosome conserved anchored tagged sequences (YCATS) for the analysis of mammalian male-specific DNA. Mol. Ecol. 12, 283–291.
Hillis, D.M., Pollock, D.D., McGuire, J.A., Zwickl, D.J., 2003. Is sparse taxon sampling a problem for phylogenetic inference? Syst. Biol. 52, 124–126.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
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., Martinkova, N., Gunduz, I., Brunhoff, C., Zima, J., Nadachowski, A., Amori, G., Bulatova, N.S., Chondropoulos, B., Fraguedakis-Tsolis, S., Gonzalez-Esteban, J., Lopez-Fuster, M.J., Kandaurov, A.S., Kefelioglu, H., Mathias, M.D., Villate, I., Searle, J.B., 2004. Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 33, 647–663.
Kerey, I.E., Meric, E., Kelling, G., Brenner, R.A., Dogan, A.U., 2004. Black Sea-Marmara Sea Quaternary connections: new data from the Bosphorus, Istanbul, Turkey. Paleogeogr. Paleoclimatol. Paleoecol. 204, 277–295.
Kocher, T.D., Thomas, W.K., Meyer, 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.
Kowalski, K., 2001. Pleistocene rodents of Europe. Folia Quaternaria 72, 1–389.
Krapp, F.V., 1982. Microtus nivalis (Martins, 1842). Schneemaus. In: Niethammer, J., Krapp, F.V. (Eds.), Handbuch der Säugetiere Europas. Akademische Verlagsgesellschaft, Wiesbaden.
Kratochvil, J., 1981. *Chionomys nivalis* (Arvicolidae, Rodentia). Acta Sci. Nat. Acad. Sci. Bohemoslov. 15, 1–62.
Kryštufek, B., 1999. Snow voles, genus *Chionomys*, of Turkey. Mammalia 63, 323–339.
Kryštufek, B., Amori, G., 2008. *Chionomys*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. www.iucnredlist.org (downloaded on 25.12.10).
Kryštufek, B., Vohralik, V., 2005. Mammals of Turkey and Cyprus. Rodentia I: Sciuridae, Dipodidae, Gliridae, Arvicolinae. Koper, University of Primorska, Slovenia.
Laurin, M., 2010. The subjective nature of Linnean categories and its impact in evolutionary biology and biodiversity studies. Contrib. Zool. 79, 131–136.
Librado, P., Rozas, J., 2009. DnaSP ver. 5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Michaux, J., Reyes, A., Catzeflis, F., 2001. Evolutionary history of the most speciose mammals: Molecular phylogeny of muroid rodents. Mol. Biol. Evol. 18, 2017–2031.
Miller, G.S., 1908. The recent voles of the *Microtus nivalis* group. Ann. Mag. Nat. History 1, 97–103.
Miller, G., 1912. Catalogue of the Mammals of Western Europe. Reprint 1966, Johnson Reprint Corporation, London.
Montuire, S., Sen, S., Michaux, J., 1994. The Middle Pleistocene mammalian fauna from Emirkaya-2, Central Anatolia (Turkey): systematics and paleoenvironment. N. Jb. Geol. Palaontol. Abhand. 193, 107–144.
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.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24, 581–583.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Rambaut, A., Drummond, A., 2007. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.
Robovsky, J., Ricankova, V., Zrzavy, J., 2008. Phylogeny of Arvicolinae (Mammalia, Cricetidae): utility of morphological and molecular data sets in a recently radiating clade. Zool. Scr. 37, 571–590.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Storch, G., 1975. Eine mittelpleistozäne Nager-Fauna von der Insel Chios, Ägäis. Senckenbergiana Biol. 56, 165–189.
Tamarin, R.H., 1985. Biology of New World Microtus. American Society of Mammalogists, Shippensburg, PA.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol 24, 1596–1599.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Triant, D.A., DeWoody, J.A., 2007. Extensive mitochondrial DNA transfer in a rapidly evolving rodent has been mediated by independent insertion events and by duplications. Gene 401, 61–70.
Triant, D.A., DeWoody, J.A., 2008. Molecular analyses of mitochondrial pseudogenes within the nuclear genome of arvicoline rodents. Genetica 132, 21–33.
Triant, D.A., DeWoody, J.A., 2009. Integrating numt pseudogenes into mitochondrial phylogenies: comment on 'Mitochondrial phylogeny of Arvicolinae using comprehensive taxonomic sampling yields new insights. Biol. J. Linn. Soc. 2009, 225–226.
Wilson, D.E., Reeder, D.-A.M. (Eds.), 2005. Mammal Species of the World. A Taxonomic and Geographic Reference, third ed. Johns Hopkins University Press, Baltimore, MA.
Woolley, S., Posada, D., Crandall, K.A., 2008. A comparison of phylogenetic network methods using computer simulation. Plos One 3, e1913.
Yannic, G., Basset, P., Hausser, J., 2008. A new perspective on the evolutionary history of Western European *Sorex araneus* group revealed by paternal and maternal molecular markers. Mol. Phylogenet. Evol. 47, 237–250.
Zima, J., Král, B., 1984. Karyotypes of European mammals. Acta Sci. Nat. Acad. Sci. Bohem. Brno 18, 1–62.