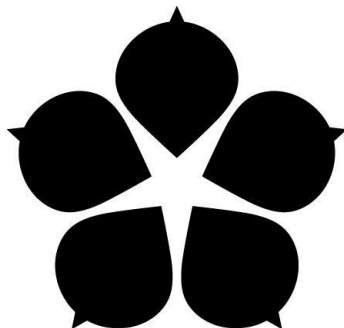


Jihočeská univerzita v Českých Budějovicích
Přírodovědecká fakulta



**Fylogeografické zhodnocení hraboše východoevropské s ohledem
k východokazašskému výskytu**

Rigorózní práce

Mgr. Tereza Holicová

České Budějovice 2019

Katedra zoologie Přf JU

Holicová T., 2019. New record of *Microtus mystacinus* in eastern Kazakhstan: phylogeographical considerations. RNDr. Thesis, University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic.

Annotation /Anotace:

The Eastern European vole (*Microtus mystacinus*) is an arvicoline rodent distributed across northern and eastern Europe, the Balkans, Turkey, Armenia, NW and N Iran, Russia as far east as the Tobol River in W Siberia, and W and N Kazakhstan. We present a novel records from eastern Kazakhstan (the village of Dzhambul – 49°14'21.3"N, 86°18'29.9"E and the village of Sekisovka – 50°21'9.18"N, 82°35'46.5"E) based on mtDNA and we discuss implications of this findings on biogeography of eastern Kazakhstan populations. Marine Isotope Stage 11 is considered an important period for the diversification of the arvalis species group. In the context of our study, it is important to analyse genetically discontinuous Siberian populations, and the current distribution of *M. mystacinus* in new localities in eastern Kazakhstan.

Prohlášení autora

Prohlašuji, že svoji rigorózní práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své rigorózní práce, a to v nezkrácené podobě Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

V Českých Budějovicích, 11. 9. 2019

Tereza Holicová

Podíl na publikaci

Autorka rigorózní práce získala pomocí molekulárních metod z poskytnutých vzorků sekvence cytochromu *b*. Tyto sekvence analyzovala a porovnávala s dříve publikovanými sekvencemi. Následně rekonstruovala fylogenezi jednotlivých geograficky definovaných populací hraboše východoevropského (*Microtus mystacinus*), včetně časů jejich divergence. Na základě výsledků analýz sepsala první verzi manuskriptu.

Finanční podpora

Spoluautoři (Macová A. a Vlček J.) byli finančně podpořeni grantem číslo 31-17-19831S.

Poděkování

Mé poděkování patří mému školiteli Janu Robovskému, a všem ostatním spoluautorům za poskytnutí odborné podpory a vzorků.

**HOLICOVÁ T., SEDLÁČEK F., MÁCOVÁ A., VLČEK J., &
ROBOVSKÝ J.**

2018

New record of *Microtus mystacinus* in eastern Kazakhstan:
phylogeographical considerations.

Zookeys (781): 67-80.

[Impact Factor: 1.143 (2019)]

New record of *Microtus mystacinus* in eastern Kazakhstan: phylogeographical considerations

Tereza Holicová¹, František Sedláček¹, Anna Mácová²,
Jakub Vlček¹, Jan Robovský¹

¹ Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

² Department of Parasitology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Corresponding author: Tereza Holicová (holic.ter@seznam.cz)

Academic editor: R. López-Antoñanzas | Received 29 March 2018 | Accepted 18 June 2018 | Published 13 August 2018

<http://zoobank.org/38FD3EFB-F36E-4E5B-84B4-FA4AD9FF5D80>

Citation: Holicová T, Sedláček F, Mácová A, Vlček J, Robovský J (2018) New record of *Microtus mystacinus* in eastern Kazakhstan: phylogeographical considerations. ZooKeys 781: 67–80. <https://doi.org/10.3897/zookeys.781.25359>

Abstract

The Eastern European vole (*Microtus mystacinus*) is an arvicoline rodent distributed across northern and eastern Europe, the Balkans, Turkey, Armenia, NW and N Iran, Russia as far east as the Tobol River in W Siberia, and W and N Kazakhstan. We present a novel records from eastern Kazakhstan (the village of Dzhambul – 49°14'21.3"N, 86°18'29.9"E and the village of Sekisovka – 50°21'9.18"N, 82°35'46.5"E) based on mtDNA and we discuss implications of this findings on biogeography of eastern Kazakhstan populations. Marine Isotope Stage 11 is considered an important period for the diversification of the *arvalis* species group. In the context of our study, it is important to analyse genetically discontinuous Siberian populations, and the current distribution of *M. mystacinus* in new localities in eastern Kazakhstan.

Keywords

Microtus mystacinus, Kazakhstan

Introduction

The Eastern European vole, *Microtus mystacinus* De Filippi, 1865, is an arvicoline rodent with an unsettled nomenclature. It has been named most commonly as *M. subarvalis* Meyer, Orlov & Skholl, 1972, *M. epiroticus* Ondrias, 1966, *M. rossiaemeridionalis*

Ognev, 1924, and *M. levis* Miller, 1908 (e.g., Musser and Carleton 2005; Kryštufek and Vohralík 2005). We adhere to the name *M. mystacinus*, following the detailed study by Mahmoudi et al. (2017) and the review of Kryštufek (2017). Despite its nomenclature instability, there is a consensus about its phylogenetic affinities: this species has been traditionally attributed to the *arvalis* species group in the subgenus *Microtus* s. str. (Musser and Carleton 2005). This view has been strongly supported by chromosomal and genetic evidence (e.g., Mazurok et al. 2001, Jaarola et al. 2004, Mahmoudi et al. 2017). According to new studies, it is related to the following species: *M. ilaeus* Thomas, 1912 (syn. *M. kirgisorum* Ognev, 1950), *M. transcaspicus* Satunin, 1905, *M. kermanensis* Roguin, 1988, *M. arvalis* (Pallas, 1778), and *M. obscurus* (Eversmann, 1841) (e.g., Golenishchev et al. 2000; Jaarola et al. 2004; Kryštufek and Vohralík 2005; Mahmoudi et al. 2017), but it is the closest relative of *M. arvalis* and *M. obscurus* based on available DNA data (cyt *b*; Mahmoudi et al. 2017).

In general, *M. mystacinus* represents one of the best cases of a cryptic species in arvicolines, because it was primarily recognized by chromosomal number (*M. mystacinus*: $2n = 54$; *M. arvalis*: $2n = 46$) (Meyer et al. 1969; Mazurok et al. 2001; Pavlova and Tchabovsky 2011). It is now generally considered a valid species of the genus *Microtus* based on hybridisation data, and chromosomal and genetic differences (for reviews see Kryštufek and Vohralík 2005 and Musser and Carleton 2005). Several authors have attempted to distinguish *M. mystacinus* from the common vole (*M. arvalis*), the Altai vole (*M. obscurus*), and the Middle Eastern vole (*M. transcaspicus*) based on morphological data (Král et al. 1981; Zagorodnyuk 1991a, b; Masing 1999; Hotzi et al. 2008; Markova et al. 2009, 2012; Markov et al. 2012; Ghorbani et al. 2015). Although some diagnostic characters have been proposed (e.g., qualitative and quantitative cranial and dental morphology) and multivariate morphometric approaches have been applied (e.g., Markov et al. 2012; Markova et al. 2012), these approaches have been lacking in diagnostic power (Kryštufek and Vohralík 2005; Markov et al. 2012), except for characters proposed by Kryštufek and Vohralík (2005).

The distribution and habitat preferences of the Eastern European vole are relatively well known due to the intensive attention devoted to it (see Kryštufek and Vohralík 2005; Musser and Carleton 2005; Shenbrot and Krasnov 2005; Kryštufek 2017, and references therein). It prefers to live in places with high and dense herbaceous or grassy vegetation, hedgerows, and stands of reeds and it avoids short-grass meadows and dry areas (Kryštufek and Vohralík 2005; Aulagnier et al. 2009; Kryštufek 2017). The distribution range of the Eastern European vole, to date, extends from southern Finland, the Baltic eastwards to western Siberia with patches in the southern Urals, the Novosibirsk suburbs to the southwest margin of Lake Baikal and Buryatia, the southern Caucasus, northern Iran to Turkey, connecting to Greece and the majority of the Balkan Peninsula to Ukraine (Baskevich 1996; Gileva et al. 1996; Yakimenko and Kryukov 1997; Musser and Carleton 2005; Shenbrot and Krasnov 2005; Pavlova and Tchabovsky 2011; Ghorbani et al. 2015; Baskevich et al. 2016; Kryštufek 2017; Moroldoev et al. 2017).

Populations occupying the Arctic Svalbard Archipelago (Fredga et al. 1990; recently extinct according to Aulagnier et al. 2009), Jan Mayen Island in the N Atlantic (Kryštufek

2017), Olkhon Island in Lake Baikal (Pavlova and Tchabovsky 2011; Kryštufek 2017) and Far Eastern Russia (Khabarovsk Krai, near Sovetskaya Gavan City, see Kartavtseva et al. 2012; Tiunov et al. 2013) are probably introduced. *M. mystacinus*, *M. arvalis*, and *M. obscurus* broadly overlap in distribution and occur sympatrically in a few regions (e.g., Meyer et al. 1996; Musser and Carleton 2005; Shenbrot and Krasnov 2005 see also Tougard et al. 2013).

When considering the distribution of *M. mystacinus* within Kazakhstan, there are records from the western or north-western parts. The easternmost record is from the Karabalyk district (Kovalskaya 1994; Meyer et al. 1996). Here, we report an additional record of *M. mystacinus* from eastern Kazakhstan and comment on it from a phylogeographic point of view.

Materials and methods

A survey of small mammals conducted in eastern Kazakhstan provided the surprising discovery of three specimens of *M. mystacinus*, that are characterized here based on molecular methods. The first sample (Kazakhstan 1) was collected in July 2006 on pasture land near the village of Dzhabul (GPS coordinates: 49°14'21.3"N, 86°18'29.9"E) by FS and two more specimens (Kazakhstan 2, 3) were collected in September 2017 near a pond not far from the village Sekisovka (GPS coordinates: 50°21'9.18"N, 82°35'46.5"E) by AM and JV.

DNA extraction was carried out using the Genomic DNA Mini Kit – tissue (Geneaid, New Taipei, Taiwan). We amplified the mitochondrial gene cytochrome *b* (cyt *b* hereinafter) using universal primers L14724, L15162, H15149 and H15915 (Irwin et al. 1991). Amplification conditions for cyt *b* consisted of 37 thermal cycles, an initial denaturation step at 94 °C for 3 min, denaturation at 94 °C for 30 seconds, annealing at 50 °C for 1 min, extension at 72 °C for 1.5 min and final extension at 72 °C for 10 min. Sequences were obtained using the Sanger sequencing (Sanger et al. 1977) services at laboratory SEQme s.r.o. (Dobříš, Czech Republic).

We obtained 1137 base pairs long sequences that satisfied the quality of base pairs (GenBank access number LT970847-LT970849). These were compared using available sequences from GenBank, specifically with 250 specimens that comprise all available sequences of *M. mystacinus* (under names *M. levis*, *M. rossiameridionalis* and *M. mystacinus*), and representative sequences of particular clades in *M. arvalis* and *M. obscurus* associated with previous studies (Baker et al. 1996a, b; Haynes et al. 2003; Fink et al. 2004; Jaarola et al. 2004; Triant and DeWoody 2007; Bužan et al. 2010; Thanou et al. 2012; Tougard et al. 2013; Stojak et al. 2016; Mahmoudi et al. 2017). Several more sequences (*M. kirgisorum*, accession number AY513809, AY513810; *M. socialis*, accession number AY513830, AY513831; and *M. transcaspius*, accession number KX581067-KX581075) were downloaded from GenBank as potentially outgroups. The obtained sequences were aligned using the ClustalW algorithm implemented in GENEIOUS v.10.0.5 (Kearse et al. 2012). We employed a likelihood (ML) and Bayes-

ian inference method (BI) for phylogenetic analyses. Likelihood phylogenetic analyses were conducted using the PhyML plugin for GENEIOUS. Final Bayesian phylogenetic analyses were conducted in BEAST 2.4.5.0 (Drummond et al. 2012), where phylogenetic relationships were reconstructed under the Yule speciation process (Steel and McKenzie 2001) with the GTR model of evolution detected in JModelTest 2.1.7 (Nylander 2004) under the Akaike Information Criterion (AIC). The nucleotide data were run for 30 000 000 generations with a sampling frequency of every 1000th generation; with final burn-in set at 20%. Time estimations were also computed in BEAST2 (Drummond et al. 2012) for the topology detected by the Bayesian phylogenetic analysis. We adopted one fossil calibration point (0.475±0.025 Mya for the origin of *M. arvalis*; Miesenheimer I; Tougaard et al. 2013) to estimate divergence time in studied taxa and to compare estimations with Mahmoudi et al. (2017) (which are based on the following proposed molecular clock rate, 3.27×10^{-7} mutations/site/year for *M. arvalis*; Martínková et al. 2013). The split time with 95% highest posterior density was applied to a relaxed-clock model assuming a constant population size. The convergence and stability of estimated parameters was checked using TRACER 1.6 (Rambaut et al. 2017) and the maximum clade credibility trees were obtained with TREEANNOTATOR 2.4.5.0, and visualized in FIGTREE 1.4.3 (Rambaut 2009).

Some analyses were applied for *M. mystacinus* only. Specifically, haplotype characteristics were identified using DnaSP version 5.0 (Rozas et al. 2003) and the degree of diversification was estimated based on average pairwise distances using the Kimura two-parameters model of substitutions in MEGA5 (Tamura et al. 2011). The detailed haplotype network was conducted in POP ART 1.7 using the median-joining method (Bandelt et al. 1999).

Results and discussion

The obtained sequences of 1137 base pairs from three specimens exhibited close relationships with available *cyt b* sequences of *Microtus mystacinus*, in all comparisons. Specifically, they were nested inside this species, so our study identified this species in eastern Kazakhstan (see also below). All sequences of *M. mystacinus* form a sister group to the *M. obscurus* + *M. arvalis*, in accordance with previous comprehensive studies (e.g., Haynes et al. 2003; Fink et al. 2004; Jaarola et al. 2004; Triant and DeWoody 2007; Tougaard et al. 2013; Stojak et al. 2015, 2016; Mahmoudi et al. 2017).

Considering the intraspecific structure in *Microtus mystacinus*, we can distinguish two deep lineages (Iran, abbreviated as IR) and the rest of populations mostly from Europe, additionally divided into several sub-lineages (TU, EU, GK), concordantly in ML and BI phylogenetic trees and the haplotype network (see Figure 1). This structure, specifically groups IR, TU, and EU, were identified firstly by Mahmoudi et al. (2017). TU lineage consists of Turkish and Armenian samples (without specimen Armenia 1), EU lineage of samples from the majority of Europe, mainly from Ukraine and Romania except for specimens from Greece, which comprise GK lineage, as well as samples

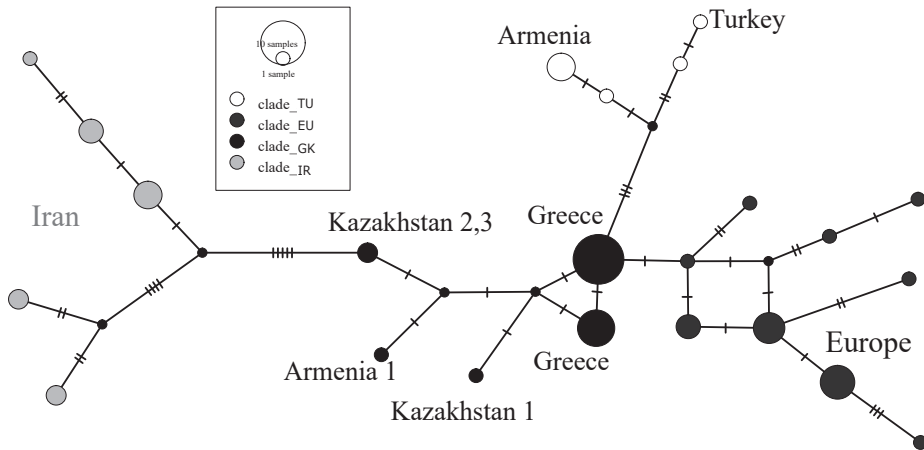


Figure 1. Median Joining Network based on the *cyt b* sequences of *M. mystacinus*.

Table 1. The K2P Inter – and intra-species average estimates of K2 genetic distance for *cyt b* in recognized lineages of *M. mystacinus* (TU – Turkey, Armenia; EU – Europe; GK – Greece, Kazakhstan; IR – Iran).

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1. TU	0.007									
2. EU	0.025	0.007								
3. GR	0.021	0.016	0.006							
4. Armenia_1	0.024	0.019	×	×						
5. Greece	0.016	0.011	×	0.009	0.001					
6. Kazakhstan	0.023	0.018	×	0.007	0.008	0.006				
7. IR	0.035	0.044	0.031	0.031	0.034	0.028	0.013			
8. <i>M. obscurus</i>	0.067	0.066	0.065	0.062	0.066	0.059	0.068	0.028		
9. <i>M. arvalis</i>	0.067	0.057	0.065	0.062	0.066	0.063	0.067	0.059	0.003	
10. <i>M. transcaspicus</i>	0.075	0.079	0.071	0.069	0.072	0.065	0.068	0.067	0.084	0.004

from eastern Kazakhstan and the specimen 1 from Armenia. This pattern indicates a complex diversification of *M. mystacinus* across its former and current distribution.

In general, *Microtus mystacinus* exhibited rather low intraspecific *cyt b* distances (except for the Iranian subset) and the obtained interspecific *cyt b* distances (see Table 1) are very similar to the values published in other studies (*M. arvalis* × *mystacinus*: Jaarola et al. (2004): 6–8%; Mahmoudi et al. (2017): 6–7%). As the intraspecific divergence for *Microtus mystacinus* and its cryptic diversity was intensively discussed by Mahmoudi et al. (2017), we would like to note only that the genetic distances cannot be presented as an absolute criterion for deciding whether two operational taxonomic units are distinct species (for detail see Groves et al. 2017), and in the case of species within the *arvalis*-group, some currently recognized species with rather low genetic distances exhibit infertile hybrids or hybrids with a reduced fertility (Meyer et al. 1985; Golenishchev et al. 2000; Jaarola et al. 2004).

The estimated clade divergence times varied substantially according to the calibration used (see Table 2). In summary, our estimations are more similar with other esti-

Table 2. Time to the most recent common ancestor (TMRCA and 95% HPD lower/upper limit – in million years) with BEAST2 for particular *Microtus* species (T – *M. transcaasicus*, M – *M. mystacinus*, O – *M. obscurus*, A – *M. arvalis*) and recognized lineages of *M. levis* (TU – Turkey, Armenia; EU – Europe; GK – Greece, Kazakhstan; IR – Iran).

Nodes	Analysis 1 – fossil calibrations		Mahmoudi et al. 2017	Tougard et al. 2013
	TMRCAs	95% HPD	TMRCAs (95%HPD)	TMRCAs (95%HPD)
a. T+M+O+A	1.102	0.77–1.28	0.238 (0.16–0.35)	–
b. M+O+A	0.797	0.60–1.05	0.217 (0.15–0.31)	0.531 (0.42–0.67)
c. O+A	0.616	0.51–0.78	0.184 (0.12–0.26)	0.478 (0.40–0.56)
d. T	0.537	0.32–0.57	0.040 (0.01–0.08)	–
e. O	0.410	0.27–0.58	0.119 (0.07–0.18)	0.173 (0.10–0.29)
f. A	0.490	0.48–0.54	0.146 (0.10–0.21)	0.446 (0.39–0.49)
g. IR+ EU+GK+TU	0.575	0.04–0.77	0.147 (0.09–0.22)	0.033 (0.00–0.08)
h. EU+GK+TU	0.408	0.28–0.57	0.092 (0.05–0.14)	–
i. EU+GK	0.332	0.23–0.47	–	–
j. TU	0.235	0.10–0.40	0.022 (0.01–0.04)	–
k. EU	0.219	0.14–0.32	0.075 (0.05–0.11)	–
l. GK	0.280	0.19–0.40	–	–
m. IR	0.390	0.24–0.47	0.117 (0.06–0.18)	–

mates based on fossil calibration points (albeit slightly higher) than with estimations based on mutation rates (see Table 2). Focusing on the most studied species, *M. arvalis*, we estimate its time to the most recent common ancestor (TMRCA) as 0.490 Mya, Tougard et al. (2008) 0.472 Mya and Tougard et al. (2013) 0.446 Mya, Stojak et al. (2015, 2016) 0.064–0.067 Myr and Mahmoudi et al. (2017) 0.146. Our estimation is similar to Tougard et al. (2008, 2013) as a logical result of the utilization of the same fossil calibration point, but all other specified estimations are much lower and associated with the same mutation rate (3.27×10^{-7} substitutions/site/year) proposed by Mar-tínková et al. (2013) specifically for *Microtus arvalis* based on a recent geological event. It is not easy to judge which values are realistic, but our estimates seem to be compatible with other phylogenetic studies (e.g., Mazurok et al. 2001; Bannikova et al. 2010) and the fossil record (e.g., Cuenca-Bescós et al. 2001; Markova et al. 2012). Based on this compatibility, we adhere to the values of our estimations. In any case, it would be worth to compare different calibrations methods under different calibrations points and proposed mutations rates in future (e.g., methods of Baker et al. 1996a; Jaarola and Searle 2002), and also to consider the potential biases of the fossil record (e.g., incomplete nature, process of geological dating, reliability of species identification; cf. Ho 2007).

Evolution and diversification of arvicoline rodents, including the *arvalis*-group, has been closely related to Quaternary climatic oscillations and the associated abiotic and biotic environmental factors (e.g., Horáček and Ložek 1988; Horáček 1990; Chaline et al. 1999; Stojak et al. 2016; Tougard 2017 and references therein). For the *arvalis*-group, interglacial periods are considered to be periods of species expansions and glacials as periods of retractions with potential survival of particular species in refu-

gia (e.g., Golenishchev et al. 2000; Tougard et al. 2008; Stojak et al. 2015; Stojak et al. 2016). Golenishchev et al. (2000) considered one of the ancient alpine glaciations as responsible for disrupting the geographic range of *M. arvalis* and *M. obscurus*, whereas Tougard et al. (2008) considered interglacials as the agents of speciation. Based on our time estimations, the diversification of *M. mystacinus* + (*M. arvalis* + *M. obscurus*) group has happened within the last 0.79 Mya, thus comprising several interglacial and glacial periods (Gates 1993; Sirocko et al. 2007; Mahmoudi et al. 2017).

In our data, we observed synchronous, deep intraspecific divergences in all three species around 0.49–0.41 Mya (see Figure 2; in *M. mystacinus* we operated with separate timelines for the Iranian lineage (IR) and the remainder (sub-lineages TU, EU, GK) because the Iranian populations are divergent from the others; pairwise distance shows significant variation, see Table 1). This interval corresponds to the Holstein interglacial period (considering the stratigraphy of Western Europe) that is considered to be equivalent to Marine Isotope Stage (MIS) 11 (Sirocko et al. 2007; see Figure 2). The influence of the Holstein on the *arvalis*-group diversification can be explained by two historical scenarios. First, the preceding period, MIS 12, was characterized by a pronounced cold period (around 0.460 Mya), during which the earliest pan-Eurasian mammoth fauna associated with tundra-steppe habitats (called mammoth steppe, see Guthrie 2001) was formed. Second, the warmest phase of MIS 11 is the phase with the highest temperatures in the last 500 thousand years, persisting, persisting two times longer than the Eemian interglacial and three times longer than the Holocene (Sirocko et al. 2007). Interglacial conditions may have disrupted the mammoth steppe biome due to an increase in precipitation, temperature, and associated forest expansions (for Late Quaternary see Řičánková et al. 2018). Tougard et al. (2008) recognized that the evolutionary history of temperate small mammals is much more complex than previously suggested. Individual species responded to various factors in multiple ways, and at different times during the Pleistocene (Lorenzen et al. 2011). Therefore, we tend to be reserved about whether observed pulses in diversification could be interpreted as expansion alongside some geographical/biotope barriers or fragmentation of some particular populations.

To conclude, our study proved an additional occurrence of *Microtus mystacinus* in Kazakhstan. The studies of Kovalskaya (1994), Meyer et al. (1996) and Okulova et al. (2014) specified the distribution of this species from western or northwestern parts of Kazakhstan, with the easternmost observation from the Karabalyk district (Kovalskaya 1994). Other localities of this species are known around Novosibirsk, several hundred kilometres away from the Kazakhstani border (Pavlova and Tchabovsky 2011). Although our material is not suitable to establish the full distribution range in Kazakhstan, it enables us to extend the range of this species further south.

The distribution of *M. mystacinus* could be partly human-induced, as documented by Tiunov et al. (2013) when regarding the railway across Siberia and the Far East of Russia (e.g., Olkhon Island, Pavlova and Tchabovsky 2011; Buryatia, Moroldoev et al. 2017). If we consider this possibility, the locality near Sekisovka is approx. 30 km distant from the nearest railway from Ust-Kamenogorsk to Ridder, but our second locality (near Dzhambul) is more than 150 km distant from the nearest

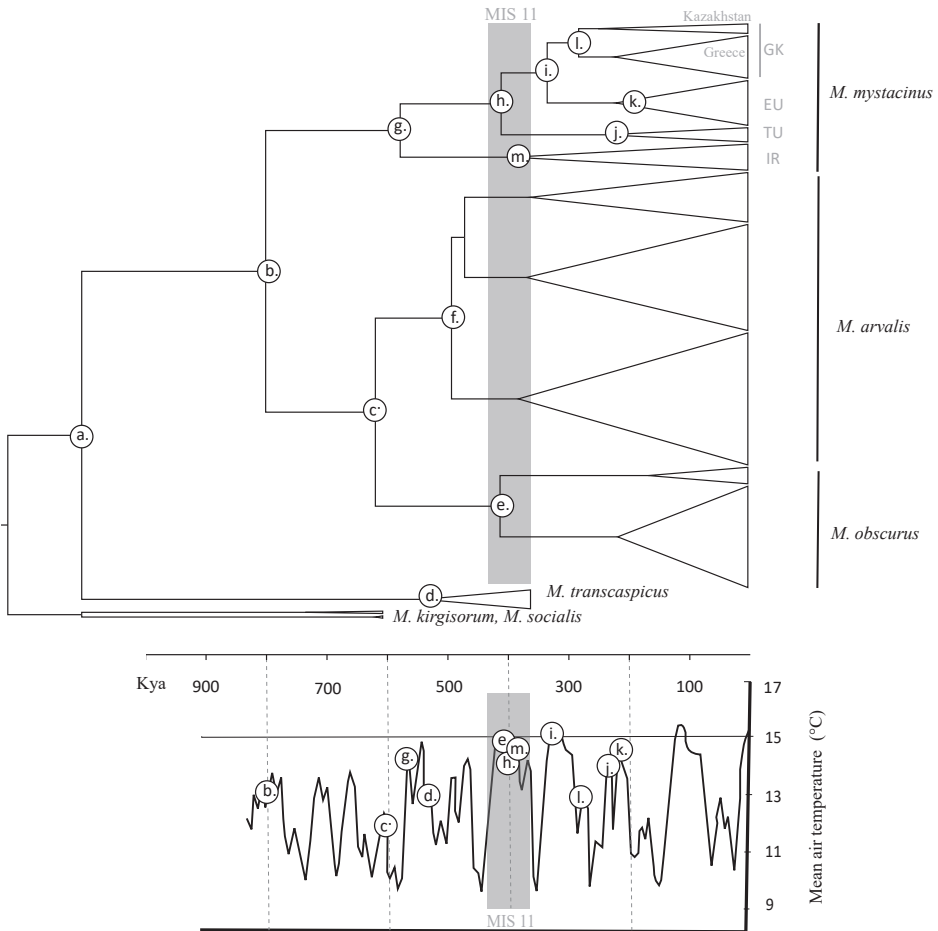


Figure 2. Time of the most recent common ancestor (TMRCA) for *Microtus* species and lineages of *M. mystacinus* using fossil calibrations. Nodes are plotted on a mean air temperature curve in last 800 thousand years (based on Gates 1993). See Table 2 for time estimates.

railway at Zyryanovsk (built after 1930; according to official web page of KTZ – КАЗАКСТАН ТЕМІР ЖОЛЫ). In Russian territory, this species shows pathways of invasion around the Transbaikalia railway and the surrounding agricultural landscape (e.g., Tiunov et al. 2013, Moroldoev et al. 2017). As the Kazakhstani specimens are significantly divergent from other available sequences (approx. 100 kya), we could consider the distribution of *M. mystacinus* in Kazakhstan as natural, but additional evidence is welcomed. Based on the presented network-phylogenetic relationship of samples it seems that a potential route of colonization for Kazakhstan populations could have originated somewhere between the Balkans and sites north of the Black

and Caspian seas, whereas populations in Turkey and parts of Armenia were colonized from a southern route.

Our study is the first genotyping of *M. mystacinus* from the eastern part of its distribution, where its occurrence is more discontinuous. In the context of our study, it is important to analyse genetically these Baikal and Far Eastern populations, and further map out the extent of *M. mystacinus* occurrence in East Kazakhstan.

Acknowledgements

We would like to thank Professor Jan Zrzavý for financial support; AM and JV were supported by grant number 31-17-19831S. We also would like to express our gratitude to Joel James Brown and Nathalie Yonow for professional language editing and to the editor and reviewers for their very valuable comments which helped to improve our manuscript.

References

- Aulagnier S, Haffner P, Mitchell-Jones AJ, Moutou F, Zima J (2009) Mammals of Europe, North Africa and the Middle East. A and C Black Publishers, London, 272 pp.
- Baker RJ, Hamilton MJ, Van Den Bussche RA, Wright AJ, Wiggins LE, Hamilton MJ, Reat EP, Smith MH, Lomakin MD, Chesser RK (1996a) Small mammals from the most radioactive sites near the Chernobyl nuclear power plant. *Journal of Mammalogy* 77(1): 155–170. <https://doi.org/10.2307/1382717>
- Baker RJ, Van Den Bussche RA, Wright AJ, Wiggins LE (1996b) High levels of genetic change in rodents of Chernobyl. *Nature* 380(6576): 707–708. <https://doi.org/10.1038/380707a0>
- Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16(1): 37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Bannikova AA, Lebedev VS, Lissovsky AA, Matrosova V, Abramson NI, Obolenskaya EV, Tesakov AS (2010) Molecular phylogeny and evolution of the Asian lineage of vole genus *Microtus* (Rodentia: Arvicolinae) inferred from mitochondrial cytochrome *b* sequence. *Biological Journal of the Linnean Society* 99(3): 595–613. <https://doi.org/10.1111/j.1095-8312.2009.01378.x>
- Baskevich MI, Mironova TA, Cherepanova EV, Krivonogov DM (2016) New data on chromosomal variability, distribution of sibling species, and hybridization of 46-chromosomal forms of *Microtus arvalis* sensu lato (Rodentia, Arvicolinae) in the Upper Volga basin. *Biology Bulletin* 43(9): 1281–1291. <https://doi.org/10.1134/S1062359016110042>
- Baskevich MI (1996) On the karyological differentiation in Caucasian population of common vole (Rodentia, Cricetidae, *Microtus*). *Zoologičeskij Žurnal* 75(2): 297–308. [In Russian]
- Bužan EV, Förster DW, Searle JB, Kryštufek B (2010) A new cytochrome *b* phylogroup of the common vole (*Microtus arvalis*) endemic to the Balkans and its implications for the evolu-

- tionary history of the species. *Biological Journal of the Linnean Society* 100(4): 788–796. <https://doi.org/10.1111/j.1095-8312.2010.01451.x>
- Chaline J, Brunet-Lecomte P, Montuire S, Viriot L, Courant F (1999) Anatomy of the arvicoline radiation (Rodentia): palaeogeographical, palaeoecological history and evolutionary data. *Annales Zoologici Fennici* 36(4): 239–267.
- Cuenca-Bescós GC, Canudo JI, Laplana C (2001) La séquence des rongeurs (Mammalia) des sites du Pléistocène inférieur et moyen d'Atapuerca (Burgos, Espagne). *Anthropologie* 105(1): 115–130. [https://doi.org/10.1016/S0003-5521\(01\)80009-1](https://doi.org/10.1016/S0003-5521(01)80009-1)
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29(8): 1969–73. <https://doi.org/10.1093/molbev/mss075>
- Fink S, Excoffier L, Heckel G (2004) Mitochondrial gene diversity in the common vole *Microtus arvalis* shaped by historical divergence and local adaptations. *Molecular Ecology* 13(11): 3501–14. <https://doi.org/10.1111/j.1365-294X.2004.02351.x>
- Fredga K, Jaarola M, Ims RA, Steen H, Yoccoz NG (1990) The common vole in Svalbard identified as *Microtus epiroticus* by chromosome analysis. *Polar Research* 8: 283–290. <https://doi.org/10.3402/polar.v8i2.6818>
- Gates DM (1993) *Climate change and its biological consequences*. Sinauer Associates, Inc. Sunderland, MA, 280 pp.
- Ghorbani F, Mohammadi Z, Darvish J, Kami HG, Siahsharvie R (2015) Morphological and morphometric characterization of the new records of the East European vole (*Microtus mystacinus* Miller, 1908) from northeast Iran. *Journal of Asia-Pacific Biodiversity* 8(3): 233–237. <https://doi.org/10.1016/j.japb.2015.07.002>
- Gileva EA, Cheprakov MI, Nochrin DY (1996) Voles of the group *Microtus arvalis* (Rodentia, Cricetidae) in Urals. *Zoologičeskij Žurnal* 75(9): 1436–1439 [in Russian].
- Golenishchev FN, Malikov VG, Bulatova NSh, Vaziri ASH, Nazari F (2000) Some new data on morphology and karyology of *Microtus kermanensis* (Rodentia, Arvicolinae) and supposed phylogeographic history of the “arvaloid” voles. In: Agadjanian AK, Orlov VN (Eds) *Systematic and Phylogeny of the Rodents and Lagomorphs*. Teriologicheskoe Obshchestvo, Moscow, 34–36.
- Groves CP, Cotterill FPD, Gippoliti S, Robovský J, Roos C, Taylor PJ, Zinners D (2017) Species definitions and conservation: a review and case studies from African mammals. *Conservation Genetics* 18(6): 1247–1256. <https://doi.org/10.1007/s10592-017-0976-0>
- Guthrie RD (2001) Origin and causes of the mammoth steppe: a story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia. *Quaternary Science Reviews* 20(1): 549–574. [https://doi.org/10.1016/S0277-3791\(00\)00099-8](https://doi.org/10.1016/S0277-3791(00)00099-8)
- Haynes S, Jaarola M, Searle JB (2003) Phylogeography of the common vole (*Microtus arvalis*) with particular emphasis on the colonization of the Orkney archipelago. *Molecular Ecology* 12(4): 951–956. <https://doi.org/10.1046/j.1365-294X.2003.01795.x>
- Ho SYW (2007) Calibrating molecular estimates of substitution rates and divergence times in birds. *Journal of Avian Biology* 38: 409–414. [10.1111/j.0908-8857.2007.04168.x](https://doi.org/10.1111/j.0908-8857.2007.04168.x)
- Horáček I (1990) On the context of Quaternary arvicolid evolution: changes in community development. In: Fejfar O, Heinrich WD (Eds) *International Symposium 'Evolution,*

- Phylogeny and Biostratigraphy of Arvicolids (Rodentia, Mammalia). Geological Survey, Prague, 201–222.
- Horáček I, Ložek V (1988) Palaeozoology and the Mid-European Quaternary past: scope of the approach and selected results. *Rozpravy ČSAV, řada matematických a přírodních věd.* 98: 1–102.
- Hotzi V, Markov G, Csorba G (2008) Taking steps to discover the East-European vole (*Microtus levis*) in Hungary. *Állattani Kozlemenyekek* 93: 47–57. [In Bulgarian]
- Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution* 32(2): 128–144. <https://doi.org/10.1007/BF02515385>
- Jaarola M, Martínková N, Gündüz I, Brunhoff C, Zima J, Nadachowski A, Amorif G, Bula-tovag NS, Chondropoulou B, Fragedakis-Tsolish S, Gonzalez-Estebani J, Lopez-Fuster MJ, Kandaurov AS, Kefelioğlu H, da Luz Mathias M, Villatei I, Searle JB (2004) Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 33(3): 647–63. <https://doi.org/10.1016/j.ympev.2004.07.015>
- Jaarola M, Searle JB (2002) Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. *Molecular Ecology* 11(12): 2613–2621. <https://doi.org/10.1046/j.1365-294X.2002.01639.x>
- Kartavtseva IV, Tiunov MP, Lapin AS, Visotchina NP, Ryabkova AV (2012) Invasion of *Microtus rossiaemeridionalis* into the territory of the Russian Far East. *Russian Journal of Biological Invasions* 3(1): 11–15. <https://doi.org/10.1134/S2075111712010031>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kovalskaya YM (1994) On the distribution of voles of the group *arvalis* (Rodentia) in Kazakhstan. *Zoologičeskij Žurnal* 73(3): 120–125. [In Russian]
- Král B, Zima J, Hrabě V, Libosvářský J, Šebela M, Červený J (1981) On the morphology of *Microtus epiroticus*. *Folia Zoologica Brno* 30(4): 317–330.
- Kryštufek B, Vohralik V (2005) Mammals of Turkey and Cyprus. Rodentia I: Sciuridae, Dipodidae, Gliridae, Arvicolinae. University of Primorska, Science and Research Centre Koper, Koper, 292 pp.
- Kryštufek B (2017) The East European Vole. In: Wilson DE, Lacher Jr TE, Mittermeier RA (Eds) *Handbook of the mammals of the World, Volume 7*. Lynx Edicions, Barcelona, 352.
- Lorenzen ED, Nogués-Bravo D, Orlando L, Weinstock J, Binladen J, Marske KA, Ugan A, Borregaard MK, Gilbert TP, Nielsen R, Ho SYW, Goebel T, Graf KE, Byers D, Stenderup JT, Rasmussen M, Campos PF, Leonard JA, Koepfli KP, Froese D, Zazula G, Stafford TV, Aaris-Sørensen K, Batra P, Haywood AM, Singarayer JS, Valdes PJ, Boeskorov G, Burns JA, Davydov SP, Haile J, Jenkins DL, Kosintsev P, Kuznetsova T, Lai X, Martin LD, McDonald HG, Mol D, Meldgaard M, Munch K, Stephan E, Sablin M, Sommer RS, Sipko T, Scott E, Suchard MA, Tikhonov A, Willerslev R, Wayne RK, Cooper A, Hofreiter M., Sher A., Shapiro B, Rahbek C, Willerslev E (2011) Species-specific responses of Late

- Quaternary megafauna to climate and humans. *Nature* 479(7373): 359–364. <https://doi.org/10.1038/nature10574>
- Mahmoudi A, Darvish J, Aliabadian M, Moghaddam FY, Kryštufek B (2017) New insight into the cradle of the grey voles (subgenus *Microtus*) inferred from mitochondrial cytochrome *b* sequences. *Mammalia* 81(6): 583–593. <https://doi.org/10.1515/mammalia-2016-0001>
- Markov G, Csorba G, Kocheva M, Gospodinova M (2012) Skull features of the common vole (*Microtus arvalis* sensu lato) from Hungary: craniometrical evidence for its taxonomic detachment. *Turkish Journal of Zoology* 36(3): 283–290. <https://doi.org/10.3906/zoo-1002-49>
- Markova E, Malygin V, Montuire S, Nadachowski A, Quéré JP, Ochman K (2009) Dental variation in sibling species *Microtus arvalis* and *M. rossiaemeridionalis* (Arvicolinae, Rodentia): between species comparisons and geography of morphotype dental patterns. *Journal of Mammalian Evolution* 17: 121–139. <https://doi.org/10.1007/s10914-009-9128-8>
- Markova E, Beeren Z, van Kolschoten T, Strukova T, Vrieling K (2012) Differentiating sibling species in the Quaternary fossil record: a comparison of morphological and molecular methods to identify *Microtus arvalis* and *M. rossiaemeridionalis* (Arvicolinae, Rodentia). *Journal of Systematic Palaeontology* 10(3): 585–597. <https://doi.org/10.1080/14772019.2011.618146>
- Martínková N, Barnett R, Cucchi T, Struchen R, Pascal M, Fischer MC, Higham T, Brace S, Ho SYW, Quéré JP, O’ Higgins P, Excoffier L, Heckel G, Hoelzel RA, Dobney KM, Searle JB (2013) Divergent evolutionary processes associated with colonization of offshore islands. *Ecology* 22(20): 5205–5220. <https://doi.org/10.1111/mec.12462>
- Masing M (1999) The skull of *Microtus levis* (Arvicolidae, Rodentia). *Folia Theriologica Estonica* 4: 76–90.
- Mazurok NA, Rubtsova NV, Isaenko AA, Pavlova ME, Slobodyanyuk SY, Nesterova TB, Zakian SM (2001) Comparative chromosome and mitochondrial DNA Analyses and phylogenetic relationships within common voles (*Microtus*, Arvicolidae). *Chromosome Research* 9(2): 107–120. <https://doi.org/10.1023/A:1009226918924>
- Meyer MN, Golenishchev FN, Radjabli SI, Sablina OV (1996) Voles (subgenus *Microtus* Schrank) of Russia and adjacent territories. *Proceedings of the Zoological Institute of the Russian Academy of Sciences* (232): 1–320. [in Russian]
- Meyer MN, Orlov VN, Scholl ED (1969) Utilization of karyological, physiological and cytological analysis for the separation of new species of rodents (Rodentia, Mammalia). *Doklady Akademii Nauk USSR* 188: 1411–1414 [in Russian].
- Meyer MN, Radjabli SI, Bulatova NS, Golenishchev FN (1985) Karyological peculiarities and probable relations of common voles of the group “arvalis” (Rodentia, Cricetidae, *Microtus*). *Zoologičeskij Žurnal* 64(3): 417–428.
- Moroldoev IV, Sheremetyeva IN, Kartavtseva IV (2017) The first finding of East European vole (*Microtus rossiaemeridionalis*) in Buryatia. *Russian Journal of Biological Invasions* 8(3): 266–271. <https://doi.org/10.1134/S2075111717030109>
- Musser GG, Carleton MD (2005) Subfamily Arvicolinae. In: Wilson DE, Reeder DM (Eds) *Mammal species of the world: a taxonomic and geographic reference*. The Johns Hopkins University Press, Baltimore, 956–1039.
- Nylander JAA (2004) MrModeltest v2. Program distributed by the Author. Evolutionary Biology Centre, Uppsala University.

- Okulova NM, Khlyap LA, Bidashko FG, Warshavskiy AA, Grazhdanov AK, Neronov VV (2014) Rodent communities of the Western Kazakhstan oblast of the Republic of Kazakhstan 1: Maps of rodent communities and zoogeographic regionalization. *Arid Ecosystems* 4(2): 75–84. <https://doi.org/10.1134/S2079096114020073>
- Pavlova SV, Tchabovskiy AV (2011) Presence of the 54-chromosome common vole (Mammalia) on Olkhon Island (Lake Baikal, East Siberia, Russia), and the occurrence of an unusual X-chromosome variant. *Comparative Cytogenetics* 5(5): 433–40. <https://doi.org/10.3897/CompCytogen.v5i5.1720>
- Rambaut A (2009) Computer program and documentation distributed by the author. <http://beast.bio.ed.ac.uk>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2017) Tracer v1.7, Available from <https://github.com/beast-dev/tracer>
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19(18): 2496. <https://doi.org/10.1093/bioinformatics/btg359>
- Řičánková VP, Horsák M, Hais M, Robovský J, Chytrý M (2018) Environmental correlates of the Late Quaternary regional extinctions of large and small Palaearctic mammals. *Ecography* 41(3): 516–527. <https://doi.org/10.1111/ecog.02851>
- Sanger FS, Nicklen S, Coulson AE (1977) DNA sequencing with chain termination inhibitors. *Proceedings of the National Academy of Sciences of the USA* 74(12): 5463–5467. <https://doi.org/10.1073/pnas.74.12.5463>
- Shenbrot GI, Krasnov BR (2005) Atlas of the geographic distribution of the arvicoline rodents of the world (Rodentia, Muridae: Arvicolinae). Pensoft, Sofia-Moscow, 336 pp.
- Sirocko F, Claussen M, Litt T, Sanchez-Goni MF (2007) The climate of past interglacials (Vol. 7). Elsevier, Amsterdam, 622 pp.
- Steel M, McKenzie A (2001) Properties of phylogenetic trees generated by Yule-type speciation models. *Mathematical Biosciences* 170(1): 91–112. [https://doi.org/10.1016/S0025-5564\(00\)00061-4](https://doi.org/10.1016/S0025-5564(00)00061-4)
- Stojak J, McDevitt AD, Herman JS, Searle JB, Wójcik JM (2015) Post-glacial colonization of eastern Europe from the Carpathian refugium: evidence from mitochondrial DNA of the common vole *Microtus arvalis*. *Biological Journal of the Linnean Society* 115(4): 927–939. <https://doi.org/10.1111/bij.12535>
- Stojak J, McDevitt AD, Herman JS, Kryštufek B, Uhlíková J, Purger JJ, Lavrenchenko LA, Searle JB, Wójcik JM (2016) Between the Balkans and the Baltic: Phylogeography of a Common Vole mitochondrial DNA lineage limited to Central Europe. *PLoS ONE* 11(12): e0168621. <https://doi.org/10.1371/journal.pone.0168621>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731–9. <https://doi.org/10.1093/molbev/msr121>
- Thanou E, Tryfonopoulos G, Chondropoulos B, Fraguadakis-Tsolis S (2012) Comparative phylogeography of the five Greek vole species infers the existence of multiple South Balkan subrefugia. *Hystrix Italian Journal of Mammalogy* 79(3): 363–376. <https://doi.org/10.1080/11250003.2011.651163>

- Tiunov MP, Kartavtseva IV, Lapin AS (2013) Morphotype analysis of the sibling vole (*Microtus rossiaemeridionalis*) casually introduced to the Russian Far East. *Acta Theriologica* 58(1): 79–82. <https://doi.org/10.1007/s13364-012-0092-y>
- Tougaard C, Renvoise E, Petitjean A, Quéré JP (2008) New insight into the colonization processes of Common Voles: Inferences from molecular and fossil evidence. *PLoS ONE* 3(10): e3532. <https://doi.org/10.1371/journal.pone.0003532>
- Tougaard C (2017) Did the Quaternary climatic fluctuations really influence the tempo and mode of diversification in European rodents? *Journal of Zoological Systematics and Evolutionary Research* 55(1): 46–56. <https://doi.org/10.1111/jzs.12152>
- Tougaard C, Montuire S, Volobouev V, Markova E, Contet J, Aniskin V, Quere JP (2013) Exploring phylogeography and species limits in the Altai vole (Rodentia: Cricetidae). *Biological Journal of the Linnean Society* 108(2): 434–452. <https://doi.org/10.1111/j.1095-8312.2012.02034.x>
- Triant DA, DeWoody JA (2007) Extensive mitochondrial DNA transfer in a rapidly evolving rodent has been mediated by independent insertion events and by duplications. *Gene* 401(1): 61–70. <https://doi.org/10.1016/j.gene.2007.07.003>
- Yakimenko LV, Kryukov AP (1997) On karyotype variation in common vole *Microtus rossiaemeridionalis* (Rodentida, Cricetidae). *Zoologičeskij Žurnal* 76(3): 375–378. [In Russian]
- Zagorodnyuk IV (1991a) Polytypical Arvicolidae in Eastern Europe: taxonomy, distribution and diagnostics. Institute of Zoology, Kiev, 63 pp.
- Zagorodnyuk IV (1991b) Systematic position of *Microtus brevisrostris* (Rodentiformes): materials toward the taxonomy and diagnostics of the "arvalis" group. *Vestnik Zoologii* 25(3): 26–35.