Analysis of “historical” DNA of museum samples resolve taxonomic, nomenclature and biogeography issues: case study of true lemmings

Nataliya Abramson[1], **Tatyana Petrova**[1], **and Nikolai Dokuchaev**[2]

[1]Zoological Institute, Russian Academy of Sciences, Universitetskaya nab., 1,
Saint Petersburg, 199034, Russian Federation
[2]Institute of Biological Problems of the North, Far East Branch of the Russian Academy of Sciences, ul. Portovaya, 18, Magadan, 685000, Russian Federation

Address correspondence and requests for materials to Nataliya Abramson,
Nataliya.Abramson@zin.ru

Abstract

Historical DNA of museum specimens is of paramount importance in elucidation of complex nomenclature issues and a priceless source of material gathered during preceding centuries in hardly accessible today sites. Here we report the results of genotyping type specimens of *Lemmus obensis bungei* Vinogradov, 1925 from the collection of the Zoological Institute RAS and specimens of lemmings from the upper streams of the Omolon River, identified as *L. amurensis* from the collection of the Institute of Systematics and Ecology of Animals, Siberian branch of RAS. The genetic profile of the type specimens in the first case was crucial for the nomenclatural solution as these specimens are from the point of the major evolutionary divergence on the Lena River. The results of the study convincingly showed that the lectotype belong to the eastern mitochondrial lineage of *L. sibiricus*. Therefore, if ever in the further studies it would be conclusively shown that this mitochondrial lineage may become a valid taxon of any rank, then names “*novosibiricus*”, “*ognevi*”, “*portenkoi*” will become younger synonyms of “*bungei*”. The new obtained sequences of the specimens from the Omolon prove initially wrong assignment and represent *L. sibiricus* eastern lineage.

Keywords: historical DNA, collection samples, *Lemmus*, type specimens, cytochrome *b*, species identification

Introduction

With the advances in DNA technologies, it has been recognized that zoological collections and herbariums have the potential to serve as a vast source of DNA (Wandeler, Hoeck and Keller, 2007). It is customary to designate the DNA that is obtained from traditional museum voucher specimens as “historical” DNA (hDNA), and it is as a rule from specimens not older than 200 hundred years old (Raxworthy and Smith, 2021). Recent hDNA studies have helped to clarify taxonomic confusion with type specimens (Abramson and Petrova, 2018; Kehlmaier et al., 2019; Petrova, Tesakov, Kowalskaya and Abramson, 2016; and others). Historical DNA of museum specimens is of paramount importance in elucidation of complex nomenclature issues. It is also a priceless source of material that was gathered during preceding centuries in the sites which are hardly accessible today due to political or economic reasons. Historical DNA obtained from museum specimens in most cases is represented by fragments of mitochondrial (mt) genome, rarely by almost complete mitogenomes. The discordances between the mt trees and nuclear gene trees are very common, and it is one of the reasons to treat mt trees and based upon them revisions in classification with caution. However,

Citation: Abramson, N., Petrova, T., and Dokuchaev, N. 2022. Analysis of “historical” DNA of museum samples resolve taxonomic, nomenclature and biogeography issues: case study of true lemmings. Bio. Comm. 67(4): 340–348. https://doi.org/10.21638/spbu03.2022.408

Authors’ information: Nataliya Abramson, PhD, Leading Researcher, orcid.org/0000-0003-3740-1388; Tatyana Petrova, PhD, Senior Researcher, orcid.org/0000-0001-6856-920X; Nikolai Dokuchaev, Dr. of Sci. in Biology, Leading Researcher, orcid.org/0000-0002-1331-4901

Manuscript Editor: Pavel Skutschas, Department of Vertebrate Zoology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

Received: July 18, 2022;

Revised: September 30, 2022;

Accepted: October 11, 2022.

Funding: This study was supported by Ministry of Science and Higher Education of the Russian Federation (Grant No. 075-15-2021-1069).

Ethics statement: This paper does not contain any studies involving human participants or animals performed by any of the authors.

Supplementary information: Supplemental material to the article is available at https://doi.org/10.21638/spbu03.2022.408. Supplementary files are published as submitted by the authors, and are not copyedited.

Competing interests: The authors have declared that no competing interests exist.

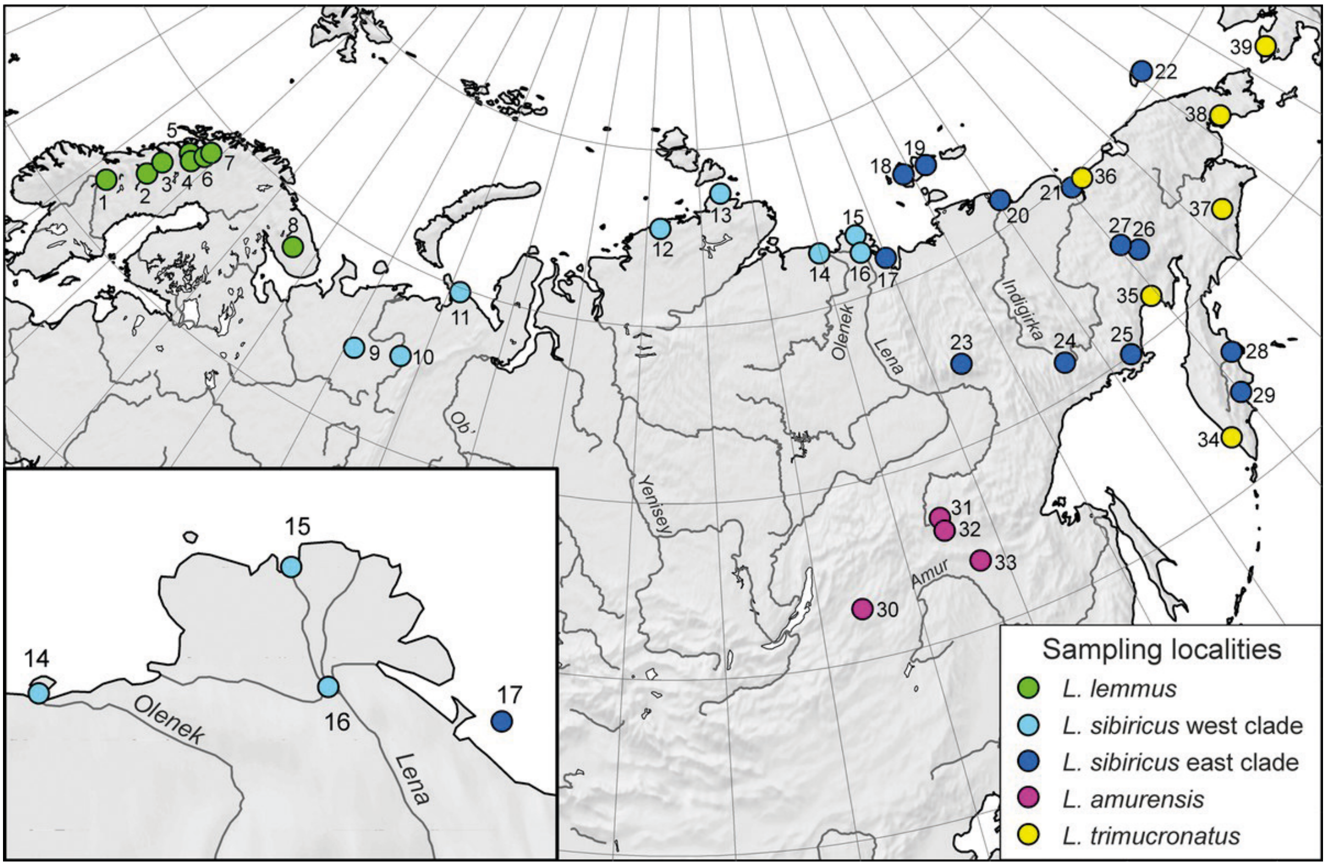

Fig. 1. Sampling localities.
Locations are colored according to cytochrome *b* lineages, as shown in Fig. 2. Points numbers 17 and 15 are the lectotype and paralectotypes of *Lemmus bungei* respectively. Locality numbers correspond to Table S1.

mtDNA may serve as a very good diagnostic feature, and as such was proposed as a DNA barcode (Hebert, Cywinska, Ball and deWaard, 2003). DNA sequencing of name-bearing type specimens is thus regarded as the gold standard for taxonomy (Renner, 2016). Such procedure enables the unambiguous assignment of extant populations to the named entity or enables a clear distinction between newly discovered forms and those that have already been described (Kehlmaier et al., 2019).

Species of the genus *Lemmus* Link, 1795, are widespread and abundant rodents in the Holarctic tundra (Shenbrot and Krasnov, 2005), and keystone herbivores in arctic ecosystems of Eurasia and North America. The studies of distribution, systematics and phylogeny of this taxon are to significant degree hampered by hard accessibility of the Arctic sites. In addition, some places from where museum samples were obtained a hundred years ago have undergone serious anthropogenic landscape transformation, and currently lemmings have totally disappeared from those sites. In this case, correct identification through genotyping specimens stored in the museum collection may help to fill the gaps in our knowledge of taxonomy and species distribution. Systematics and nomenclature of true lemmings abound with unsettled issues, reflecting sampling gaps on the one hand, and different views on species delimitation on the other. After decades of passionate research on morphology, karyology and mitochondrial sequences, using interpopulation hybridization (Abramson, Kostygov and Rodchenkova, 2008; Abramson and Petrova, 2018; Chernyavsky et al., 1993; Chernyavsky and Kartavtseva, 1999; Fedorov, Goropashnaya, Jarrell and Fredga, 1999; Fedorov, Goropashnaya, Jaarola and Cook, 2003; Fredga, Fedorov, Jarrell and Jonson, 1999; Gileva, Kuznetsova and Cheprakov, 1984; Pokrovski, Kuznetsova and Cheprakov, 1984; Rausch and Rausch, 1975), the two recent-most revisions still do not agree on the number of species in *Lemmus*, recognizing either two (Lissovsky et al., 2019), three (Krystufek and Shenbrot, 2022) or four species of lemmings (Abramson and Lissovsky, 2012; Pardiñas et al., 2017). Putting aside a well-differentiated trans-Beringian *Lemmus trimucronatus* Richardson, 1825, the disputes are over the Palearctic representatives. Phylogeographic studies revealed that the conventional species delimitation does not match the mitochondrial genetic clades. A widespread *L. sibiricus* Kerr, 1792 appeared to be a composite of two deeply divergent mitochondrial lineages presumably separated by the Lena River (Fedorov, Goropashnaya, Jarrell and Fredga, 1999; Fredga, Fedorov, Jarrell and Jonson, 1999). The lineage westward of the Lena River is genetically close to a richly colored *L. lemmus* Linnaeus, 1758 from Fennoscandia (Fig. 1). The eastern lineage, in its turn, splits into two deeply divergent branches, one of which represents dwarf *L. amurensis* Vinogradov, 1924. It is a small lemming with a typical stripe at the back, distributed from Transbaikal region (where it is most likely extinct) to the South Yakutia. The other branch represents motley-colored lemmings of the so-called eastern lineage of *L. sibiricus* (Abramson and Petrova, 2018) occurring in the north east of the Lena River delta and up to the left bank of the Kolyma River. The isolated populations of this lineage also occur at Novosibirskie Islands, Wrangel Island and east of Sredinnyi Range at Kamchatka Peninsula (Fig. 1). However, the south-eastern border of the eastern *L. sibiricus* lineage and *L. trimucronatus* remain obscure. In our previous work (Abramson and Petrova, 2018) it was shown that all the small lemmings from the Kolyma highlands that were referred to as *L. amurensis* (Carleton and Musser, 2005; Chernyavsky, 1984; Kostenko, 2000; Shenbrot and Krasnov, 2005) are misidentified and should be assigned to eastern lineage of *L. sibiricus*. However, while the border line between the distribution of *L. sibiricus* eastern mt lineage and *L. trimucronatus* at the north is clearly designated by the mouth of the Kolyma River, the interrelationships and distribution of both species at the vast territory along the north-western coast of the Sea of Okhotsk and Kolyma Highland are poorly known.

Systematic arrangement of lemmings is further complicated by the uncertain taxonomic status of the species group named *bungei*, which prevents a sound nomenclatural solution at the crucial point of the major evolutionary divergence at the Lena River. Unambiguous taxonomy is crucial in this case because *L. obensis bungei* Vinogradov, 1925, is the oldest available name for lemmings of Siberia, next to *Mus Lemmus sibiricus* Kerr, 1792. The name *bungei* was once suggested as the appropriate name for the eastern mt lineage of *sibiricus*, ranked either a species (Fredga, Fedorov, Jarrell and Jonson, 1999) or a subspecies (Pardiñas et al., 2017). The type locality of *Lemmus obensis bungei* was designated as “the lower course of the Lena River (Sagystyr Island, range Khara-Ulakh)” (Vinogradov, 1925: 56). The sequences of the mt cytochrome *b* (*cytb*) of lemmings trapped at the Samoilovskiy Island that is in the middle of the Lena River delta, and thus considered as topotypes, were earlier compared with the sequences of lemmings from other regions of the range (Abramson, Kostygov and Rodchenkova, 2008). The results obtained in the cited study clearly pointed out that lemmings from Samoilovskiy Island belong to the western clade whereas Fredga, Fedorov, Jarrell and Jonson (1999) supposed the name “*bungei*” for the eastern clade. However, the specimens from the type series have not yet been studied, and the proper use of species names depends entirely on verifying whether newly analyzed specimens are conspecific with the type material.

The aim of our study is by applying the hDNA technique (1) to define the taxonomic identity of type specimens *L. obensis bungei*, and (2) check the taxonomic assignment of small lemmings occurring east of the Kolyma River in an area where one can expect either the eastern lineage of *L. sibiricus* or *L. trimucronatus*.

Materials and methods

Material

The material for this study consisted of nine museum samples (including four specimens of the type series of *L. obensis bungei*) originating from four localities in

Table 1. Material used in the study

Species names as labeled	Taxonomic status	Specimen voucher	Locality (map)	Collection year	GenBank accession No
L. obensis bungei	lectotype	ZISP 11028	Russia, Yakutia, Lena River delta, Muostakh Island, 71.6° N, 129.98° E (7)	1883	MN542670
L. bungei	paralectotype	ZISP 6726	Russia, Yakutia, Lena River delta, Sagastyr Island, 73.387° N, 126.615° E (5)	1883	MN542671
L. bungei	paralectotype	ZISP 11025		1883	MN542672
L. bungei	paralectotype	ZISP 11026		1883	MN542673
L. amurensis		ISEA 49007	Russia, Magadan Region, Kegali, 64.362° N, 161.968° E (16)	1969	MN542674
L. amurensis		ISEA 49037		1969	MN542675
L. amurensis		ISEA 49039		1969	MN542676
L. amurensis		ZMMU S-124117	Russia, , Omolon Village, 65.234° N, 160.550° E (17)	1978	OL519590
L. amurensis		ZMMU S-124118		1978	OL519591

Museum names are abbreviated as follows: ZISP, Zoological Institute of the RAS (Saint Petersburg); ISEA, Institute of Systematics and Ecology of Animals of the Siberian Branch of the RAS (Novosibirsk); ZMMU, Zoological Museum of Moscow State University (Moscow). Locality numbers (in brackets) correspond to Fig. 1.

eastern Siberia, Russian Federation (Table 1). Until further genomic studies using nuclear data, we keep using conventional species recognition and use the nomenclature as in Abramson and Lissovsky (2012) or Pardiñas et al. (2017) with a notion that these names do not always embrace monophyletic taxa as evidenced by mitochondrial *cytb* gene. In addition to our new samples, a further 79 *cytb* sequences were downloaded from the GenBank database (Table S1). The information on the geographic origin and the year of collection of the studied museum specimens is given in Table 1.

DNA extraction, amplification, and sequencing

DNA from museum skin samples was isolated using Qiagen's QIAamp Tissue Kit. A 337 bp fragment of the *cytb* gene was amplified in three overlapping fragments using primers and PCR conditions published in Lagerholm et al. (2014). Both DNA isolation and PCR with museum samples was conducted in the room isolated from post-PCR facilities using a PCR Workstation (LAMSYSTEMS CC) and the working surface, all instruments and plastics were sterilized with UV light and chloramine-T to avoid contamination. The PCR products were purified with Omnix kit columns (Omnix, St. Petersburg) and sequenced in both directions using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3130 (Applied Biosystems Inc.). Sequences were edited, assembled and aligned with BioEdit (Hall, 1999). Newly determined sequences were deposited in GenBank under accession numbers MN542670 — MN542676, OL519590, OL519591 (Table 1).

Phylogenetic tree and network

To verify the position of animals under study among the extant lemmings' mitochondrial lineages we analyzed 88 *Lemmus* sequences (Table S1). The final alignment comprised the 313 bp *cytb* fragment (it was shortened in order to use previously published data). Phylogenetic re-

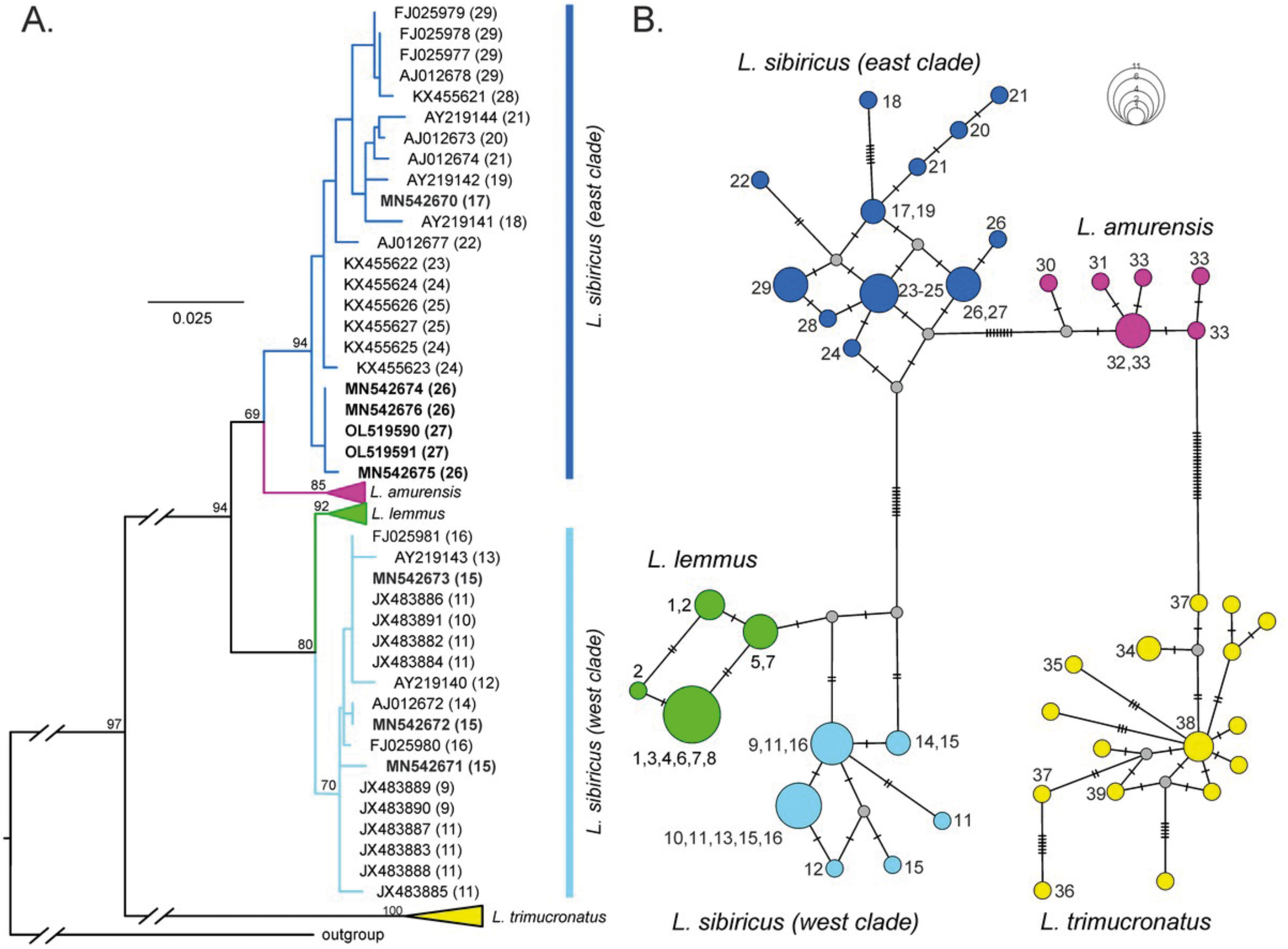

Fig. 2. Maximum Likelihood phylogenetic reconstruction and median-joining network based on the *Lemmus* cytochrome *b* sequences. A. Maximum Likelihood phylogenetic reconstruction. Node labels display ultrafast ML bootstrap above 50 %. B. Median-joining network. For haplotype labels, refer to Fig. 1, Tables 1 and S1. Size of circles diameter is proportional to haplotype frequency; branch length is shown with hatch marks.

construction was performed with Maximum Likelihood (ML) method using IQ-TREE web server (Trifinopoulos, Nguyen, Haeseler and Minh, 2016) with 10,000 ultrafast bootstrap replicates (Hoang et al., 2018). Three specimens were used as an outgroup (*Myopus schisticolor* EU165208, *Dicrostonyx torquatus* AF119275 and *Synaptomys cooperi* DQ323957). Median-joining network (Bandelt, Forster and Röhl, 1999) was conducted in PopART 1.7 (http://popart.otago.ac.nz).

Results

Our study retrieved nine new specimens, generating a total dataset of 44 different lemming *cytb* haplotypes. Within the 313-bp long fragments considered here, 65 polymorphic sites were found, 46 of which were parsimony informative. No stop codons, insertions, or deletions were observed in the alignment. The ML tree (Fig. 2A) reconstructed a topology shown earlier (Abramson and Petrova, 2018), where Palearctic branch is divided in two well supported clades each of which is further subdivided. These final four supported clades within Palearctic *Lemmus* do not correspond to conventional species delimitation, and *L. sibiricus* thus appeared to be paraphyletic at the mitochondrial tree. Populations west of the Lena River mouth are sister to *L. lemmus,* and populations east of the Lena River mouth including Novosibirskie Islands and Wrangel Island, east shore of Kamchatka and Verkhoyanskiy Ridge are sister to *L. amurensis*. No subdivisions within the clades are supported, and genetic variation within clade is clearly smaller than between clade (Fig. 2B). The lectotype of *L. obensis bungei* (taxonomy is given as at the label) is clearly within the eastern clade of *L. sibiricus*. However, all paralectotypes studied are found within the western clade of *L. sibiricus.*

The median-joining haplotype network (Fig. 2B) clearly showed the same four clusters, which were already recognized at the tree and in earlier studies. New specimens from "*bungei*" type series fell into both clusters of *L. sibiricus* in accordance with their geographical origin but not with the previous morphology-based taxonomic identification. Those from Sagastyr Island (Fig. 1, pt. 15) were in the western clade, and the lectotype from Muostakh Island (Fig. 1, pt. 17) was in the eastern clade of *L. sibiricus*. It is noteworthy that the haplotype of lemming from Moustakh Island is identical to one from Novosibirskie Island and very close (1 substitution) to one from the left bank of the Kolyma River.

Lemmings from Kegali and Omolon (Fig. 1, pt. 26 and 27), which were initially classified as *L. amurensis*, genetically clustered with *L. sibiricus* east and not with *L. amurensis* haplotypes from the Far East. The identification of these samples unambiguously refers them to the Palearctic branch of *Lemmus*, not to *L. trimucronatus*.

Discussion

Nomenclature issues related to *Lemmus obensis bungei* Vinogradov, 1925

The results of genotyping type series of *Lemmus obensis bungei* revealed the situation when holotype / lectotype and paratypes / paralectotypes belong to genetically divergent mitochondrial lineages not for the first time. We faced a similar situation when genotyping type material on *Stenocranius gregalis raddei* Poljakov, 1881 (Petrova, Tesakov, Kowalskaya and Abramson, 2016) and in case with type material of *Lemmus flavescens* Vinogradov, 1925 (Abramson and Petrova, 2018). For the nomenclatural decisions, the genetic identity of the name bearing specimen, that is the holotype / lectotype, is of primary importance, as already underlined.

In his paper of 1924, Vinogradov proposed two new lemmings (*Lemmus amurensis* and *Lemmus obensis novosibiricus*), and introduced still another name under remarks to *novosibiricus* (p. 189). The following year Vinogradov (1925) published again *L. obensis bungei* as a new subspecies and provided a detailed description (type locality and diagnosis are in English), measurements and drawings of the skull in dorsal and ventral views. Thus, Vinogradov 1925 paper is the first proper naming of *bungei*. Vouchers used by Vinogradov are deposited in Zoological Institute in Saint Petersburg and were listed on p. 56 of his 1925 paper. They originate from two islands (Sagastyr and Muostakh) and further two mainland sites on the right (Kharaulakhskiy ridge) and the left bank of the Lena River (village Bulun), respectively. Among these syntypes, Baranova and Gromov (2003: 65) designated a voucher ZISP 11028 from "Island Muostakh east of the estuary of the Lena River (Yakutia)" as the lectotype. In the case of *bungei* the locality of the lectotype does not fully coincide with the one designated as type locality in Vinogradov (1925). However, according to the Article 73.2.3 "If a lectotype is subsequently designated, the type locality is the place of origin of the lectotype (Art. 76.2). "The choice for the designation of the lectotype was in full accordance with the Recommendation 74B of the Code: Preference for illustrated specimens ("other things being equal, an author who designates a lectotype should give preference to a syntype of which an illustration has been published"). The only skull of the series that was intact and therefore chosen for illustration was the specimen from Moustakh, the island that is eastward of the Lena delta (Fig. 1, pt. 17). Thus, Baranova and Gromov (2003) strictly followed the recommendation of the Code. The taxonomic identity of the lectotype is unambiguously evident from the sequenced *cytb* fragment which is deposited in the GenBank database (accession No MN542670) and applies to the eastern lineage of *L. sibiricus*. However, the rest of the series, properly paralectotypes, from Sagastyr Island and Khara-Ulakh range clearly fall within the western lineage of the *L. sibiricus*.

Our results thus clarify the application of the name *bungei* in the contact zone of two mitochondrial lineages (*sibiricus* west and *sibiricus* east) which are classified either as a distinct species (Fredga, Fedorov, Jarrell and Jonson, 1999) or as subspecies (Pardiñas et al., 2017). We want to underline here that we do not imply that mitochondrial lineage is equal to taxon of any rank. For correct decisions on the number and rank of taxa, first of all, the robust phylogeny is required. No doubt that this goal may be achieved only when using both mitochondrial and nuclear data. The best results in this case, in our viewpoint, may be achieved using modern NGS data. Until now for the genus *Lemmus* only data from mitochondrial *cytb* are available, and, as we mentioned above, the discordance between mitochondrial and nuclear trees is a very common case. In our opinion the name "*bungei*" has little chance to ever become valid, though such attempts have been made (Fredga, Fedorov, Jarrell and Jonson, 1999). However, already now it is necessary to have clear attribution of diagnostic features of the type specimens that were ever described for a correct compilation of synonymy list and for any further discussion of nomenclature issues that may arise. Thus, leaving aside the issue of species recognition within the Palearctic *Lemmus* and focusing exclusively on the nomenclature issue, the name for eastern mitochondrial lineage if it ever coincides with proper taxonomic delineation would be *bungei* Vinogradov, 1925. Following this logic, if ever in the further studies it would be conclusively shown that this mitochondrial lineage may become a valid taxon of any rank, then names "*novosibiricus*", "*ognevi*", "*portenkoi*" will become younger synonyms of "*bungei*".

Distribution and taxonomy of true lemmings from the Kolyma basin

Morphological and cytogenetic characteristics of lemmings inhabiting the Omolon basin, southern territories of the Magadan area, southern Yakutia and Kamchatka allowed referring all these populations to Amur lemming (Chernyavsky et al., 1980; 1993). However, genotyping of specimens from all these populations showed that the distribution range of *L. amurensis* is confined to Transbaikal region (where it highly likely got extinct), Amur region and South Yakutia (Abramson and Petrova, 2018), whereas all populations from the regions north of South Yakutia actually should be assigned to east mitochondrial lineage of *L. sibiricus*. At the same time, it was shown that at the western coast of Kamchatka Peninsula and in Gizhiga Bay, of the Magadan area (Abramson, Dokuchaev and Petrova, 2018; Abramson and Petrova, 2018) *L. trimucronatus* (Fig. 1) was recorded. Thus, in the Omolon basin one can expect the presence of either *L. sibiricus* of eastern lineage or *L. trimucronatus*. The data presented here unambiguously show that the basin of Omolon is populated by *L. sibiricus* of eastern lineage (Fig. 1). In the upstream of Kolyma, Omolon and Yana rivers remained only isolated populations of *L. sibiricus* and it is easternmost records of its distribution in the mainland part of the range.

Disputable issues of *Lemmus* taxonomy and nomenclature

As — mentioned earlier, the main goal of our study was to perform using DNA diagnostic an identification of specimens from type series and remote and poorly studied area of northeast Siberia where distribution of particular species is questionable. We have already underlined that it is impossible to retrieve robust phylogeny and consequently build a solid classification having only mitochondrial data. It is the main reason why we retain the "old" conventional taxonomy and consider any changes in *Lemmus* classification at this step of knowledge to be hasty. For the same reason, we first wanted to avoid a broad discussion on *Lemmus* systematics. At the same time, in several last publications significant amendments to *Lemmus* taxonomy (Krystufek and Shenbrot, 2022; Spitsyn et al., 2021) were made proceeding only from results of new and published mitochondrial *cytb* trees. These amendments, among others, included introduction of new taxa both of species (*L. nigripes* True, 1894) and subspecies (*L. lemmus chernovi* Spitsyn et al., 2021; *L. l. kamchaticus* Krystufek and Shenbrot, 2022) rank and could not be ignored. Below we shortly want to comment on these changes and substantiate our vision in favor of conventional *Lemmus* taxonomy.

As to the decision to unite all lemmings from Fennoskandia up to the left bank of the Kolyma River, Arctic islands, Kolyma Highlands, Amur area, Transbaikal and south Yakutia (i.e. *L. lemmus, L. sibiricus, L. amurensis*) in one species *L. lemmus* (Krystufek and Shenbrot, 2022; Lissovsky et al., 2019), our arguments against are the following: highly isolated allopatric populations often retain fertility and give offspring in laboratory experiments. This fact alone does not prove their conspecific, but only indicates their relatedness. *L. lemmus* and *L. sibiricus* are highly isolated by the White Sea at least since the end of the LGM, and currently no mixing or gene flow is possible. These populations are very easily diagnosable by DNA and morphology, and our hypothesis is that further genomic studies may show a deeper divergence and discordance with mitochondrial data, as it was already shown for many taxa (Kutschera et al., 2014; Shaw, 2002; Toews and Brelsford, 2012). For the same reason, we consider the description of a new taxon *L. l. chernovi* to be preliminary, though the finding is very interesting for the understanding of *Lemmus* biogeographic history. Following the logic, we need to rename all bank voles (*Clethrionomys glareolus*) inhabiting north Karelia and northern Finland, Sweden and partly Urals as red vole (*Cl. rutilus*), as they all have the introgressed mitochondrial "*rutilus*" lineage (Abramson et al., 2009; Deffontaine et al., 2005). Moreover, we need to rename *Ursus maritimus* and consider it as a synonym of *Ursus arctos*, overweighting the feature of common mitochondrial haplotypes over morphological, behavioral, physiological and ecological features which undoubtedly have genetic basis (Korsten et al., 2009). The interspecies hybridization in mammals with the following complete introgression of mitochondrial genome is a very common case, and it is another reason why additional nuclear data for correct phylogeny is necessary.

As related to the deep mitochondrial split at the Lena River mouth, we observe quite an opposite situation. There are no current geographical barriers, lemming populations from both riverbanks, having a very large radius of activity, most likely interbreed, and constant gene flow exists nowadays. Large genetic distance in mitochondrial markers serves first of all as evidence for events of range fragmentation in the past but not necessarily for speciation events, as further recolonization from refugiums may lead to the mixing of earlier isolated populations, but due to uniparental heredity, the deep divergence in mitochondrial genes will remain. In case of the split along the Lena River, we have the consequences of past fragmentation of lemming populations by the Verkhoyanskiy Range as the borderline of *Lemmus* distribution in Pleistocene was shifted very far to the south. We assume that further genomic studies will either prove or discard this hypothesis, and thus it is preliminary to make taxonomic divisions at this point.

L. amurensis is a very remote and isolated form, evidently Pleistocene relict populations, very distinct in color pattern. In addition, even the distance in mitochondrial markers is not smaller than the split along the Lena River. We assume that genomic studies here will certainly reveal the distinct position of "*amurensis*". Noteworthy reminding that even in hybridological experiments (Pokrovski, Kuznetsova and Cheprakov, 1984) the hybrids showed reduced fertility. Summing up, we think that until further studies that are in progress, it is rational to keep the conventional taxonomy distinguishing Palearctic species group "*lemmus*" with three species *lemmus, sibiricus* and *amurensis* and Nearctic "*trimucronatus*", with one species, that is as given in Abramson and Lissovsky (2012), Pardinas et al. (2017).

The establishing of so called "subspecies groups" (Krystufek and Shenbrot, 2022) does not have any logical or phylogenetically meaningful basement. The subspecies is the smallest taxonomic category according to the ICZN. When we unite very closely related or recently diverged species into a group, there is some evolutionary and phylogenetic sense. For instance, the "*arvalis*" species group unites several related species undistinguishable morphologically but genetically highly diverged and easily identified by karyotype or sequences. They form distinct sister clades at the phylogenetic tree. But what does a group of subspecies mean? Subspecies is a further indivisible taxon, and a group of subspecies may form only the species, but could not be united as a group, as then the distinguishing of subspecies loses any sense turning them into local races. The concept of subspecies itself is in some crisis since phylogenetic thinking (tree-thinking) penetrated within species level. The bases for subspecies recognition conventionally were small morphological differences such as size or color patterns between populations restricted to certain geographical regions. No surprise that practically in majority of taxa subspecies boundaries did not coincide with phylogenetic units at the intraspecies trees in the course of phylogeographic studies. The translation of a phylogenetic tree into a classification is a disputable matter and the same tree may result in different number of recognizable taxa and ranking. Moreover, the more robust and detailed phylogeny we have, the more acute is the issue of the insufficient number of taxonomic categories in Linnaean hierarchy for naming each of the clade. Anyway, the classification suggested by Krystufek and Shenbrot (2022) seems illogical in their treatment of the mitochondrial tree given in Abramson and Petrova (2018). For instance, there is no phylogenetic base for distinguishing the new subspecies such as *L. l. kamchaticus*. Genetically the lemmings from Uzon are very close to lemmings from the Wrangel Island, morphological similarity in dental features was also shown earlier (Chernyavsky et al., 1993). They form one cluster and, what is even more important, no branches within the so-called *L. sibiricus* East clade are somehow supported in any statistical analysis (ML or BI), and actually separate terminal nodes are divided by minimal genetic distance, what is even more visible at the net (Fig. 2B). All listed subspecies under the subspecies group "*amurensis*" differ only by overall dimensions. These differences in size dimensions show cline variation obeying the Bergman rule: northern and island populations being larger, southern smaller. Within this cluster, the holotype of *L. s. ognevi* differs from the new subspecies from Kamchatka by one-two substitutions (Fig. 2B), moreover, they are very similar in size and color pattern. The restriction of the range of the new subspecies only by the caldera of Uzon volcano (Fig. 1, pt. 29) is quite absurd, whereas tens km northeast (Fig. 1, pt. 28) the same lemming differing by one substitution was recorded. Therefore, it is very incorrect to interpret the tree as: "In the molecular tree, *ognevi* holds a sister position to the clade of *novosibiricus* + *portenkoi* + *kamchaticus* ssp" (Krystufek and Shenbrot, 2022: 46), as these actually are false clades, as was underlined above, having no support. In practice, cited authors combine the old approach, distinguishing subspecies on the basis of single highly variable characteristic such as size and color shades with largely misinterpreted scarce molecular data. However, even if to proceed from the mt tree alone, it is impossible to consider equally ranked subspecies *amurensis, novosibiricus, portenkoi*, and *kamchaticus.* We have two sister clades, one "*amurensis*" and the other clade unites all forms described once under the names "*novosibiricus, bungei, portenkoi*". That means, if we want to infer any classification from the given mt tree, these clades should be treated equally as species or subspecies and the earliest name for the second clade is in this case "*bungei*". Subspecies is the lowest category and there is no sense to split it further. Then, proceeding from the same logic, we have several options to translate this mitochondrial tree to taxonomy: a) if we recognize one species *L. lemmus*, we may further have only two subspecies *L. l. lemmus* and *L. l. amurensis* corresponding to two sister clades which should be in one rank (among available names for the second clade "*amurensis*" is the earliest). In our opinion, such classification corresponds less to the objective pattern of biodiversity as it does not describe explicit subdivisions within these two clades/subspecies; b) we may also recognize superspecies *L. lemmus* with four species: *L. lemmus*, *L. sibiricus, L. bungei* and *L. amurensis* that would be a better decision, but this taxonomy may appear to be true only in the case when the mitochondrial tree and the species tree in further studies will coincide.

Acknowledgments

The authors thank the administration of the Institute of Systematics and Ecology of Animals Siberian Branch Academy of Sciences Yu. N. Litvinov for the possibility to study lemmings' specimens. Authors are very grateful to Boris Krystufek, Slovenian Museum of Natural History, for the heated discussions on lemming nomenclature and systematics that stimulated this study.

References

Abramson, N. I., Kostygov, A. Yu., and Rodchenkova, E. N. 2008. The taxonomy and phylogeography of Palaearctic true lemmings (*Lemmus*, Cricetidae, Rodentia): new insights from cyt *b* data. *Russian Journal Theriology* 7:17–23. https://doi.org/10.15298/rusjtheriol.7.1.03

Abramson, N. I. and Lissovsky, A. A. 2012. Subfamily Arvicolinae; pp. 127–141 in I. Y. Pavlinov and A. A. Lissovsky (eds), The mammals of Russia: A taxonomic and geographic reference. KMK Scientific Press, Moscow.

Abramson, N. I. and Petrova, T. V. 2018. Genetic analysis of type material of the Amur lemming resolves nomenclature issues and creates challenges for the taxonomy of true lemmings (*Lemmus*, Rodentia: Cricetidae) in the eastern Palearctic. *Zoological Journal of the Linnean Society* 182(2):465–477. https://doi.org/10.1093/zoolinnean/zlx044

Abramson, N. I., Dokuchaev, N. E., and Petrova, T. V. 2018. Long-standing taxonomic and nomenclature issue of *Lemmus obensis chrysogaster* Allen, 1903 (Rodentia, Cricetidae) resolved. *Mammalia* 82(2):167–172. https://doi.org/10.1515/mammalia-2016-0081

Bandelt, H., Forster, P., and 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

Baranova, G. I. and Gromov, I. M. 2003. Catalogue of type specimens in the collection of the Zoological Institute. Mammalia. No. 4. Rodentia. 100 pp. Izdatelstvo Zoologicheskogo instituta RAS, Saint Petersburg. (In Russian)

Carleton, M. D. and Musser, G. G. 2005. Order Rodentia; pp. 745–752 in D. E. Wilson and D. M. Reeder (eds), Mammal species of the world, 3rd ed. Johns Hopkins University Press, Baltimore.

Chernyavsky, F. B. 1984. The mammals of the extreme North-East Siberia. 388 pp. Nauka, Moscow. (In Russian)

Chernyavsky, F. B., Krivosheev, V. G., Revin, Yu. V., Khvorostyanskaya, L. P., and Orlov, V. N. 1980. On distribution, taxonomy and biology of the Amur lemming (*Lemmus amurensis*). *Zoologichesky Zhurnal* 59(7):1077–1084. (In Russian)

Chernyavsky, F. B., Abramson, N. I., Tsvetkova, A. A., Anbinder, E. M., and Kurysheva, L. P. 1993. On systematics and zoogeography of true lemming of the genus *Lemmus* (Rodentia, Cricetidae) of Beringia. *Zoologichesky Zhurnal* 72(8):111–122. (In Russian)

Chernyavsky, F. B. and Kartavtseva, I. V. 1999. On systematic status of lemmings (Rodentia, Cricetidae, *Lemmus*) from the Kolyma lowland. *Zoologichesky Zhurnal* 78(10):1237–1244. (In Russian)

Deffontaine, V., Libois, R., Kotlik, P., Sommer, R., Nieberding, C., Paradis, E., Searle, J. B., and Michaux J. R. 2005. Beyond the Mediterranean peninsulas: evidence of central European glacial refugia for a temperate forest mammal species, the bank vole (*Clethrionomys glareolus*). *Molecular Ecology* 14:1727–1739. https://doi.org/10.1111/j.1365-294X.2005.02506.x

Ellerman, J. R. and Morrison-Scott, R. C. S. 1951. Cheeklist of Palearctic and Indian Mammals. British Museum of Natural History, London.

Fedorov, V., Goropashnaya, A., Jarrell, G. H., and Fredga, K. 1999. Phylogeographic structure and mitochondria1 DNA variation in true lemmings (*Lemmus*) from the Eurasian Arctic. *Biological Journal of the Linnean Society* 66:357–371. https://doi.org/10.1111/j.1095-8312.1999.tb01896.x

Fedorov, V. B., Goropashnaya, A. V., Jaarola, M., and Cook, J. A. 2003. Phylogeography of lemmings (*Lemmus*): no evidence for postglacial colonization of Arctic from the Beringian refugium. *Molecular Ecology* 12:725–731. https://doi.org/10.1046/j.1365-294X.2003.01776.x

Fredga, K., Fedorov, V., Jarrell, G., and Jonson, L. 1999. Genetic diversity in Arctic Lemmings. *AMBIO* 28(3):261–269.

Gileva, E. A., Kuznetsova, I. A., and Cheprakov, M. I. 1984. Chromosome sets and taxonomy of the true lemmings (genus *Lemmus*). *Zoologichesky Zhurnal* 63:105–114. (In Russian)

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.

Hebert, P. D., Cywinska, A., Ball, S. L., and deWaard, J. R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270(1512):313–321. https://doi.org/10.1098/rspb.2002.2218

Hinton, M. A. C. 1926. Monograph of the voles and lemmings (Microtinae) living and extinct. British Museum Natural History, London. https://doi.org/10.5962/bhl.title.8319

Hoang, D. T, Chernomor, O., von Haeseler, A., Minh, B. Q., and Vinh, L. S. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35: 518–522. https://doi.org/10.1093/molbev/msx281

Jarrell, G. H. and Fredga, K. 1993. How many kinds of lemmings? A taxonomic overview; pp. 45–57 in N. C. Stenseth and R. A. Ims (eds), The Biology of Lemmings. Academic Press, London.

Kehlmaier, C., Zhang, X., Georges, A, Campbell, P. D., Thomson S., and Fritz U. 2019. Mitogenomics of historical type specimens of Australasian turtles: clarification of taxonomic confusion and old mitochondrial introgression. *Scientific Reports* 9:5841. https://doi.org/10.1038/s41598-019-42310-x

Korsten, M., Ho, S. Y. W., Davison, J., Pähn, B., Vulla, E., Roht, M., Tumanov, I. L., Kojola, I., Andersone-Lilley, Z., Ozolins, J., Pilot, M., Mertzanis, Y., Giannakopoulos, A., Vorobiev, A. A., Markov, N. I., Saveljev, A. P., Lyapunova, E. A., Abramov, A. V., Männil, P., Valdmann, H., Pazetnov, S. V., Pazetnov, V. S., Rõkov, A. M., and Saarma, U. 2009. Sudden expansion of a single brown bear maternal lineage across northern continental Eurasia after the last ice age: a general demographic model for mammals? *Molecular Ecology* 18:1963–1979. https://doi.org/10.1111/j.1365-294X.2009.04163.x

Kostenko, V. A. 2000. Rodents (Rodentia) of the Russian Far East. 207 pp. Dalnauka Publ., Vladivostok. (In Russian)

Krystufek, B. and Shenbrot, G. I. 2022. Voles and Lemmings (Arvicolinae) of the Palaearctic Region. 449 pp. University Press, Maribor. https://doi.org/10.18690/um.fnm.2.2022

Kutschera, V. E., Bidon, T., Hailer, F., Rodi, J. L., Fain, S. R., and Janke, A. 2014. Bears in a forest of gene trees: phylogenetic inference is complicated by incomplete lineage sorting and gene flow. *Molecular Biology and Evolution* 31(8):2004–2017. https://doi.org/10.1093/molbev/msu186

Lagerholm, V. K., Sandoval-Castellanos, E., Ehrich, D., Abramson, N. I., Nadachowski, A., Kalthoff, D. C., Germonpré, M., Angerbjörn, A., Stewart, J. R., and Dalén, L. 2014. On the origin of the Norwegian lemming. *Molecular Ecology* 23:2060–2071. https://doi.org/10.1111/mec.12698

Lissovsky, A. A., Sheftel, B. I., Saveljev, A. P., Ermakov, O. A., Kozlov, Yu. A., Smirnov, D. G., Stakheev, V. V., and Glazov, D. M. 2019. Mammals of Russia: species list and applied issues. *Archives of Zoological Museum of Moscow State University* 56:1–191.

Ognev, S. I. 1948. Mammals of the USSR and Adjacent Territories. Vol. 6. Izdatelstvo Akademii nauk SSSR, Moskva-Leningrad. (In Russian)

Pavlinov, I. Ya. and Rossolimo, O. L. 1987. Systematics of mammals of the USSR. Moscow University Press, Moscow.

Pardiñas, U. F. J., Myers, P., León-Paniagua, L., Ordóñez Garza, N., Cook, J. A., Kryštufek, B., Haslauer, R., Bradley, R. D., Shenbrot, G. I., and Patton, J. L. 2017. Family Cricetidae (true hamsters, voles, lemmings and New World rats and mice); pp. 204–535 in D. E. Wilson, Jr. T. E. Lacher and R. A. Mittermeier (eds), Handbook of the mammals of the world. Vol. 7. Rodents II. Lynx Edicions, Barcelona.

Petrova, T. V., Tesakov, A. S., Kowalskaya, Y. M., and Abramson, N. I. 2016. Cryptic speciation in the narrow-headed vole, *Lasiopodomys* (*Stenocranius*) *gregalis*, (Rodentia: Cricetidae). *Zoologica Scripta* 45(6):618–629. https://doi.org/10.1111/zsc.12176

Pokrovski, A. V., Kuznetsova, I. A., and Cheprakov, M. I. 1984. Hybridological studies of reproductive isolation of the *Lemmus* Palearctic species (Rodentia, Cricetidae). *Zoologichesky Zhurnal* 63(6):904–911. (In Russian)

Raxworthy, C. J. and Smith, B. T. 2021. Mining museums for historical DNA: advances and challenges in museomics. *Trends in Ecology & Evolution* 36(11):1049–1060. https://doi.org/10.1016/j.tree.2021.07.009

Rausch, R. L. and Rausch, V. R. 1975. Taxonomy and zoogeography of *Lemmus* spp. (Rodentia, Arvicolinae) with notes on laboratory-reared lemmings. *Ibidem* 40:8–34.

Renner, S. S. 2016. A return to Linnaeus's focus on diagnosis, not description: the use of DNA characters in the formal naming of species. *Systematic Biology* 65(6):1085–1095. https://doi.org/10.1093/sysbio/syw032

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *PNAS* 99 (25):16122–16127. https://doi.org/10.1073/pnas.242585899

Shenbrot, G. I. and Krasnov, B. R. 2005. An atlas of the geographic distribution of the arvicoline rodents of the world (Rodentia, Muridae: Arvicolinae). Pensoft Publishing, Sofia.

Spitsyn, V. M., Bolotov, I. N., Kondakov, A. V., Klass, A. L., Mizin, I. A., Tomilova, A. A., Zubrii, N. A., and Gofarov, M. Y. 2021. A new Norwegian Lemming subspecies from Novaya Zemlya, Arctic Russia. *Ecologica Montenegrina* 40:93–117. https://doi.org/10.37828/em.2021.40.8

Toews, D. P. and Brelsford, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* 21(16):3907–3930. https://doi.org/10.1111/j.1365-294X. 2012.05664.x

Trifinopoulos J., Nguyen L.-T., von Haeseler A., and Minh B. Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: W232–W235. https://doi.org/10.1093/nar/gkw256

Vinogradov, B. S. 1924. Two new interesting lemmings from Siberia. *The Annals and Magazine of Natural History* 14:186–189. https://doi.org/10.1080/00222932408633106

Vinogradov, B. S. 1925. Materials on taxonomy and morphology of rodents. III. Notes on Palaearctic lemmings (genus *Lemmus*). *Ezhegodnik Zoologicheskogo Museya AN SSSR* 26:51–73. (In Russian)

Wandeler, P., Hoeck, P. E. A. and Keller, L. F. 2007. Back to the future: museum specimens in population genetics. *Trends in Ecology & Evolution* 22(12):634–642. https://doi.org/10.1016/j.tree.2007.08.017