Музейные коллекции и ДНК

Соловьева Е.Н.

«Лаборатория Исторической ДНК»

Зоологический музей МГУ имени М.В. Ломоносова

Зоологические коллекции как источник ДНК

- Генетические исследования типовых экземпляров необходимы при выяснении сложных таксономических и номенклатурных вопросов.
- Музейный материал позволяет исследовать генетическую изменчивость не только видов под угрозой исчезновения, но и исчезнувших популяций и видов; кроме того, работа с такой ДНК открывает возможность сравнительного изучения прошлого и настоящего популяций.
- Траты на экспедиции по сбору новых, свежих экземпляров зачастую оказываются дороже расходов на работу с архивной ДНК и к тому же не гарантируют успеха.

Типы зоологических коллекций

- Влажные коллекции:
- Этанол
- Формалин (фрагментализация, депуринизация, модификация)
- Сухие коллекции:
- Снятые шкуры, чучела
- Кости
- Энтомологические коллекции

Проблемы работы с «музейной» ДНК

- низкая копийность и деградация исторической ДНК (Zimmermmann et al., 2008);
- химическая модификация (Mitchell et al., 2005; Green et al., 2008);
- загрязненность микробной ДНК и вероятность
- контаминации экзогенной ДНК (Hofreiter et al., 2001; 2008, etc.);
- Присутствие ингибиторов ПЦР (Cooper, Wayne 1998; Yoder, Delefosse, 2002).

Как бороться с этими проблемами?

Работа с ДНК музейных коллекций

- Главное – предотвратить контаминацию!
- Система вентиляции с фильтрами
- Предбанник
- Компартментализация помещений
- Комбинезоны, маски, бахилы
- Постоянное очищение пространства

Работа с ДНК музейных коллекций

- Главное – предотвратить контаминацию!
- УФ-лампы
- Работа готовыми наборами (с изменениями протоколов)
- …И другие особенности лабораторной работы

MOLECULAR ECOLOGY
RESOURCES

Molecular Ecology Resources (2017) doi: 10.1111/1755-0998.12655

Phylogenomics using formalin-fixed and 100+ year-old intractable natural history specimens

SARA RUANE*[ID] and CHRISTOPHER C. AUSTIN*†

*Museum of Natural Science, Louisiana State University, 119 Foster Hall, Baton Rouge LA, 70803, USA, †Department of Biological Sciences, Louisiana State University, 202 Life Science Building, 119 Foster Hall, Baton Rouge LA, 70803, USA

Abstract

Museum specimens provide a wealth of information to biologists, but obtaining genetic data from formalin-fixed and fluid-preserved specimens remains challenging. While DNA sequences have been recovered from such specimens, most approaches are time-consuming and produce low data quality and quantity. Here, we use a modified DNA extraction protocol combined with high-throughput sequencing to recover DNA from formalin-fixed and fluid-preserved snakes that were collected over a century ago and for which little or no modern genetic materials exist in public collections. We successfully extracted DNA and sequenced ultraconserved elements ($\bar{x}$ = 2318 loci) from 10 fluid-preserved snakes and included them in a phylogeny with modern samples. This phylogeny demonstrates the general use of such specimens in phylogenomic studies and provides evidence for the placement of enigmatic snakes, such as the rare and never-before sequenced Indian *Xylophis stenorhynchus*. Our study emphasizes the relevance of museum collections in modern research and simultaneously provides a protocol that may prove useful for specimens that have been previously intractable for DNA sequencing.

Keywords: museum collections, next-generation sequencing, phylogeny, sequence capture, snakes, ultraconserved

Damaged by time and formalin: Using ancient DNA technology to recover century-old snake mitogenomes

Morten E. Allentoft[1], Arne Redsted[2] & Hans Viborg Kristensen[3]

[1] *Centre for GeoGenetics, Natural History Museum, University of Copenhagen. Øster Voldgade 5-7, 1350 Copenhagen K, Denmark*

[2] *The Royal Danish Academy of Fine Arts, School of Conservation, Esplanaden 34, 1263 København K, Denmark*

[3] *Naturhistorisk Museum, Wilhelm Meyers Allé 210, Universitetsparken 8000, Aarhus C, Denmark*

E-mail: meallentoft@snm.ku.dk

The Natural History Museum of Denmark is housing a remarkable herpetological collection with many thousands of specimens collected across centuries. A large proportion of the animals have, however, been formalin-fixed during preservation which is known to seriously compromise the potential for molecular research.

Using carefully optimized protocols normally applied to ancient DNA research (Allentoft et al. 2015), combined with Next Generation Sequencing, we have recently received highly encouraging results from a number of such specimens. We present the methodology and showcase genetic results from the danish population of Smooth Snake (*Coronella austriaca*) that went locally extinct in the early 20th century.

Despite displaying severely degraded DNA (av. fragment length <50bp, C-T damage rates >10%), we managed to assemble the complete mitochondrial genomes of three danish *C. austriaca* individuals, and combined these data with mitogenomes of >30 modern individuals from across Europe, sequenced specifically for this study. Phylogenetic and network-based analyses showed that the danish population had very high genetic similarity with the current Scandinavian populations (posterior clade probability = 1) and was thus not related with the *C. austriaca* lineages present today in Central-Western Europe. We discuss the phylogeographic implications of these findings. More broadly, we discuss the potential for analysing highly degraded DNA (whether damaged by time or formalin or both) obtained from herpetological tissues.

Методы извлечения ДНК из старых насекомых

OPEN ACCESS Freely available online

PLoS one

Non-Destructive Sampling of Ancient Insect

Philip Francis Thomsen[1], Scott Elias[2], M. Thomas P. Gilbert[1], James Haile[3], Kaspe
Kuzmina[5], Duane G. Froese[5], Andrei Sher[6‡], Richard N. Holdaway[7,8], Eske Willersl

1 Centre for Ancient Genetics and Environments, Natural History Museum and Institute of Biology, University of Copenhagen, Co
Department, Royal Holloway, University of London, Egham, Surrey, United Kingdom, **3** Henry Wellcome Ancient Biomolecules Centre,
of Oxford, Oxford, United Kingdom, **4** Department of Integrative Biology, University of California, Berkeley, California, United States of A
Atmospheric Sciences, University of Alberta, Edmonton, Alberta, Canada, **6** Severtsov Institute of Ecology and Evolution, Russian Aca
7 Palaecol Research Ltd, Christchurch, New Zealand, **8** School of Biological Sciences, University of Canterbury, Christchurch, New Zea

Abstract

Background: A major challenge for ancient DNA (aDNA) studies on insect remains is that sampling least partial destruction of the specimens. A recent extraction protocol reveals the possibility of ob insect remains without causing visual morphological damage. We test the applicability of this proto beetle specimens dating back to AD 1820 and on ancient beetle chitin remains from permafrost (p dating back more than 47,000 years. Finally, we test the possibility of obtaining ancient insect DNA d sediments deposited 3280-1800 years ago - an alternative approach that also does not involve material.

Methodology/Principal Findings: The success of the methodological approaches are tested by PCR and 16S mitochondrial DNA (mtDNA) fragments of 77–204 base pairs (-bp) in size using species-spe primers.

Conclusion/Significance: The applied non-destructive DNA extraction method shows promisin museum specimens of historical age as far back as AD 1820, but less so on the ancient permafrost-preserved insect fossil remains tested, where DNA was obtained from samples up to ca. 26,000 years old. The non-frozen sediment DNA approach appears to have great potential for recording the former presence of insect taxa not normally preserved as macrofossils and opens new frontiers in research on ancient biodiversity.

DNA Extraction and PCR

DNA extraction and PCR setup was carried out in dedicated aDNA clean-laboratories [2]. DNA was extracted from museum specimens and macrofossils using the non-destructive method [16]: Whole specimens were placed in 2 ml Eppendorf Biopur tubes, fully immersed in digestion buffer (volume dependent on specimen size, 0.5–1.5 ml in this study), and incubated overnight at 55°C with gentle agitation. The buffer consisted of 3 mM $CaCl_2$, 2% sodium dodecyl sulphate (SDS), 40 mM dithiotreitol (DTT), 250 mg/ml proteinase K, 100 mM Tris buffer pH 8 and 100 mM NaCl (final concentrations). After incubating with gentle agitation for 16–20 hours, specimens were removed from the buffer, placed in 100% EtOH for 2–4 hours to stop further digestion, air-dried, and replaced in their collections. Nucleic acids were purified from the digestion buffer using a Qiagen PCR purification kit (QIAquick).

DNA from the sediments was extracted using the procedure described in [22] and [23].

Methods

A single tibia was removed from each of 133 air-dried adult *C. mercuriale* that were collected between 1924 and 1989 (Table 1). We are not aware that any special preservation treatment had been applied to any of the samples. Standard precautions to minimise contamination were employed throughout, such as using dedicated pipettes with anti-aerosol tips, bleaching forceps/pestles and processing samples in a room that had not been used previously for *C. mercuriale* genotyping. Legs were ground with a pestle in microcentrifuge tubes under liquid nitrogen and the DNA was extracted using a DNeasy tissue kit (Qiagen) following the protocol for animal tissue but with an overnight incubation (55°C) in buffer ATL/proteinase-K and a final elution volume of 140 µl.

High sensitivity DNA quantification was achieved from the sample fluorescence with PicoGreen® (Molecular Probes) relative to a DNA standard using a FLUOstar OPTIMA spectrofluorimeter (BMG Labtech) that was integrated onto a Microlab STAR robotic deck (Hamilton). Not all samples were quantified as some were exhausted during laboratory evaluation of other microsatellite loci (that ultimately proved unreliable for PCR with museum DNA). The 'quality' of the genetic material was examined by PCR amplification at 10 microsatellite loci (LIST4-002, LIST4-023, LIST4-024, LIST4-031, LIST4-034, LIST4-035,

11:195–198
-9024-y

UNICATION

s DNA extracted from the legs of archived insects llite-based population genetic analyses?

vid J. Thompson ·
Stephen J. Kemp

6 / Accepted: 23 May 2006 / Published online: 25 November 2006

tained from museum specimens perspective on levels of genetic samples are irreplaceable so it is parts of the specimens are used, amount of DNA obtained from , at present there are no quantiof DNA from such samples. In this the amount of DNA that may be gs of museum-archived specimens be obtained by routine PCR. Thus, air-dried insect legs more than 50 years old appear to have limited usefulness for studies that seek to amplify many nuclear loci without the use of other techniques that may be used to increase the possible low-quantities of template DNA present.

Keywords DNA extraction · Genotyping · Historic DNA · Microsatellite · Non-destructive sampling

Table 1 Sample information, yield of DNA obtained (from single damselfly legs) and the percentage of positive genotypes that were identical (i.e. excluding all allelic dropouts & one or more PCR failures) across two separate rounds of PCR at 10 microsatellite loci

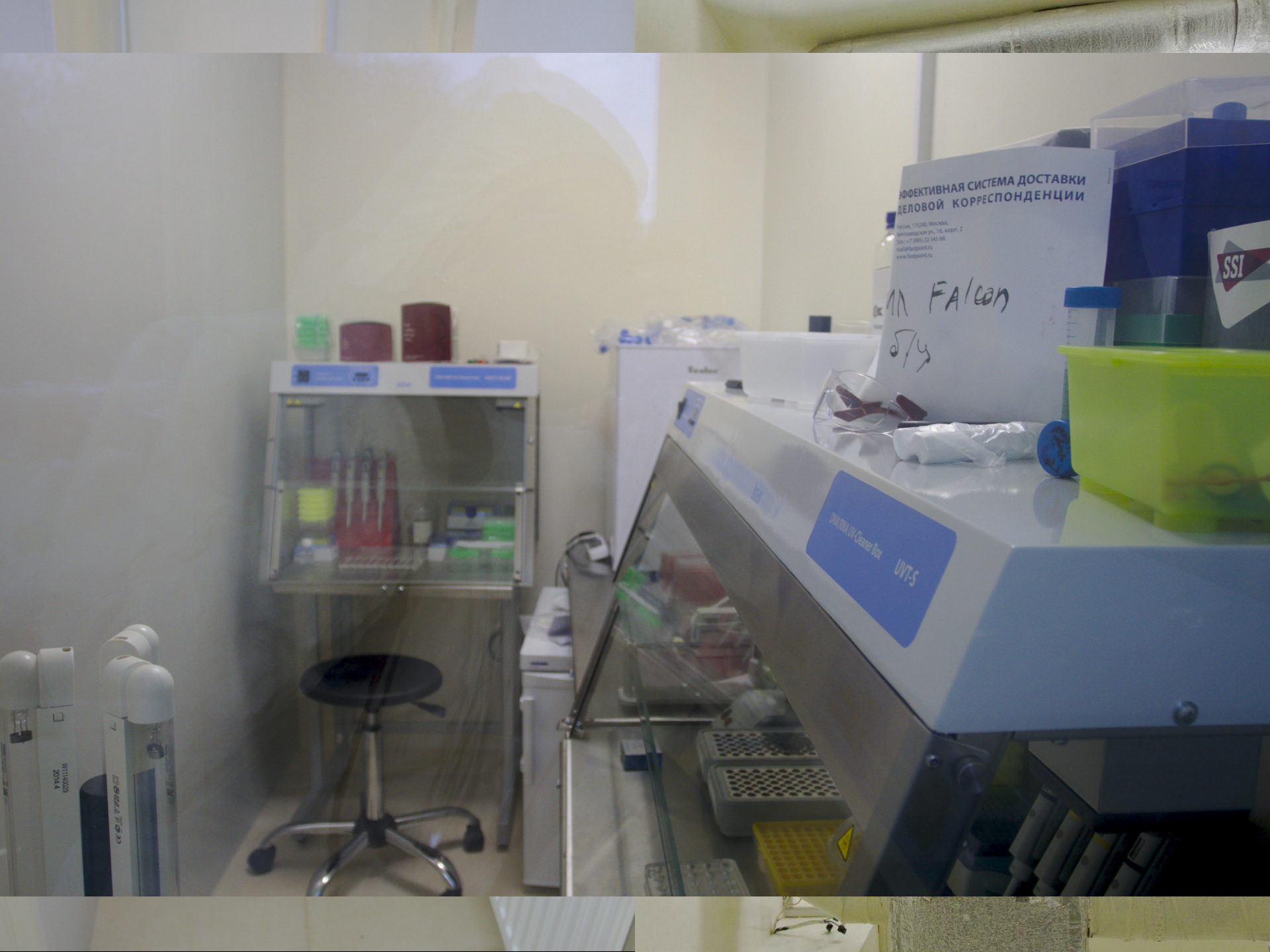
Sample information		*n*	DNA concentration		PCR success
Date	Location		ng μl^{-1}	SE	%
1989	Hampshire, England	3			100.0
1982	Rochebeaucount, France	6			83.3
1981	Rochebeaucount, France	5	3.30	0.78	68.0
1968	Spain	14	0.74	0.12	97.1
1965	Devon, England	1	0.34	–	90.0
1963	Devon, England	5	0.44	0.22	82.0
1962	Devon, England	1	0.75	–	90.0
1958	Dorset, England	1			60.0
1957	Cornwall, England	31	0.29	0.07	71.5
1954	Hampshire, England	7	0.18	0.17	77.1
1953	Hampshire, England	3	<0.01	–	0.0
1952	Pembrokeshire, Wales	28	0.10	0.03	0.0
1951	Hampshire, England	2	<0.01	–	0.0
1949	Hampshire, England	6	<0.02	–	0.0
1946	Hampshire, England	2	<0.01	–	0.0
1943	Hampshire, England	2			0.0
1942	Hampshire, England	8	<0.01	–	0.0
1924	Hampshire, England	8			0.0
	Total	133			

n, sample size; SE, standard error

Российские лаборатории, работающие с древней и старой ДНК

- Лаб. палеогенетики населения Евразии, Институт цитологии и генетики СО РАН (руководитель к.б.н. Пилипенко А.С.)
- Лаб. эволюционной геномики, Институт общей генетики им. Вавилова РАН. (руководитель: д.б.н., профессор Рогаев Е.И.)
- Лаборатория исторической генетики, радиоуглеродного анализа и прикладной физики, МФТИ (руководитель Мустафин Х.Х.)
- Лаб. генетики человека, Институт общей генетики им. Вавилова РАН. (руководитель: д.б.н, доцент Жукова О.В.)
- Лаб. геномики и биоинформатики, НИЦ «Курчатовский институт. (руководитель к.б.н. А.В. Недолужко)
- «Лаб. Исторической ДНК» ЗММУ

Лаборатория исторической ДНК Зоологического музея МГУ


ЭФФЕКТИВНАЯ СИСТЕМА ДОСТАВКИ
ДЕЛОВОЙ КОРРЕСПОНДЕНЦИИ
SSI
Falcon
DNA DNA UV-Cleaner Box
UVT-S


LEDENEV


PCR
TUBES
RACKS
TIPS
biofan
DNA/RNA UV-Cleaner Box
UVC/T-M-AR


Tesler


Breezart
Breezart

Кратко о методах

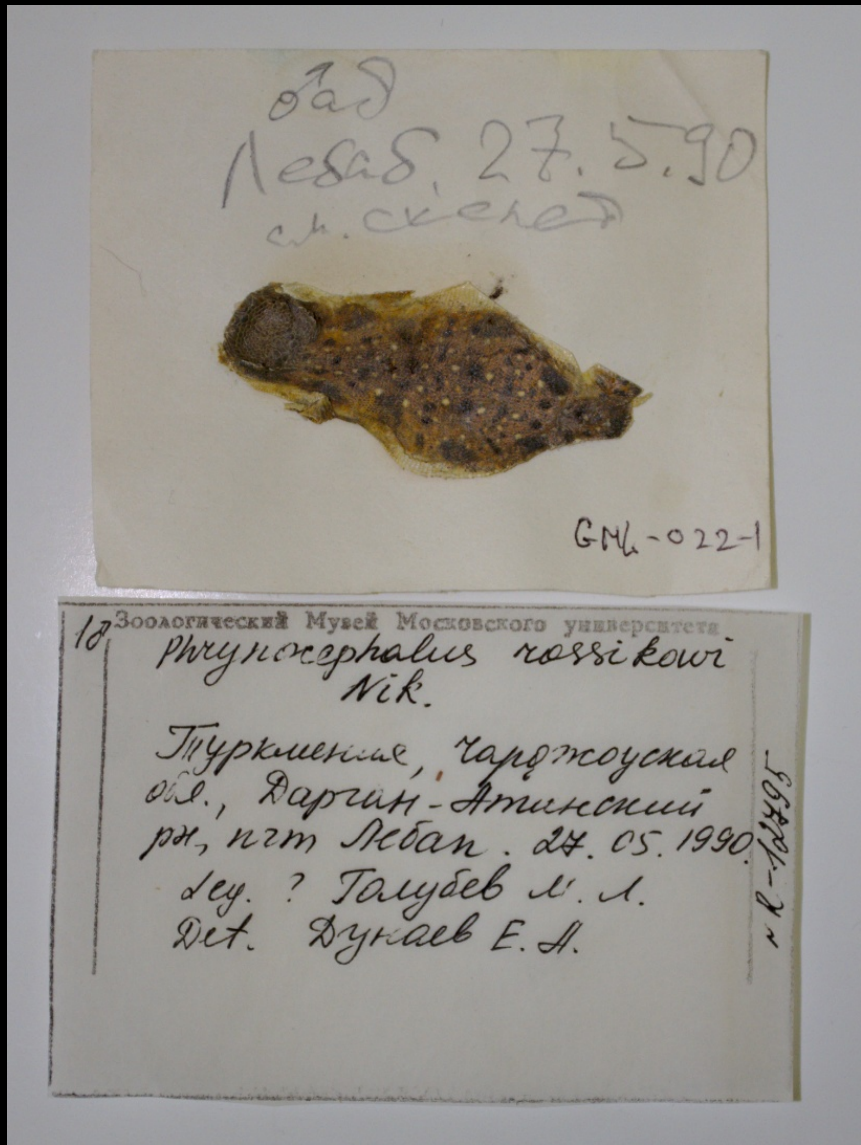
- Выделение ДНК наборами Qiagen с модификациями.
- Измерение выделенной ДНК на аппарате Nanodrop Light.
- Для каждого объекта подбирают специфические пары праймеров на короткие перекрывающиеся фрагменты (100-200 п.н.) ДНК.
- Контроль качества ПЦР-продуктов осуществляют в 1% агарозном геле на базе «Лаборатории молекулярных методов в зоологии» Биологического факультета МГУ.
- Для секвенирования ПЦР-продукты передают в ЗАО «Евроген».

Phrynocephalus rossicowi Nikolsky, 1898 – хентаунская круглоголовка

- Материал: сухая шкурка

- Малоизученный вид, обитающий на территории Туркменистана и на граничащих с Туркменистаном территориях южного Узбекистана.

- В коллекции Зоомузея депонирован только один экземпляр, с которым и проводилась работа.
- Дата сбора: 1990 г.

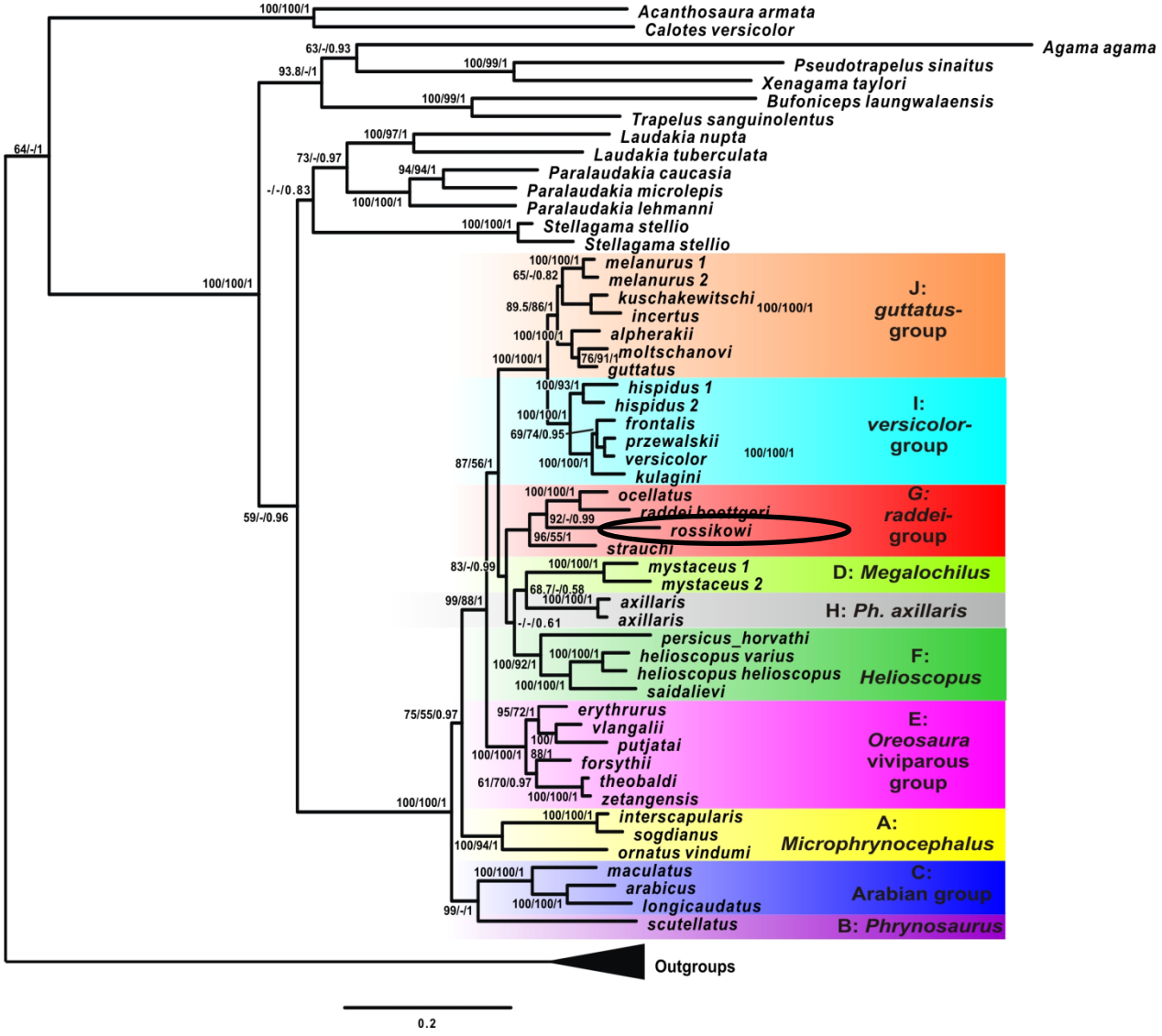


Phrynocephalus rossicowi Nikolsky, 1898 – хентаунская круглоголовка

Solovyeva E.N., 2017. The position of *Phrynocephalus rossikowi* (Reptilia, Agamidae) according to mtDNA from old museum specimen // SEH 19th OGM, programs and abstracts, 18 September - 24 September 2017.

Solovyeva et al., 2018. Cenozoic aridization in Central Eurasiashaped diversification of toad-headedagamas (Phrynocephalus; Agamidae,Reptilia) // PeerJ, DOI 10.7717/peerj.4543

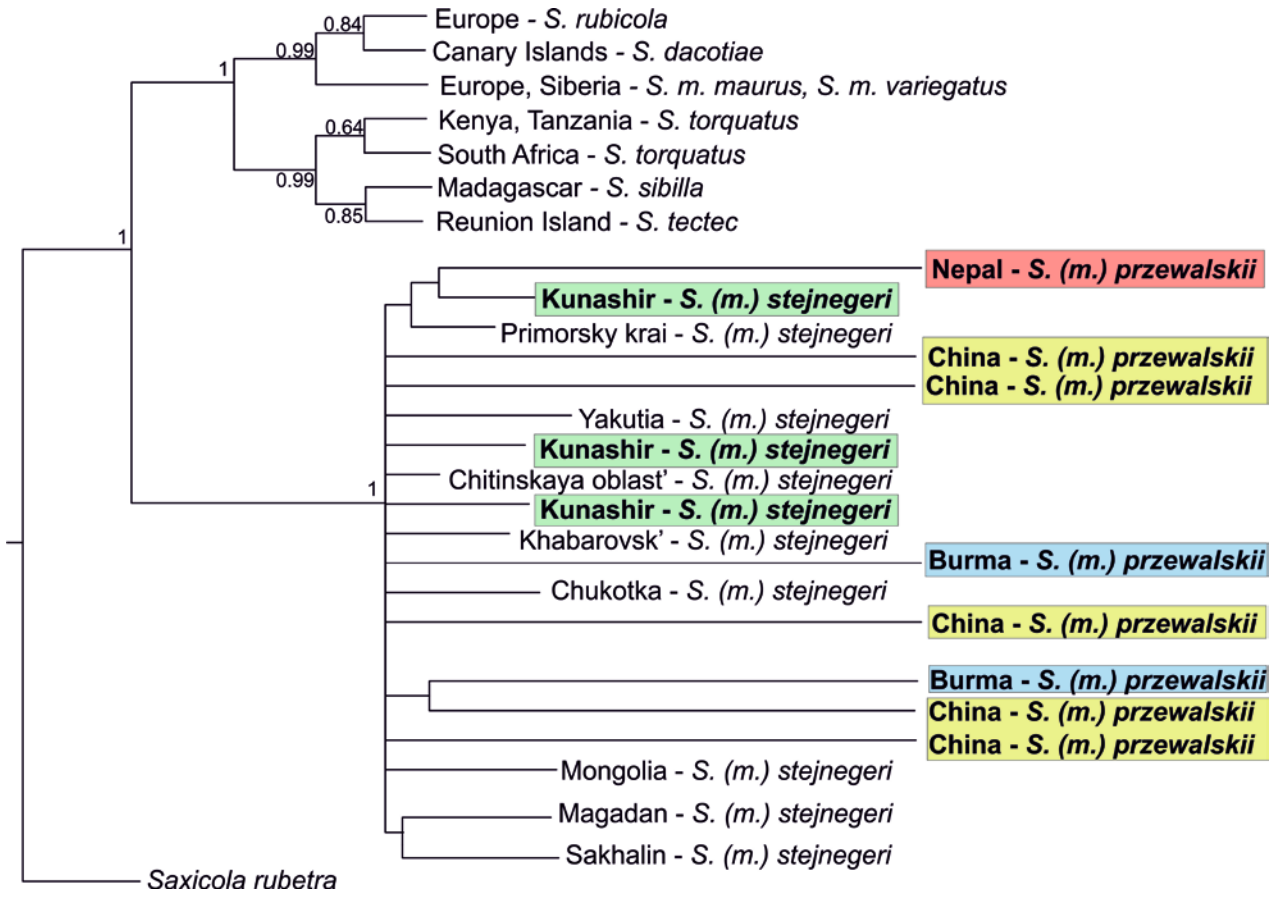
Phrynocephalus rossicowi Nikolsky, 1898 – хентаунская круглоголовка



Saxicola przewalskii (Pleske 1889) – чекан Пржевальского

- 11 проб возрастом 130-150 лет:
- 5 проб представителей формы спорного статуса *Saxicola przewalskii* (=*S. maurus przewalskii*), обитающей в Южном Китае, а также 6 проб *S. stejnegeri*.

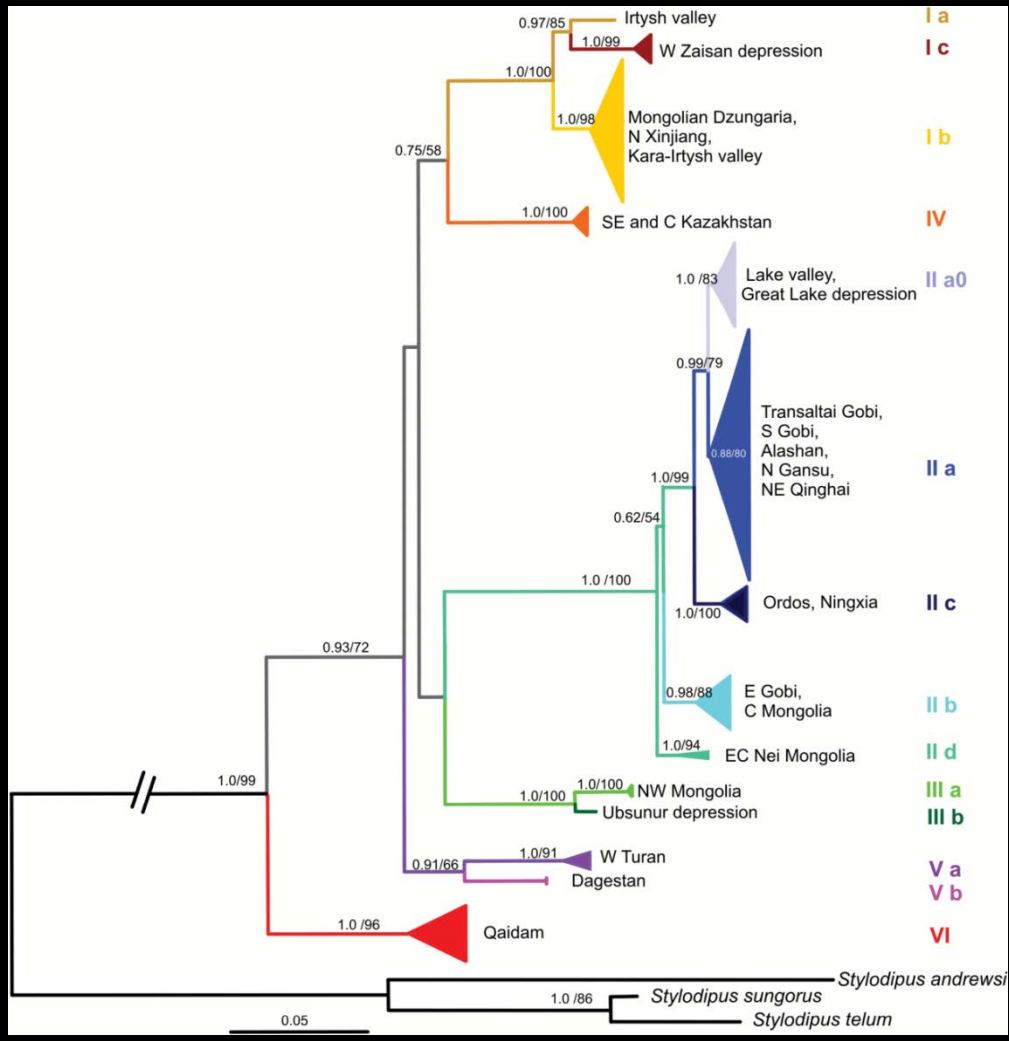
Saxicola przewalskii (Pleske 1889) – чекан Пржевальского



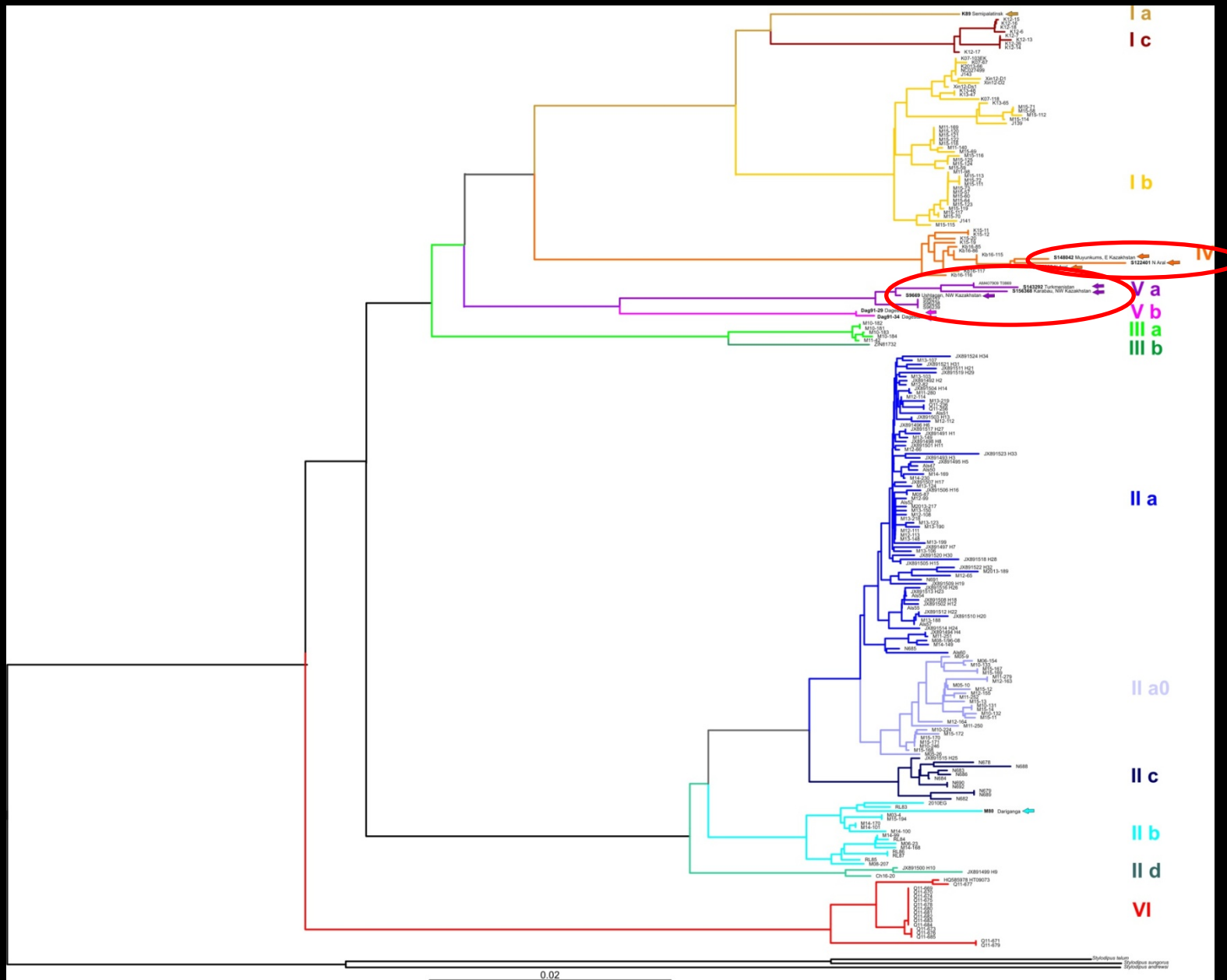
Фрагменты гена ND2 длиной около 700 п.о. получены для 7 особей, для 4 особей получены более короткие фрагменты. Построены филогенетические деревья (BI и ML).

Тушканчики рода *Dipus*

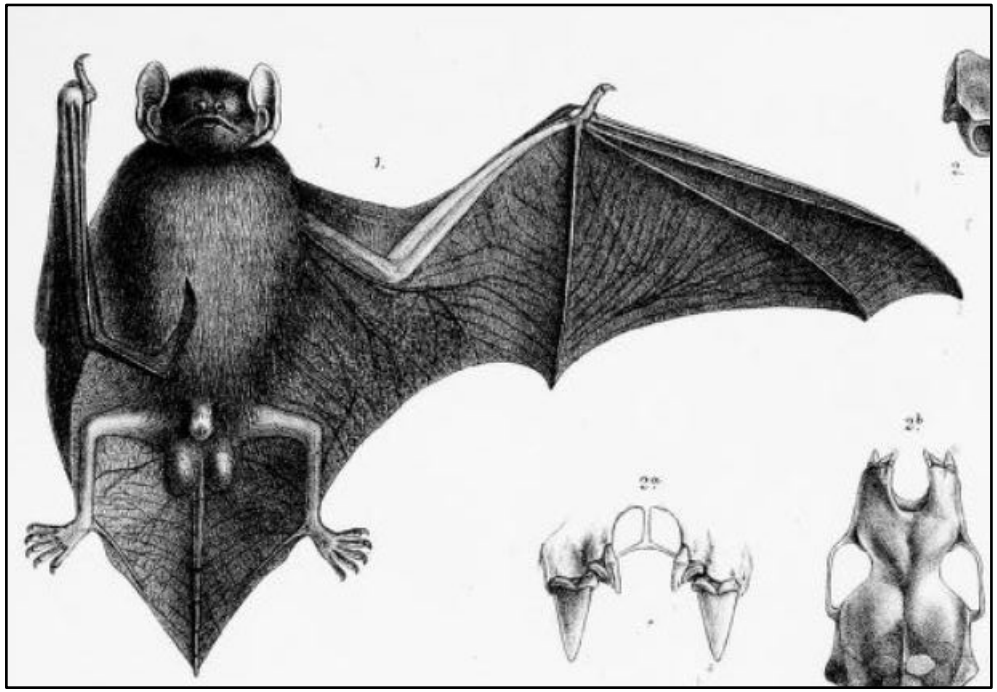
Выделено ДНК из 5 проб (ZMMU S-152695, S-143292, S-122385, S-156368, S-148042) тушканчиков, получены сиквенсы фрагмента cytb длиной около 300 п.о. Произведен филогенетический анализ, полученные результаты опубликованы в статье «Phylogeographical study reveals high genetic diversity in a widespread desert rodent, *Dipus sagitta* (Dipodidae: Rodentia)».



Тушканчики рода *Dipus*



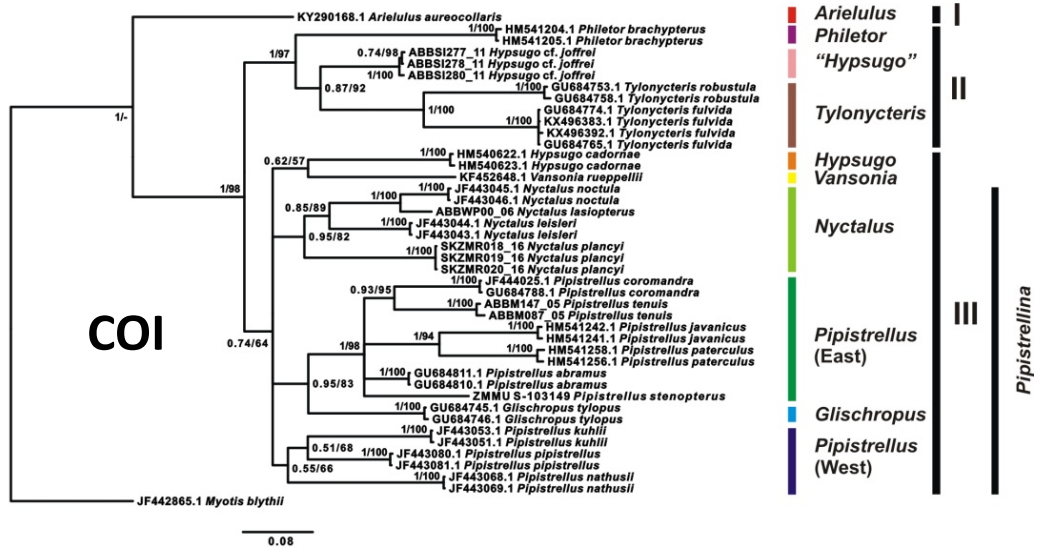
Pipistrellus stenopterus (Mammalia, Vespertilionidae)

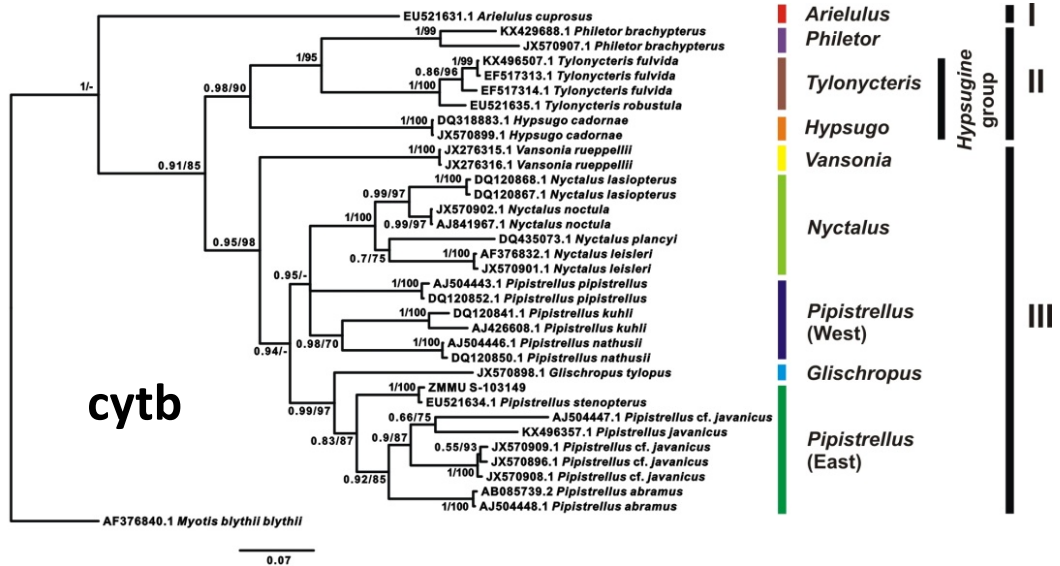


“What adaptations may come: taxonomic position of the Malayan noctule (Vespertilionidae; Chiroptera)“

Данные и митохондриальной (404 п.о. cytb, 615 п.о. COI), и ядерной ДНК (723 п.о. RAG2) демонстрируют близость *P. stenopterus* азиатским пипистреллюсам, скорее всего это сестринская ветвь группе “*javanicus*”.

Pipistrellus stenopterus (Mammalia, Vespertilionidae)



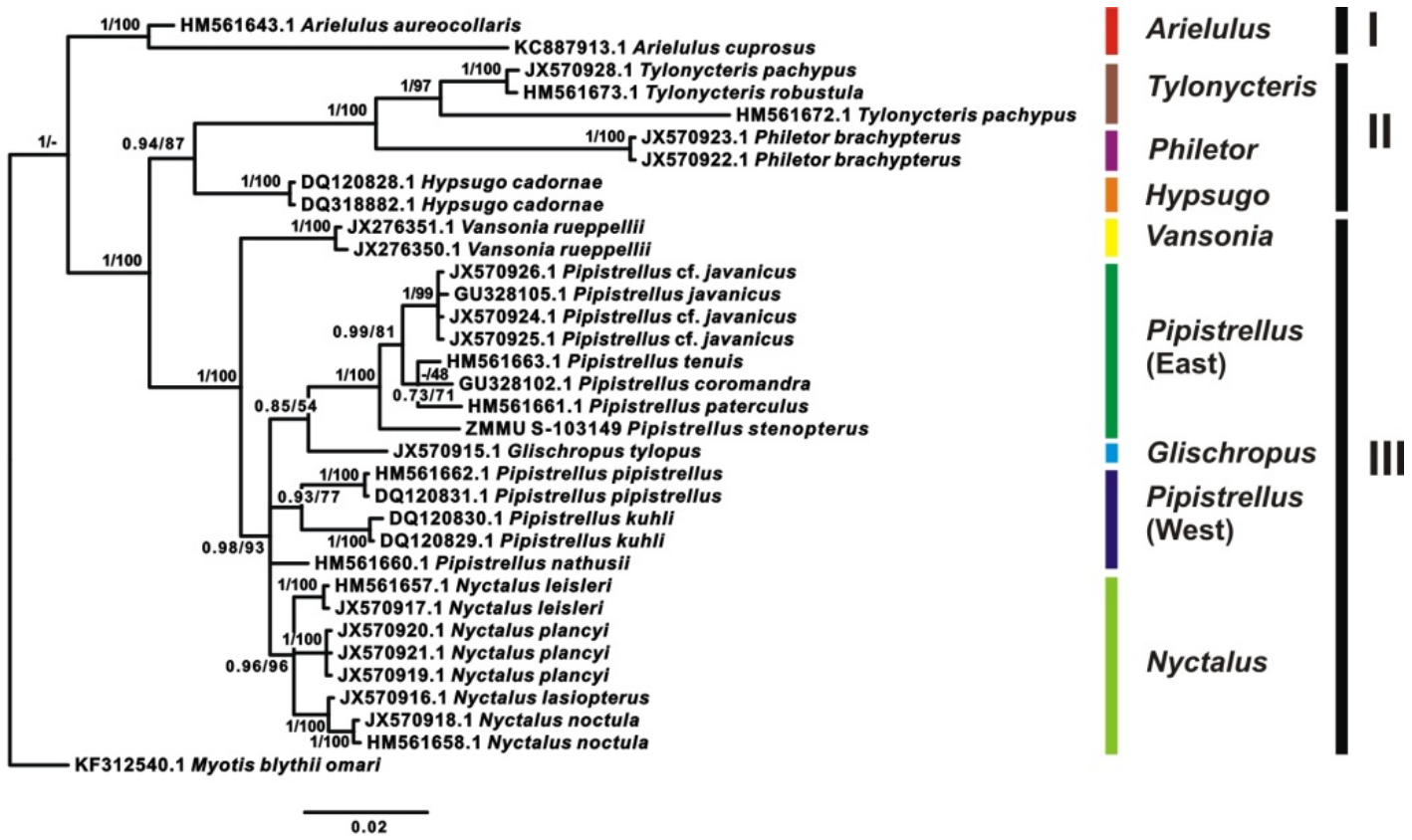


What adaptations may come: taxonomic position of the Malayan noctule (Vespertilionidae; Chiroptera)"

Данные митохондриальной (404 п.о. cytb, 615 п.о. COI), и ядерной ДНК (723 п.о. RAG2) демонстрируют близость *P. stenopterus* азиатским пипистреллюсам, скорее всего это сестринская ветвь группе "*javanicus*".

Pipistrellus stenopterus (Mammalia, Vespertilionidae)

RAG-2



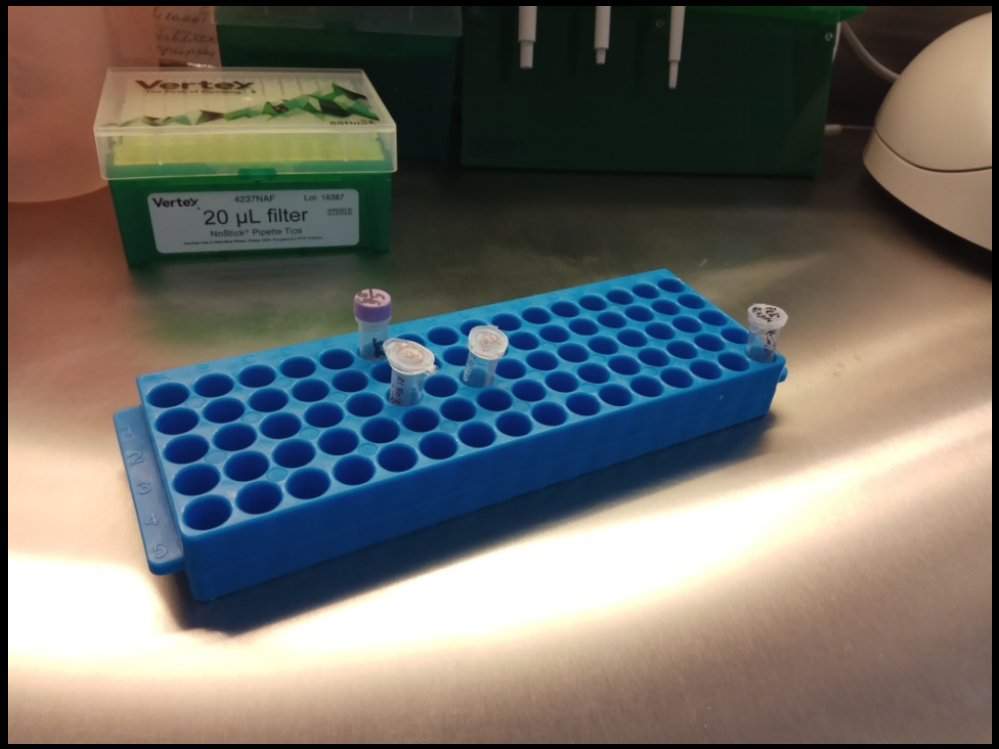
В статье предлагается выделение *P. stenopterus* в отдельный подрод *Alionoctule*.

Также подтверждается парафилия гена *Pipistrellus*.

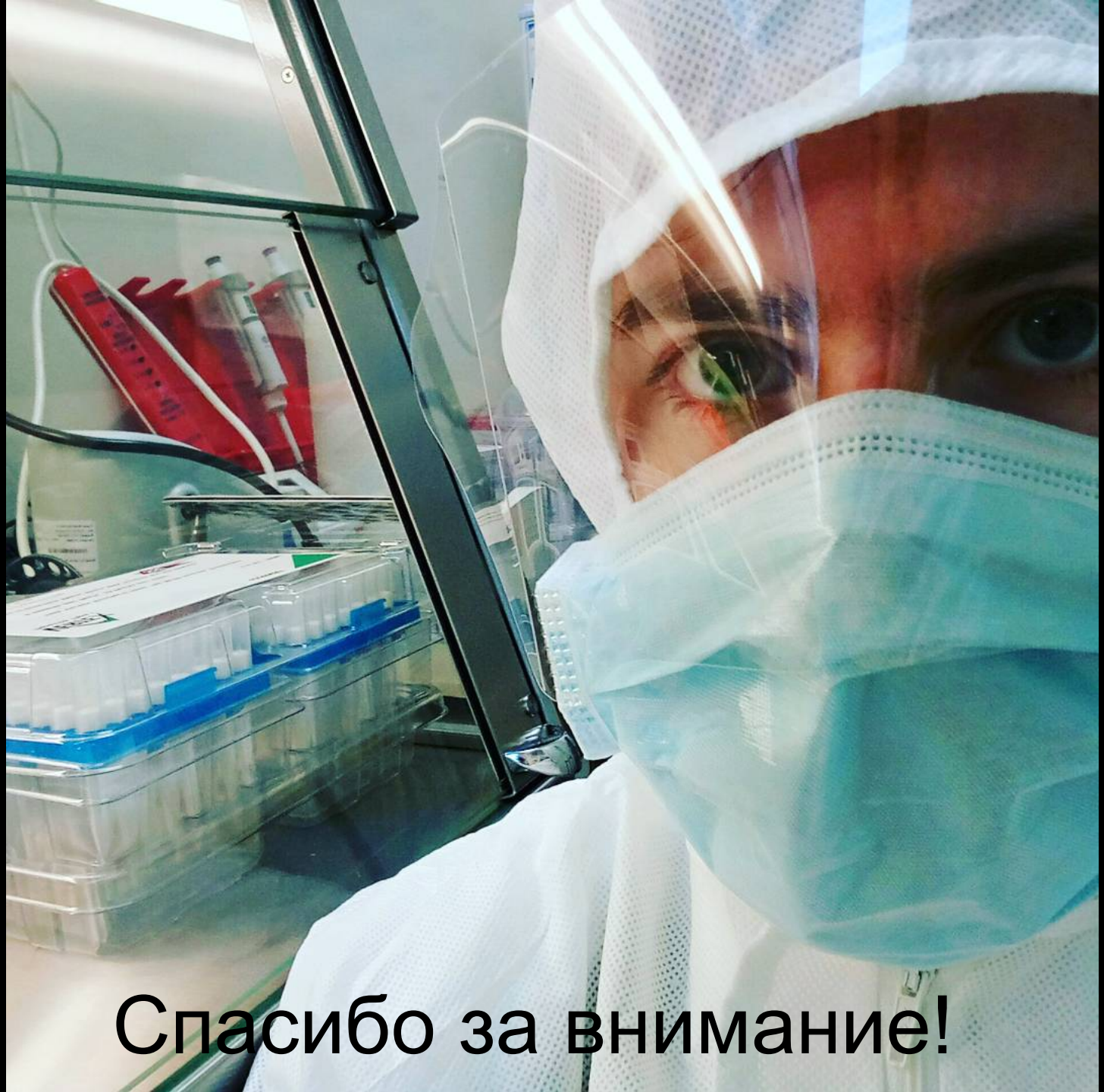
Другие проекты

- Кокшальская ящурка *Eremias kokshaalinensis* (Reptilia, Lacertidae)
- Тушканчик Виноградова *Allactaga vinogradovi* (Mammalia, Dipodidae).
- *Mogera robusta* (Mammalia, Talpidae).
- *Microtus shantaricus* (Mammalia, *Cricetidae)*.
- Геном тарпана *Equus gmelini* (Mammalia, Equidae).
- И это еще не всё!

Другие проекты



- Пока неудачные…:
- *Cobitis satunini* (Actinopterygii, Cobitidae) – формалин!



Спасибо за внимание!

Naturhistoriska riksmuseet: Ancient DNA laboratory

Лаборатория Древней ДНК отдела биоинформатики и генетики стокгольмского музея естественной истории

Please cite this article in press as: Pečnerová et al., Genome-Based Sexing Provides Clues about Behavior and Social Structure in the Woolly Mammoth, Current Biology (2017), https://doi.org/10.1016/j.cub.2017.09.064

Current Biology
Report

CellPress

Genome-Based Sexing Provides Clues about Behavior and Social Structure in the Woolly Mammoth

Patrícia Pečnerová,[1,2,*] David Díez-del-Molino,[1] Nicolas Dussex,[1] Tatiana Feuerborn,[1,3] Johanna von Seth,[1,2] Johannes van der Plicht,[4,5] Pavel Nikolskiy,[6] Alexei Tikhonov,[7,8] Sergey Vartanyan,[9] and Love Dalén[1,10,*]
[1]Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Box 50007, 10405 Stockholm, Sweden
[2]Department of Zoology, Stockholm University, 10691 Stockholm, Sweden
[3]Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen K, Denmark
[4]Centre for Isotope Research, Groningen University, Nijenborgh 4, 9747 AG Groningen, the Netherlands
[5]Faculty of Archaeology, Leiden University, Postbus 9514, 2300 RA Leiden, the Netherlands
[6]Geological Institute of the Russian Academy of Sciences, Pyzhevsky pereulok 7, Moscow 119017, Russia
[7]Zoological Institute of Russian Academy of Sciences, Universitetskaya nab. 1, 199034 Saint Petersburg, Russia
[8]Institute of the Applied Ecology of the North, North-Eastern Federal University, Lenina 1, 677000 Yakutsk, Russia
[9]North-East Interdisciplinary Scientific Research Institute N.A.N.A. Shilo, Far East Branch, Russian Academy of Sciences (NEISRI FEB RAS), Magadan, Russia
[10]Lead Contact
*Correspondence: patricia.pecnerova@nrm.se (P.P.), love.dalen@nrm.se (L.D.)
https://doi.org/10.1016/j.cub.2017.09.064

SUMMARY

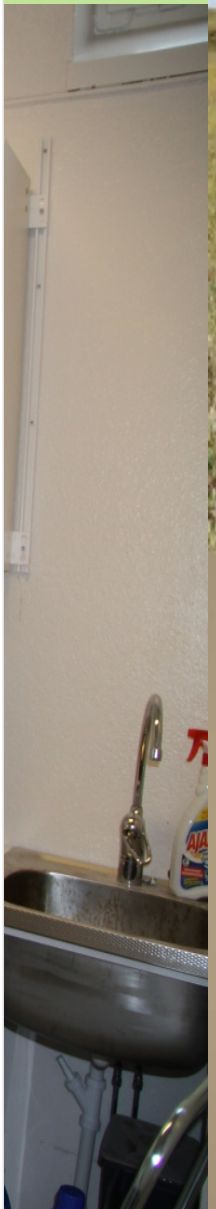
While present-day taxa are valuable proxies for understanding the biology of extinct species, it is also crucial to examine physical remains in order to obtain a more comprehensive view of their behavior, social structure, and life histories [1, 2]. For example, information on demographic parameters such as age distribution and sex ratios in fossil assemblages can be used to accurately infer socioecological patterns (e.g., [3]). Here we use genomic data to determine the sex of 98 woolly mammoth (*Mammuthus primigenius*) specimens in order to infer social and behavioral patterns in the last 60,000 years of the species' existence. We report a significant excess of males among the identified samples (69% versus 31%; $p < 0.0002$). We argue that this male bias among mammoth remains is best explained by males more often being caught in natural traps that favor preservation. We hypothesize that this is a consequence of social structure in proboscideans, which is characterized by matriarchal hierarchy and sex segregation. Without the experience associated with living in a matriarchal family group, or a bachelor group with an experienced bull, young or solitary males may have been more prone to die in natural traps where good preservation is more likely.

ples collected at various locations throughout Siberia (Figure 1; Table S1). The samples mostly comprise individual fragments found in river basins and along coastlines and lake shores, where they have been redeposited after erosion from permafrost sediments. DNA was extracted using a silica-based method [4, 5], converted into indexed libraries [6], and sequenced on an Illumina HiSeq 2500 platform. Additionally, we included previously published whole-genome shotgun data from mammoth hair shafts [7, 8] to generate a final dataset of 98 mammoth samples. Sequence reads were mapped against the genome assembly of the African savannah elephant (*Loxodonta africana*). The number of reads mapping to chromosome X and 8, respectively, were used to determine the sex of each specimen (for details, see STAR Methods). In total, 66 specimens were identified as males and 29 as females (Figure 2).

Causes for a Biased Sex Ratio

All samples were collected opportunistically and do not originate from fossil assemblages and can thus be considered a random sample of the available fossil record. In the absence of other factors, this sampling scheme would be expected to yield a sex ratio equal to the natal sex ratio, which is usually balanced in mammal populations [9]. Furthermore, the natal sex ratios in both the wild Asian elephant (*Elephas maximus*) and the African savannah elephant are close to 1:1 [10], suggesting that the natal sex ratio was most likely also balanced in the woolly mammoth.

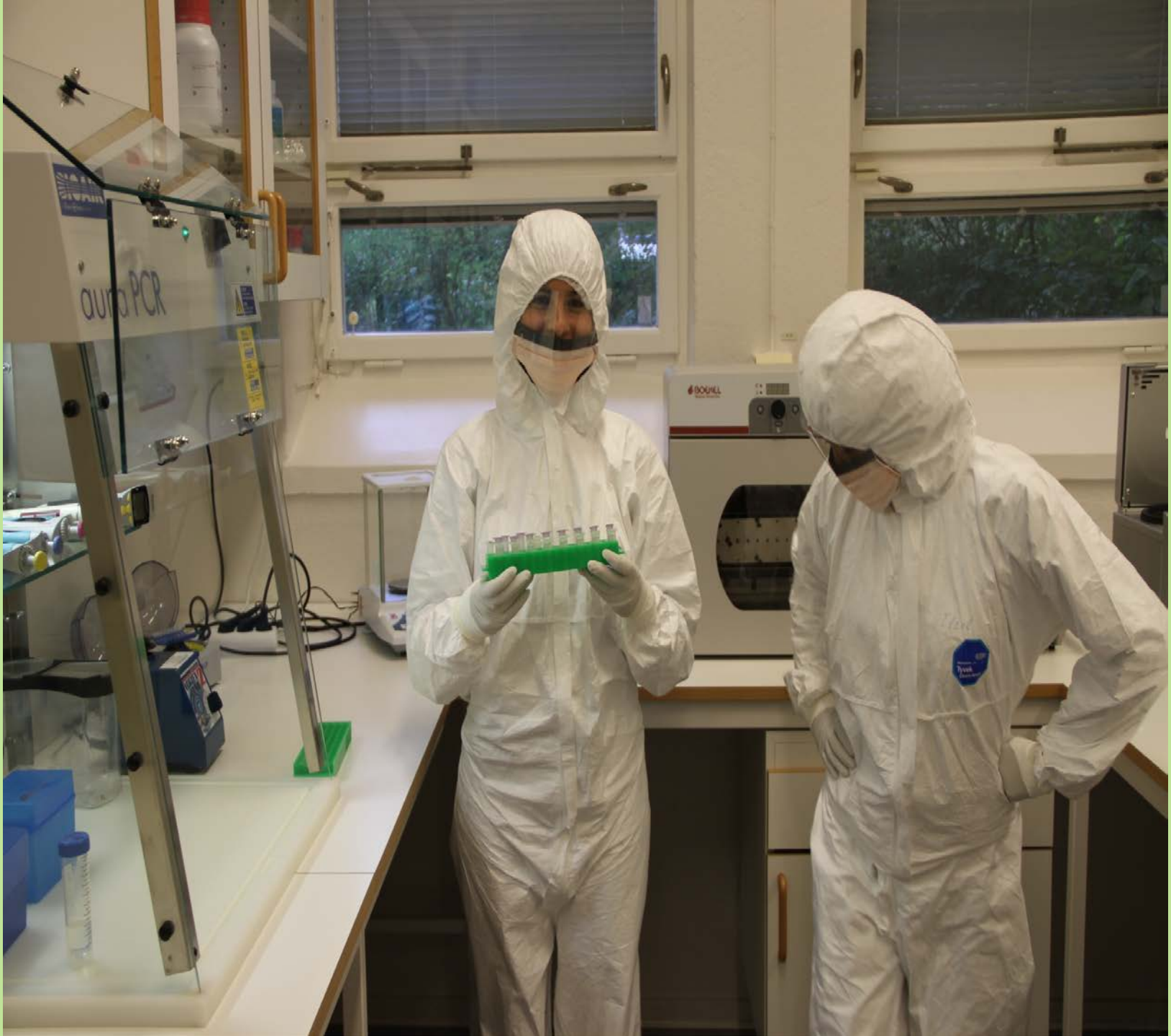
We find a role of sexual dimorphism unlikely in explaining the observed skew in sex ratio. In sexually dimorphic species, taphonomic processes such as scavenging, decomposition, and



- 2016:

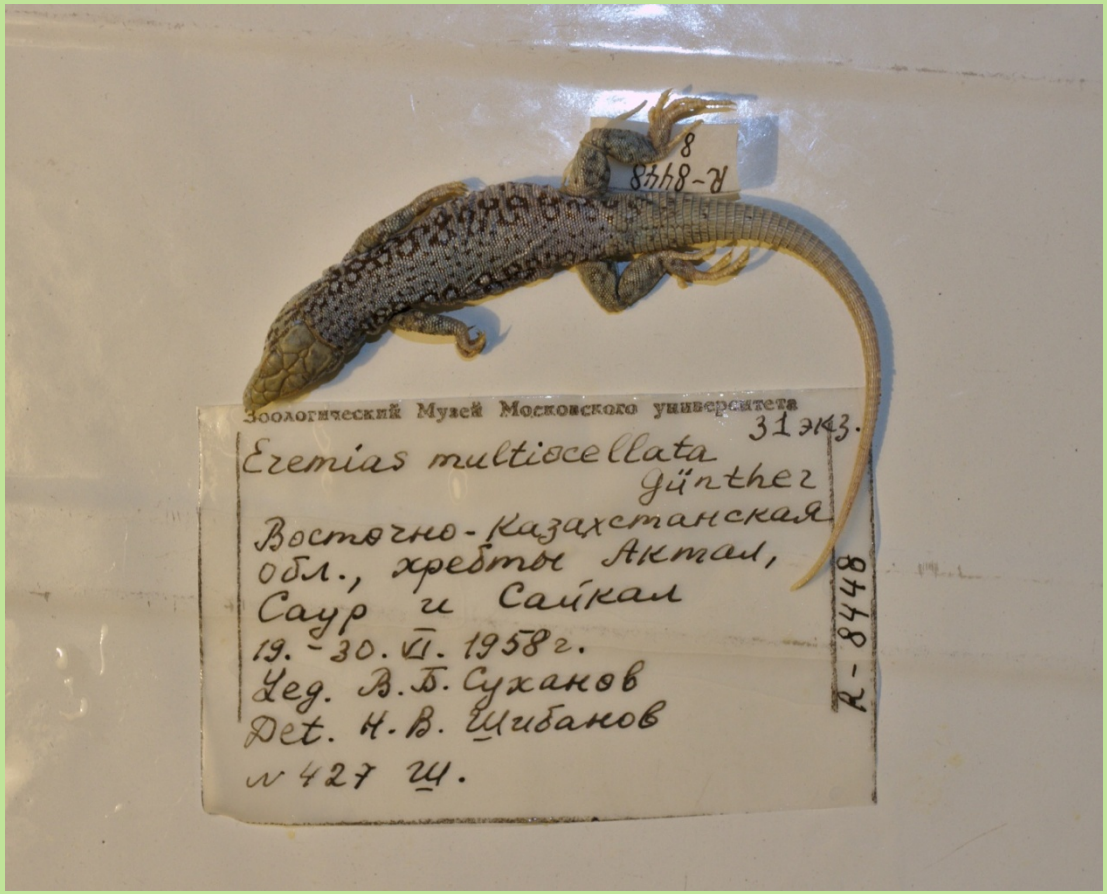
- участие в проекте по мегатериевой фауне: выделение ДНК из костей, генотипирование по 16S;

- выделение и ПЦР ДНК из старых образцов коллекции Зоологического музея МГУ им. М.В. Ломоносова: ящурка *Eremias multiocellata*, чекан Пржевальского *Saxicola przewalskii*, красный волк из вымершей киргизской популяции *Cuon alpinus*, возраст коллекционного материала от 1876 до 1958 г.) и получены фрагменты последовательностей гена COI этих образцов. Не удалось выделить ДНК из пробы загадочного *Ranodon cf. kozhewnikowi*.


auto PCR

Eremias cf. multiocellata Günther, 1872

- Материал: спиртовые коллекции.
- Популяция *E. cf. multiocellata* из окр. хребтов Актал, Саур и Сайкал (Восточно-Казахстанская обл.) (Reptilia, Lacertidae). Успешно выделена ДНК из пробы *E. cf. multiocellata* ZMMU R-8448-8 (1958 г.)



Saxicola przewalskii (Pleske 1889) – чекан Пржевальского

- Материал: тушка, проба – кусочек кожи
- Представители формы спорного статуса (=*S. maurus przewalskii*), обитающей в Южном Китае
- Дата сбора экземпляров: 1885 и 1876 гг. (140 и 130 лет)
- На данный момент в лаборатории ЗММУ еще 11 экземпляров

Cuon alpinus (Pallas, 1811) – красный волк

- Материал: шкура
- Шкура принадлежит особи из Киргизии, где на данный момент красные волки считаются вымершими.
- Дата сбора: зима 1937-38 г.

(78-79 лет)

Результаты ПЦР
сторической ДНК
из ЗММУ

азмер фрагментов
~150 bp