

## The northernmost Palearctic scorpion

### Самый северный скорпион Палеарктики

Nikita M. Poverennyi<sup>1</sup>, Matthew R. Graham<sup>2</sup>, Victor Ya. Fet<sup>3</sup>  
Н.М. Поверенныи<sup>1</sup>, М.Р. Грэхэм<sup>2</sup>, В.Я. Фет<sup>3</sup>

<sup>1</sup> Department of Biology, N.G. Chernyshevsky Saratov State University, Astrakhanskaya Str. 93, Saratov, 410012 Russian Federation. E-mail: nikitapov64@yandex.ru

Биологический факультет, Саратовский гос. университет им. Н.Г. Чернышевского, ул. Астраханская, 93, Саратов, 410012 Россия.

<sup>2</sup> Department of Biology, Eastern Connecticut State University, Willimantic, Connecticut 06226, USA. E-mail: grahamm@easternct.edu

<sup>3</sup> Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755–2510, USA. E-mail: fet@marshall.edu  
(Corresponding author)

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КЛЮЧЕВЫЕ СЛОВА: *Mesobuthus*, Buthidae, скорпионы, северная граница ареала, Палеарктика, Оренбург, Айтуйар.

**ABSTRACT.** This note addresses the northernmost (above 51°N) Palearctic scorpion population of the Aituar Steppe in the Orenburg Nature Reserve, Russia. According to our original *COI* mtDNA analysis, this population is *Mesobuthus bogdoensis* (Birula, 1896) (Buthidae).

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**РЕЗЮМЕ.** Данная заметка приводит новые данные о самой северной (выше 51° с.ш.) популяции скорпионов в Палеарктике, Айтуйарская степь, заповедник «Оренбургский», Оренбургская обл., Россия. Согласно нашим новым данным, полученным в ходе анализа мтДНК-маркера *COI*, эта популяция относится к виду *Mesobuthus bogdoensis* (Birula, 1896) (Buthidae).

### Introduction

The Asian genus *Mesobuthus* Vachon, 1950, s.str. (Buthidae) includes the most common scorpion species found from Turkey to China. A most recent, extensive revision (Kovařík *et al.*, 2022), based on new collections, mainly from Central Asia, Turkey, and Iran, used both morphological and *COI* mtDNA marker data. Currently, the genus includes 29 valid species. The type species, *Mesobuthus eupeus* (C.L. Koch, 1839), s.str., is restricted to the Caucasus Mts.

The identity of scorpions of the genus *Mesobuthus* that inhabit northwestern Kazakhstan and adjacent areas of Russian Federation (Orenburg Province) is currently unclear. This note focuses on the northernmost known scorpion population of the Palearctic (above 51°N), from the Aituar Steppe in the Orenburg Nature

Reserve. Molecular data indicate that the populations is *M. bogdoensis* (Birula, 1896).

### Material and methods

The field collection was performed by the first author (N.P.). On 13 August 2021, 10 scorpion specimens were collected with the help of UV light at night on a slope of the Karagashta Ravine, 1 km from Aituar Village, Kuvandyk District, Orenburg Province, Russian Federation (51.1072°N, 57.6606°E), within the Aituar Steppe area of the Orenburg Nature Reserve. The specimens were found in dry grass-tussock stony steppe habitat (Fig. 2). DNA extraction, amplification, and sequencing of the *COI* gene for this population were also performed by the first author (N.P.) according to the methods published earlier [Poverennyi, Anikin, 2020a, b]. The new single Aituar *COI* sequence was deposited in GenBank under accession number OM905082. This sequence was added to 23 sequences belonging to five other congeneric species, which represented a subset of 98 sequences recently used for revision of genus *Mesobuthus* [Kovařík *et al.*, 2022] (see Fig. 1 legend for details). The phylogenetic analysis of the combined dataset was performed by the second author (M.R.G.). The mitochondrial *COI* sequence data were aligned using MUSCLE [Edgar, 2004] in the AliView 1.7.1 software package [Larsson, 2014] using default parameters. Successful alignment of the sequences was confirmed visually and ends were trimmed manually in AliView. Phylogenetic relationships were estimated by implementing the criteria of Maximum Likelihood (ML) and Bayesian Inference (BI). In the ML analysis, IQTREE version 1.6.6 [Nguyen *et al.*, 2015] was used, allowing the software to select optimum substitution models with ModelFinder [Kalyaanamoorthy *et al.*, 2017]. Node support was calculated

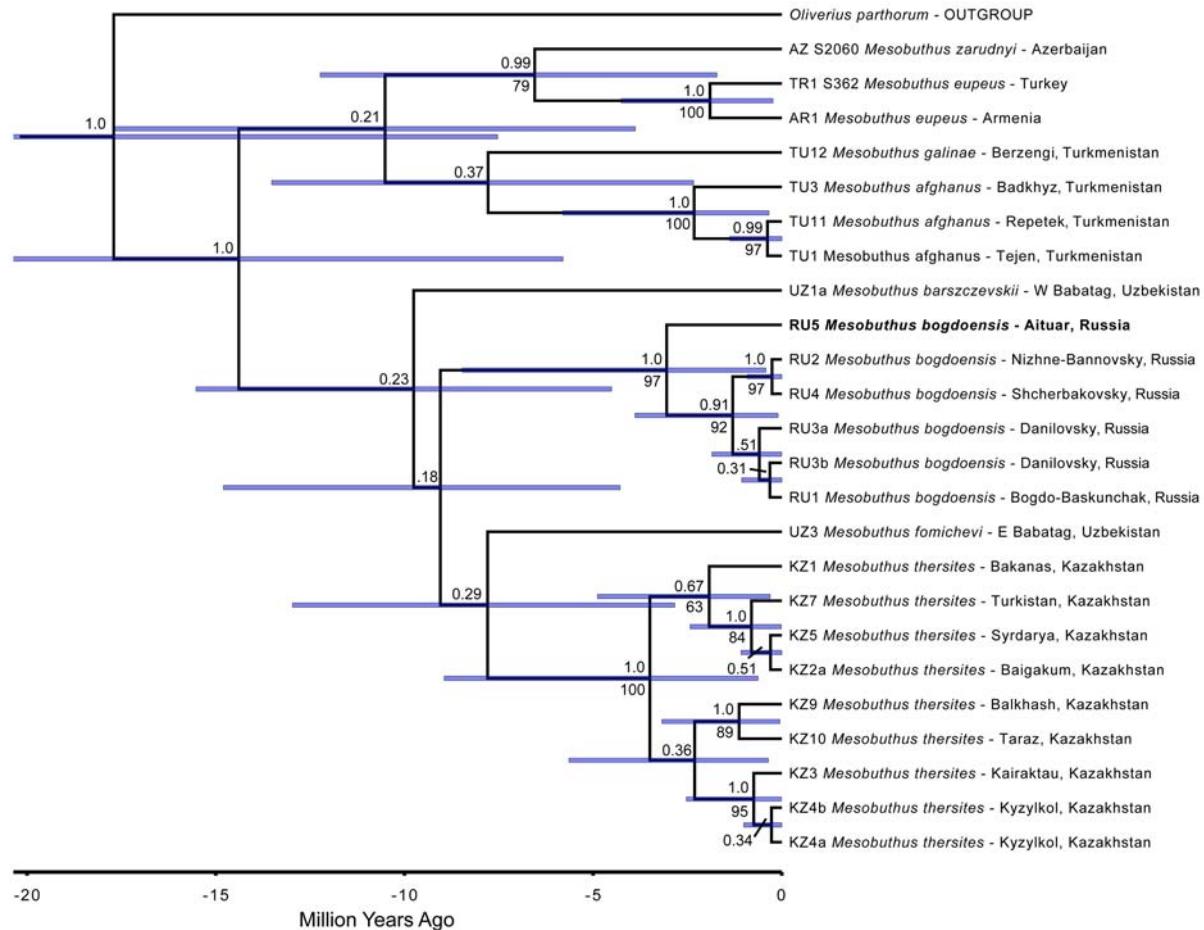


Fig. 1. A time-calibrated phylogenetic tree generated using Bayesian Inference (BI). Values above nodes are posterior probabilities from the BI analysis. Values below nodes indicate bootstrap values, where applicable, from a Maximum Likelihood (ML) analysis of the same samples. The species of *Mesobuthus* included in this analysis are: *M. afghanus* (Pocock, 1889) (Turkmenistan, TU1, TU3, TU11-12), *M. barsczewskii* (Birula, 1904) (Uzbekistan, UZ1a), *M. bogdoensis* (Birula, 1896) (Russia; RU1-RU5), *M. epeus* (C.L. Koch, 1839), s.str. (Armenia, AR1; Turkey, TR1), *M. fomichevi* Kovářík *et al.*, 2022 (Uzbekistan, UZ3), *M. thersites* (C.L. Koch, 1839) (Kazakhstan, KZ1, KZ2a, KZ3, KZ4a-b, KZ5, KZ7, KZ9, KZ10), *M. zarudnyi* Navruzov *et al.*, 2022 (Azerbaijan, AZ). Outgroup: *Olivierius parthorum* (Pocock, 1889). The northernmost sample from the Aituar Steppe is in bold. For more detail, see Kovářík *et al.* [2022].

Рис. 1. Филогенетическое дерево, полученное методами максимального правдоподобия (ML) и Байеса (BI) (консенсус). Виды рода *Mesobuthus*, включенные в анализ: *M. afghanus* (Pocock, 1889) (Туркменистан, TU1, TU3, TU11-12), *M. barsczewskii* (Birula, 1904) (Узбекистан, UZ1a), *M. bogdoensis* (Birula, 1896) (Россия; RU1-RU5), *M. epeus* (C.L. Koch, 1839), s.str. (Армения, AR1; Турция, TR1), *M. fomichevi* Kovářík *et al.*, 2022 (Узбекистан, UZ3), *M. thersites* (C.L. Koch, 1839) (Казахстан, KZ1, KZ2a, KZ3, KZ4a-b, KZ5, KZ7, KZ9, KZ10), *M. zarudnyi* Navruzov *et al.*, 2022 (Азербайджан, AZ). Аутгруппа: *Olivierius parthorum* (Pocock, 1889). Подробнее см. Kovářík *et al.* [2022].

using ultrafast bootstrap resampling [Hoang *et al.*, 2018]. The BI analyses followed that of Kovářík *et al.* [2022]. MEGA X [Kumar *et al.*, 2018] was used to determine the best-fit substitution model (HKY+G), which was used in BEAST 1.8.0 [Drummond *et al.*, 2012] to conduct two MCMC runs of 50 million generations each, sampling every 5,000 generations. The Yule tree prior was selected and an uncorrelated log-normal clock was calibrated by using a normal mean rate prior (ucl.d.mean) with the mean set to 0.007 and Stdev set at 0.00146. Tracer 1.7 [Rambaut *et al.*, 2018] was used to confirm convergence among runs and adequate ESS values, and tree files were combined with TreeAnnotater (BEAST package). The consensus

tree (Fig. 1) was visualized with Figtree 1.4.0 (<http://tree.bio.ed.ac.uk/software/>).

## Results and Discussion

According to our phylogenetic analysis (Fig. 1), the Aituar population clearly belongs to *Mesobuthus bogdoensis* (Birula, 1896) (Buthidae), forming a lineage that is sister to four lower Volga populations of this species analyzed recently in our phylogeny of the genus [Kovářík *et al.*, 2022]. The sample from Aituar represents a unique haplotype but is only slightly divergent. Uncorrected p-distances between this sample and the other *M. bogdoensis* haplotypes range from



Fig. 2. The collection locality of the northernmost Palearctic scorpion (Aituar Steppe, Orenburg Nature Reserve). Photo by Nikita M. Poverennyi, 13 May 2021.

Рис. 2. Местообитание самого северного скorpиона Палеарктики — Айтурская степь, заповедник «Оренбургский». Фото: Н.М. Поверенный, 13.05.2021.

0.012 to 0.016, which is consistent with levels of within species haplotype diversity in other buthid scorpions (i.e. Pedroso *et al.* [2013]; Coelho *et al.* [2014]; Alqahtani & Badry [2020]).

*Mesobuthus bogdoensis* was originally described from the Maloe Bogdo hill (now in Bokeyorda District, West Kazakhstan Province, Kazakhstan) ( $48.46^{\circ}\text{N}$ ,  $47.08^{\circ}\text{E}$ ). For a detailed history of study and discussion of this species and its congeners, see Kovařík *et al.* [2022]. *Mesobuthus bogdoensis* was represented in the DNA phylogeny of Kovařík *et al.* [2022] by five sequences from Russia (Volgograd and Saratov provinces; see also Anikin & Poverennyi [2017]; Poverennyi & Anikin [2020a, b]). This species was listed (as either *M. eupeus* or *M. e. volgensis*) in the Red Data Books of endangered species of the Astrakhan, Saratov, and Volgograd Provinces of Russia. It is protected

there in the Shcherbakovsky Nature Park, Nizhne-Bannovsky Reserve, and Bogdo-Baskunchak Reserve, all three localities represented in our DNA data (see also Kovařík *et al.* [2022]).

The existence of scorpions in the Orenburg Province of Russia has been confirmed in literature only recently [Davygora, Rusakov, 2001, as “*M. eupeus*”], although a sample allegedly from the Guberli Mts (in the east of the Orenburg Province) has been known for more than a century [Fet, 1989]. The presence of a population in the Aituar Steppe (as “*M. eupeus*”) was confirmed by Fet [2010], after a personal communication by Sergei Esyunin.

The Aituar record of *Mesobuthus bogdoensis* extends the range of this species considerably to the northeast. Most likely, *Mesobuthus* records forming a continuous range from western Kazakhstan between

Volga and Ural Rivers (West Kazakhstan and Atyrau Provinces of Kazakhstan) [Fet, 1989] belong to the same species. *Mesobuthus bogdoensis* appears to represent an interesting palaeo-Caspian relict; its closest extant relatives are *M. barsczewskii* (Birula, 1904) and *M. fomichevi* Kovařík *et al.*, 2022 from southern Uzbekistan (Fig. 1).

It should be noted that the Aituar population represents the northernmost (above 51°N) scorpion record in the Palearctic realm. This could be also the northernmost boundary of the order Scorpiones worldwide, depending on the imprecisely defined northern range of *Paruroctonus boreus* (Girard, 1854) (Vaejovidae) in the Nearctic [Johnson, 2004; Fet, 2010].

#### Conflict of Interests

The authors declare no potential conflict of interest.

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