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MOLECULAR-GENETIC CHARACTERIZATION OF THE SPECIES *ERYX MILIARIS* (SQUAMATA: BOIDAE) DISTRIBUTED IN UZBEKISTAN

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Summary

The article presents a study on the molecular genetic characterization of the widespread Eryx miliaris (Pallas, 1773) species in Uzbekistan, the species is endemic to Asia. In Uzbekistan, two species belonging to the genus Eryx, E. miliaris and E. tataricus, are widespread, and their taxonomic status is considered problematic. According to the results of the molecular genetic studies, the nucleotide sequence of the mtDNA 16S rRNA domain of the E. miliaris, stored in the collection of the Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan, was studied. This species was studied by comparing it with the nucleotides of its species in the National Center for Biotechnology Information (NCBI) gene bank. According to the results of the bioinformatic studies, it was determined that this species has a 99% close identity to the E. miliaris (MN338670) species in the gene bank. In the phylogenetic tree, species belonging to the genus Eryx were grouped into 4 groups, and the E. miliaris species E. tataricus and E.f. jaculus were grouped together with 98.4% bootstrap support from the phylogenetic point of view, and the E. miliaris_Uz sample was found to be closely related to the E. miliaris species distributed in Iran. The mtDNA 16S rRNA region was found to be one of the key genes in the identification of species belonging to the genus Erys (Erycidae).

Keywords: *molecular genetic characterization, Eryx miliaris, Eryx tataricus, Uzbekistan, mtDNA 16S rRNA, phylogenetic analysis, taxonomic status, bioinformatics, gene bank, NCBI, species identification, Erycidae.*

Introduction. The fauna of Uzbekistan has an ancient history, which is connected by very complex relationships. In this region, endemic and autochthonous species of Turan and Turkestan play a significant role. Throughout history, various animals from Central Asia, India-China, the areas surrounding the Mediterranean Sea, and the Eurasian steppes have arrived here. At the same time, part of the fauna consists of species introduced from the Far East, the Caucasus, Europe, and America, either acclimatized or accidentally introduced. The modern fauna of Uzbekistan includes 706 species of vertebrates, of which 107 are mammals, 460 are birds, 64 are reptiles, 3 are amphibians, and 76 are fish. The number of invertebrates reaches more than 15 thousand species [1].

Recent studies have documented 13 species within the subfamily Erycinae, genus Eryx Daudin,1803, distributed globally: *E. borrii* Lanza & Nistri, 2005, *E. colubrinus* (Linnaeus, 1758), *E. conicus* (Schneider, 1801), *E. elegans* (Gray, 1849), *E. jaculus* (Linnaeus, 1758), *E. jayakari* Boulenger, 1888, *E. johnii* (Russell, 1801), *E. miliaris* (Pallas, 1773), *E. muelleri* (Boulenger, 1892), *E. somalicus* Scortecci, 1939, *E. tataricus* (Lichtenstein, 1823), *E. vittatus* Chernov, 1959 and *E. whitakeri* Das, 1991. These species are distributed across diverse biogeographical regions, including North and East Africa, Europe, the Middle East, and South and Central Asia [2]. Regionally, members of this genus are recorded in Russia (*E. miliaris),* Iran *(E. elegans, E. jaculus, E. tataricus, E. johnii, E. sistanensis),* Kazakhstan (*E. miliaris)* [3, 4].

Phylogenetic studies of *E. milliaris* specimens from Chine, based on mitochondrial DNA (mtDNA) sequences of the 16S rRNA and COI gene regions, have provided insights into the evolutionary relationships of this species with other ophidian taxa [5].

In Uzbekistan, two species of the genus *Eryx, E. miliaris* (Pallas,1773) and *E. tataricus* (Lichtenstein, 1823), are widely distributed [6]. However, morphological and molecular analyses have demonstrated limitations in differentiating these species with high confidence [7, 8]. Further integrative taxonomic approaches, incorporating genetic, ecological, and morphometric data, are necessary to resolve species delineation within the genus *Eryx*.

This study aims to conduct a molecular-genetic characterization of the species *Eryx miliaris*, which belongs to the genus Eryx and is stored in the collection of the Institute of Zoology of the Academy of Sciences of Uzbekistan.

Materials and methods. ***Data collection and DNA extraction*** Specimens were collected from the Institute of Zoology at the Academy of Sciences of the Republic of Uzbekistan. Total genomic DNA was extracted from these genetic samples using the DNeasy Blood and Tissue Kit (Qiagen Inc., November 2023). To analyze the nucleotide sequences of the mitochondrial 16S rRNA region of *Eryx miliaris*, we used the primers 16SA (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SB (5′-CCCGTCT GAAC TCAGATCACG-3′), which are commonly employed in the taxonomy of Squamata [9].

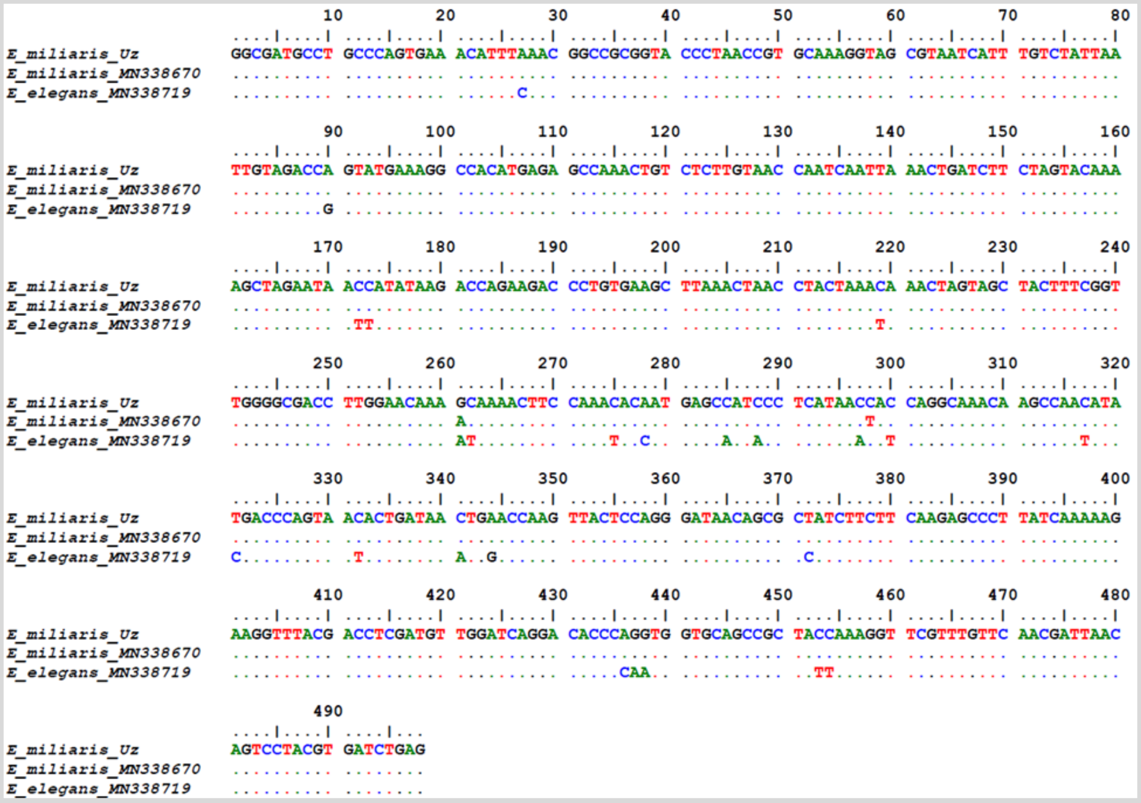
Polymerase chain reaction (PCR) amplification was performed under the following conditions: an initial denaturation at 95°C for 15 minutes, followed by a 30-second denaturation at 94°C, primer annealing at 53°C for 45 seconds, and elongation at 72°C for 1 minute and 40 seconds. A final elongation step was conducted at 72°C for 5 minutes. Steps two through four were repeated for up to 35 cycles in a thermal cycling process.

The presence of DNA in the PCR products was confirmed using electrophoresis on a 1.0% agarose gel at 120 V. DNA amplification and gel purification were performed according to the manufacturer's instructions with the reagent kit provided by "Silex M" (Moscow, Russia).

DNA sequencing was carried out using the ABI PRISM® BigDye™ Terminator v. 3.1 Cycle Sequencing Kit, and the sequencing reactions were analyzed on an ABI PRISM 3100-Avant Genetic Analyzer (Moscow, Russia).

Phylogenetic analysis. The DNA sequence obtained from the quality analysis was used for species identification via the BLAST program, confirming that it corresponds to Eryx miliaris. This specimen was aligned with closely related species retrieved from the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/) using the MAFFT program [10] and the presence of stop codons was checked with the AliView software. A phylogenetic tree was constructed using the Maximum Likelihood (ML) method with bioinformatics tools such as FastTree [11] and IQ-Tree v1.6.12 [12]. The nucleotide substitution model was selected using the ModelFinder Plus (MFP) function [13]. Phylogenetic analysis was conducted based on the EryxOR21.fasta nucleotide sequences, with the following computational parameters: automatic core detection (AUTO), SPRNG random number generator (Seed:834048), and optimized calculations using AVX+FMA processor support.

Results and discussion. According to the results of the molecular-genetic analysis, the 498 base pairs of nucleotide sequences belonging to the mtDNA 16s rRNA region of Eryx miliaris were compared to samples in the GenBank database. As a result, a 99% similarity was determined, confirming that the analyzed *E. miliaris* specimen belongs to the E. miliaris species in the GenBank database based on molecular methods. A comparison between the analyzed *Eryx miliaris*_Uz sample and *Eryx miliaris* sequences from GenBank revealed two nucleotide differences. Specifically, at the 261st nucleotide position, the *E. miliaris*_Uz sample contained G (guanine), whereas the *E. miliaris*_MN338670 sample from GenBank stored a (adenine). Additionally, at the 298th nucleotide position, the *E. miliaris*_Uz sample contained s (cytosine), while the *E. miliaris*_MN338670 sample from GenBank stored T (thymine) (Figure 1). These mutations are phylogenetically significant and may reflect genetic differentiation between Eryx miliaris populations. A comparison between the *Eryx miliaris*_Uz sample and E. elegans_MN338719 from GenBank showed 24 nucleotide differences, with an overall nucleotide similarity of 95.2%.



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                             10         20         30         40         50         60         70         80
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_miliaris_Uz        GGCGATGCCT GCCCAGTGAA ACATTTAAAC GGCCGCGGTA CCCTAACCGT GCAAAGGTAG CGTAATCATT TGTCTATTAA
E_miliaris_MN338670  .......... .......... .......... .......... .......... .......... .......... ..........
E_elegans_MN338719   .......... .......... ......C... .......... .......... .......... .......... ..........

                             90        100        110        120        130        140        150        160
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_miliaris_Uz        TTGTAGACCA GTATGAAAGG CCACATGAGA GCCAAACTGT CTCTTGTAAC CAATCAATTA AACTGATCTT CTAGTACAAA
E_miliaris_MN338670  .......... .......... .......... .......... .......... .......... .......... ..........
E_elegans_MN338719   .........G .......... .......... .......... .......... .......... .......... ..........

                            170        180        190        200        210        220        230        240
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_miliaris_Uz        AGCTAGAATA ACCATATAAG ACCAGAAGAC CCTGTGAAGC TTAAACTAAC CTACTAAACA AACTAGTAGC TACTTTCGGT
E_miliaris_MN338670  .......... .......... .......... .......... .......... .......... .......... ..........
E_elegans_MN338719   .......... .TT....... .......... .......... .......... ........T. .......... ..........

                            250        260        270        280        290        300        310        320
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_miliaris_Uz        TGGGGCGACC TTGGAACAAA GCAAAACTTC CAAACACAAT GAGCCATCCC TCATAACCAC CAGGCAAACA AGCCAACATA
E_miliaris_MN338670  .......... .......... A......... .......... .......... .......T.. .......... ..........
E_elegans_MN338719   .......... .......... AT........ ....T..C.. ....A..A.. ......A..T .......... ......T...

                            330        340        350        360        370        380        390        400
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_miliaris_Uz        TGACCCAGTA ACACTGATAA CTGAACCAAG TTACTCCAGG GATAACAGCG CTATCTTCTT CAAGAGCCCT TATCAAAAAG
E_miliaris_MN338670  .......... .......... .......... .......... .......... .......... .......... ..........
E_elegans_MN338719   C......... .T........ A..G...... .......... .......... .C........ .......... ..........

                            410        420        430        440        450        460        470        480
                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
E_miliaris_Uz        AAGGTTTACG ACCTCGATGT TGGATCAGGA CACCCAGGTG GTGCAGCCGC TACCAAAGGT TCGTTTGTTC AACGATTAAC
E_miliaris_MN338670  .......... .......... .......... .......... .......... .......... .......... ..........
E_elegans_MN338719   .......... .......... .......... .....CAA.. .......... ..TT...... .......... ..........

                            490
                     ....|....| ....|...
E_miliaris_Uz        AGTCCTACGT GATCTGAG
E_miliaris_MN338670  .......... ........
E_elegans_MN338719   .......... ........
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Figure 1. Comparison of Eryx milliaris species samples with nucleotide sequences

A cross-phylogenetic tree was created with this identified species and other specimens from GenBank (Figure 2).

From the phylogenetic tree, we observe that it was constructed based on the 16S rRNA gene sequences of snake species belonging to the genera *Acanthophis* (*A.*) (Elapidae), *Epicrates* (*E.*), *Boa* (*B.*), and *Eryx* (*E.*) (Boidae). Bootstrap values indicate the statistical reliability of the tree branches. These values are displayed in the upper left corner of the tree, ranging from 50.01 to 100. Higher bootstrap values (closer to 100) signify stronger statistical support for the evolutionary relationships depicted in the tree. The tree distinctly highlights the following clusters:

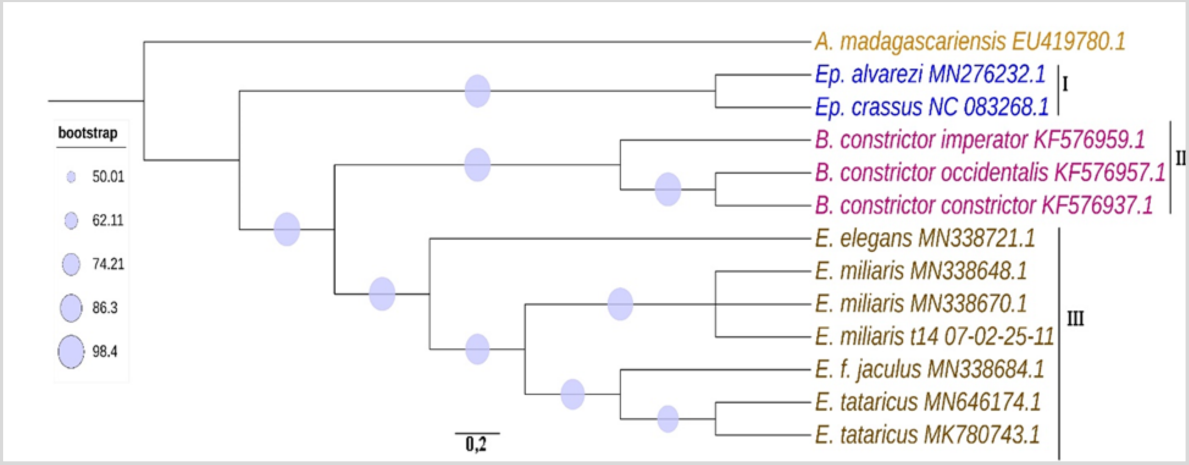

Figure 2. Phylogenetic tree of Eryx species with other snakes based on the Maximum Likelihood method

Outgroup clade: *Acanthophis madagascariensis* (EU419786.1) is positioned at the most distant evolutionary branch, serving as the outgroup.

I. *Epicrates* clade (green cluster): *Epicrates alvarezi* (MN276232) and *Epicrates crassus* (NC083268) formed a distinct cluster with a bootstrap value of 96.5%.

II. *Boa* clade: *Boa constrictor occidentalis* (KF576957) and *Boa constrictor constrictor* (KF576937) formed a well-supported subclade with a bootstrap value of 98.4%. The inclusion of the *Boa constrictor imperator* (KF576959) resulted in a larger clade with a bootstrap value of 98.3%.

III. *Eryx* clade (main internal group): *Eryx tataricus* (MN39174) and *Eryx tataricus* (MK780743) formed a separate clade with a bootstrap value of 84.3%, indicating differentiation from *E. miliaris*. The samples *Eryx miliaris* (MN39848, MN39870, and LN476152) clustered together with our *E. miliaris* sample, forming a subclade with 96.5% bootstrap support. Additionally, *Eryx elegans* (MN39873) grouped within a larger clade with a bootstrap value of 98.4%.

The phylogenetic tree reveals the following evolutionary relationships: all *Eryx* species form a monophyletic group, indicating a shared common ancestor. The genus *Boa* is shown to be more closely related to *Eryx* than to *Epicrates*. Within the *Eryx* clade, the following subclades can be observed: A subclade containing *Eryx elegans*. A subclade including multiple samples of *Eryx miliaris*. A subclade comprising *Eryx jaculus* and *Eryx tataricus*. The genus *Epicrates* represents a distinct evolutionary lineage that diverged earlier than the *Boa* and *Eryx* lineages. Based on this phylogenetic analysis, we can draw taxonomic conclusions that support the current classification of these snake genera and provide insights into their evolutionary relationships. The tree designates *Acanthophis* (*Elapidae*) as the outgroup, highlighting it as the most distantly related genus among the analyzed taxa. *Boa* and *Eryx* share a more recent common ancestor, while *Epicrates* occupies an intermediate position in terms of evolutionary divergence. The presence of multiple samples for certain species (*E. miliaris* and *E. tataricus*, in particular) allows for the assessment of intraspecific variability and helps confirm species boundaries.

According to the literature, the taxonomic status of *Eryx tataricus* and *Eryx miliaris* has been a subject of debate in previous molecular studies, with their relationship being hypothesized to be at the subspecific level [2].

Conclusion. According to the results of the molecular-genetic research, the mtDNA 16S rRNA region nucleotide sequence of the *Eryx miliaris* (Pallas, 1773) species, which belongs to the genus *Eryx* and is preserved in the collection of the Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, was studied. This species was compared with nucleotide sequences of similar species in the National Biotechnology Information Center's GenBank. Based on the obtained bioinformatic analysis, it was confirmed that this species is 99% similar to *E. miliaris* (MN338670) in the GenBank database. In the phylogenetic tree, species belonging to the genus *Eryx* are clustered into four groups, with *E. miliaris*, *E. tataricus*, and *E. f. jaculus* forming a group with 98.4% bootstrap support. The *E. miliaris_Uz* sample was found to be closely related to *E. miliaris* from Iran. The mtDNA 16S rRNA region was confirmed to be one of the key genes for the identification and study of phylogenetic relationships in the genus *Eremias*.

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Material received on 22.02.25

Өзбекстанда таралған Eryx miliaris (Squamata: Boidae) түрінің молекулалық-генетикалық сипаттамасы

Аңдатпа

Мақалада Азия аймағына тән эндемик түр болып табылатын Eryx miliaris (Pallas, 1773) жыланының Өзбекстандағы кең таралған популяциясына жүргізілген молекулалық-генетикалық сипаттамасы ұсынылады. Өзбекстанда Eryx туысына жататын екі түр — E. miliaris пен E. tataricus кең таралған, алайда олардың таксономиялық мәртебесі әлі де нақты анықталмаған. Зерттеу аясында Өзбекстан Республикасы Ғылым академиясының Зоология институты жинағында сақталған E. miliaris түрінің митохондриялық ДНҚ-сындағы 16S рРНҚ доменінің нуклеотидтік тізбегі зерттелді. Бұл тізбек Ұлттық биотехнологиялық ақпарат орталығының (NCBI) ген банкіндегі мәліметтермен салыстырылды. Биоинформатикалық зерттеулер нәтижесінде бұл түрдің ген банкте тіркелген E. miliaris (MN338670) түрімен 99% ұқсастығы анықталды. Филогенетикалық ағашта Eryx туысына жататын түрлер төрт топқа біріктірілді, ал E. miliaris, E. tataricus және E.f. jaculus бір топқа 98.4% бутстрэп қолдауымен кірді. E. miliaris_Uz үлгісі Иранда таралған E. miliaris түрлерімен жақын туыстық қатынаста екені анықталды. Митохондриялық ДНҚ-ның 16S рРНҚ аймағы Eryx туысына жататын түрлерді анықтауда негізгі гендердің бірі ретінде белгіленді (Erycidae тұқымдасы).

Түйінді сөздер: *молекулалық-генетикалық сипаттама, Eryx miliaris, Eryx tataricus, Өзбекстан, mtDNA 16S рРНҚ, филогенетикалық талдау, таксономиялық мәртебе, биоинформатика, ген банкі, NCBI, түрді анықтау, Erycidae.*

Материал баспаға 22.02.25 түсті

Молекулярно-генетическая характеристика вида Eryx miliaris (Squamata: Boidae), распространённого в Узбекистане

Аннотация

В статье представлено исследование молекулярно-генетической характеристики широко распространённого вида Eryx miliaris (Pallas, 1773) на территории Узбекистана. Вид является эндемиком Азии. В Узбекистане широко распространены два вида рода Eryx — E. miliaris и E. tataricus, при этом их таксономический статус считается проблематичным. В рамках молекулярно-генетических исследований была изучена нуклеотидная последовательность домена 16S рРНК митохондриальной ДНК (mtDNA) у E. miliaris, хранящегося в коллекции Института зоологии Академии наук Республики Узбекистан. Данный вид сравнивался с нуклеотидными последовательностями, представленными в генофонде Национального центра биотехнологической информации (NCBI). По результатам биоинформатических анализов установлено, что данный вид имеет 99% сходства с образцом E. miliaris (MN338670), зарегистрированным в генбанке. На филогенетическом дереве представители рода Eryx были сгруппированы в четыре кластера, при этом E. miliaris, E. tataricus и E.f. jaculus были объединены в одну группу с поддержкой бутстрэпа 98,4%. Образец E. miliaris_Uz оказался близкородственным популяциям E. miliaris, распространённым в Иране. Область 16S рРНК митохондриальной ДНК была определена как один из ключевых генов для идентификации видов рода Eryx (семейство Erycidae).

Ключевые слова: *молекулярно-генетическая характеристика, Eryx miliaris, Eryx tataricus, Узбекистан, mtDNA 16S рРНК, филогенетический анализ, таксономический статус, биоинформатика, генбанк, NCBI, идентификация видов, Erycidae*

Материал поступил в редакцию 22.02.2025

Authors' contribution. The largest contribution is distributed as follows:

A.E. Kuchboev and **O.O.Amirov** - conceived the idea, designed the experiments and carried them out.

O.O.Amirov and **A.A.Yuldoshkhonov** - performed, carried out the genetic characterization and phylogenetic study

A.E. Kuchboev and **O.O.Amirov** - wrote the manuscript.

All authors reviewed and approved the current version of the manuscript.

Conflict of interest. The authors declare no conflict of interest.