ISSN 1022-7954, Russian Journal of Genetics, 2017, Vol. 53, No. 4, pp. 472–482. © Pleiades Publishing, Inc., 2017.
Original Russian Text © A.E. Girnyk, A.A. Vergun, A.V. Omelchenko, V.G. Petrosyan, V.I. Korchagin, A.P. Ryskov, 2017, published in Genetika, 2017, Vol. 53, No. 4, pp. 468–479.

ANIMAL GENETICS

Molecular and Genetic Characterization of the Allelic Variants of Du215, Du281, Du323, and Du47G Microsatellite Loci in Parthenogenetic Lizard *Darevskia armeniaca* (Lacertidae)

A. E. Girnyk[a, *], A. A. Vergun[a, b], A. V. Omelchenko[c], V. G. Petrosyan[c], V. I. Korchagin[a], and A. P. Ryskov[a, **]

[a] *Institute of Gene Biology, Russian Academy of Sciences, Moscow, 119334 Russia*
[b] *Department of Biochemistry, Molecular Biology, and Genetics, Moscow State Pedagogical University, Moscow, 119991 Russia*
[c] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 119071 Russia*
**e-mail: nasstenochka@mail.ru*
***e-mail: ryskov@mail.ru*

Received May 25, 2016; in final form, June 14, 2016

Abstract—A key issue in the study of unisexual (parthenogenetic) vertebrate species is the determination of their genetic and clonal diversity. In pursuing this aim, various markers of nuclear and mitochondrial genomes can be used. The most effective genetic markers include microsatellite DNA, characterized by high variability. The development and characterization of such markers is a necessary step in the genetic studies of parthenogenetic species. In the present study, using locus-specific PCR, for the first time, an analysis of allelic polymorphism of four microsatellite loci is performed in the populations of parthenogenetic species *Darevskia armeniaca*. In the studied populations, allelic variants of each locus are identified, and the nucleotide sequences of each allele are determined. It is demonstrated that allele differences are associated with the variation in the structure of microsatellite clusters and single nucleotide substitutions at fixed distances in flanking DNA regions. Structural allele variations form haplotype markers that are specific to each allele and are inherited from their parental bisexual species. It is established which of the parental alleles of each locus were inherited by the parthenogenetic species. The characteristics of the distribution and frequency of the alleles of microsatellite loci in the populations of *D. armeniaca* determining specific features of each population are obtained. The observed heterozygosity of the populations at the studied loci and the mutation rates in genome regions, as well as Nei's genetic distances between the studied populations, are determined, and the phylogenetic relationships between them are established.

Keywords: parthenogenesis, unisexual and bisexual lizards of the genus *Darevskia*, microsatellites, allele polymorphism, population genetic parameters, phylogenetic relationships

DOI: 10.1134/S1022795417040068

INTRODUCTION

The discovery of unisexual (parthenogenetic) species of lizards by I.S. Darevsky in 1958 posed a number of general biological issues on the mechanisms of clonal reproduction and formation of genetic and clonal diversity, as well as on the origin and evolution of these forms of vertebrates. Allozyme and mitochondrial DNA data analysis showed that parthenogenetic species of the genus *Darevskia*, like the vast majority of unisexual vertebrates, originated from interspecific hybridization between bisexual species [1].

The genus *Darevskia* contains seven parthenogenetic species, including *D. armeniaca*. It was demonstrated that *D. armeniaca* originated from the hybridization between the males of *D. valentini* and females of *D. mixta* [2–4]. Moreover, these bisexual parent species belong to different phylogenetic clades of the genus *Darevskia* [2, 4]. Maternal species belong to the caucasica clade, while paternal species belong to the rudis clade. Note that phylogenetic factors restricting the formation of parthenogenetic species are also described in other lizards [5, 6].

Biogeographic studies showed that *D. armeniaca* was distributed in the mountainous regions of Central Transcaucasia at elevations between 1400 and 2000 m above sea level [1]. The southern boundary of the range runs along the northern and northeastern slopes of the Mount Aragats in Armenia, and the northern boundary runs along the southern spurs of the Trialeti Range in Georgia and the northern foothills of the Lesser Caucasus in West Azerbaijan. *D. armeniaca*, like other parthenogenetic species of the genus *Darevskia*, is often found in areas that are unfavorable for the habitation of bisexual forms and adapts rather

well to new habitats. This is evidenced by the existence for over 50 years of the introduced population of *D. armeniaca* in Ukraine, which is separated from the main range of this species living in the mountains of Northern Armenia [7].

Initially, using the allozyme analysis of 35 loci in 75 individuals of *D. armeniaca* from seven populations, three clones were detected, including one major clone found in five populations and two rare clones. However, unlike other parthenogenetic species of this group, one of the rare clones comprised most individuals of the same population (Papanino, 19 out of 27 animals), and in another population (Kuchak), 16 of the 22 studied lizards belonged to a rare clone. More recent studies using additional population samples revealed the existence of four allozyme clones in *D. armeniaca*, two of which were major, and other two were minor [8–10]. New information on the genetic and clonal diversity of *D. armeniaca* can be obtained by using more effective markers such as microsatellite DNA. The first step in this direction is the identification and molecular genetic characterization of the allelic variants of the loci containing microsatellite DNA in the populations of this species.

This study presents data on cloning, sequencing, and genetic characterization of the alleles of Du215, Du281, Du323, and Du47G microsatellite loci in parthenogenetic species *D. armeniaca*. In addition, on the basis of these data, the genetic estimates of the studied populations and the parameters of the phylogenetic relationships between them were obtained.

MATERIALS AND METHODS

The experiments were performed using a collection of DNA samples of *D. armeniaca* (127 individuals). The specimens of parthenogenetic species *D. armeniaca* represented 14 populations from Armenia (Alaverdi, $n = 3$; Artic, $n = 18$; Kuchak, $n = 7$; Lchap, $n = 1$; Lchashen, $n = 1$; Megradzor, $n = 9$; Medved-gora, $n = 12$; Papanino, $n = 4$; Pushkinskiy Pass, $n = 7$; Semenovskiy Pass, $n = 8$; Sotk, $n = 3$; Stepanavan, $n = 9$; Takyarlu, $n = 21$; Tezh, $n = 8$) and one population from the Ukraine ($n = 16$). Location of the studied population is shown in Fig. 1. Monolocus PCR analysis was carried out with primers designed previously for the Du215, Du281, and Du323 loci [11] and the Du47G locus of parthenogenetic species *D. unisexualis* (F: 5'-GATGGAACGACTCACCTATG-3'; R: 5'-ATACAACCGCAATAACAACAATA-3').

PCR was carried out in a total volume of 20 μL with 50 ng of genomic DNA as a template using a GenePak® PCR Core kit (Isogene Lab. Ltd., Russia). Amplification was run on a Tertsik TP4-PCR-01 four-channel thermal cycler (DNA-Technology, Russia) under the temperature conditions described in [11] ($t_{annealing}$ of the primers for the Du47G locus was 54°C). Amplification products were fractionated on 8% neutral polyacrylamide gel (PAGE), excised from the gel, and cloned using a pGEM®-T Easy Vector System I plasmid vector (Promega, United States) according to the protocol of the manufacturer. DNA sequencing was performed according to the Sanger method using a ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit (GE Healthcare, United States) on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, United States). DNA sequences were aligned using the Clustal W algorithm of the MEGA 6.0.6 software program [12]. Heterozygosity of the loci in populations and the phylogenetic tree parameters were determined using Web version of the POPTREEW software program (http://www.med.kagawa-u.ac.jp/~genomelb/takezaki/poptreew/index.html). We used Nei's distance D_A as a measure of genetic distance, because it is best fitting for treating the microsatellite data [13]. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used as the clustering algorithm. The bootstrap analysis with 1000 iterations was used. The phylogenetic trees were also constructed in the MEGA 6.0.6 software program. The mutation rate was calculated using the classical functional relationship between the genetic distance and time of divergence [14]:

$$D_A = 2\mu t,$$

where D_A is Nei's genetic distance, μ is the mutation rate [number of mutations/generation], and t is the divergence time [years].

The validity of our application of the formula should be noted. It is based on a mathematical model of genetic variability [14] suggesting that populations diverge because of genetic drift and mutations, which fully meets the conditions of existence, genetics, and ecology of parthenogenetic species *D. armeniaca*, since these lizards lack combinative variation inherent in sexual reproduction.

The correlation between genetic distances and geographical distances was assessed as implemented in the STATISTICA 7 software program. The Mantel test and determination of the parameters of regression relationships of genetic and geographical distances were performed with 1000 bootstrap iterations in the Web version of the Genepop 4.2 software program (http://genepop.curtin.edu.au).

The determined DNA sequences were deposited in GenBank (GU972533–GU972535; HM070259–HM070264; HM013992–HM013994; KT070998–KT071004; GU972553; HM013997; GU972551; KM573717–KM573727).

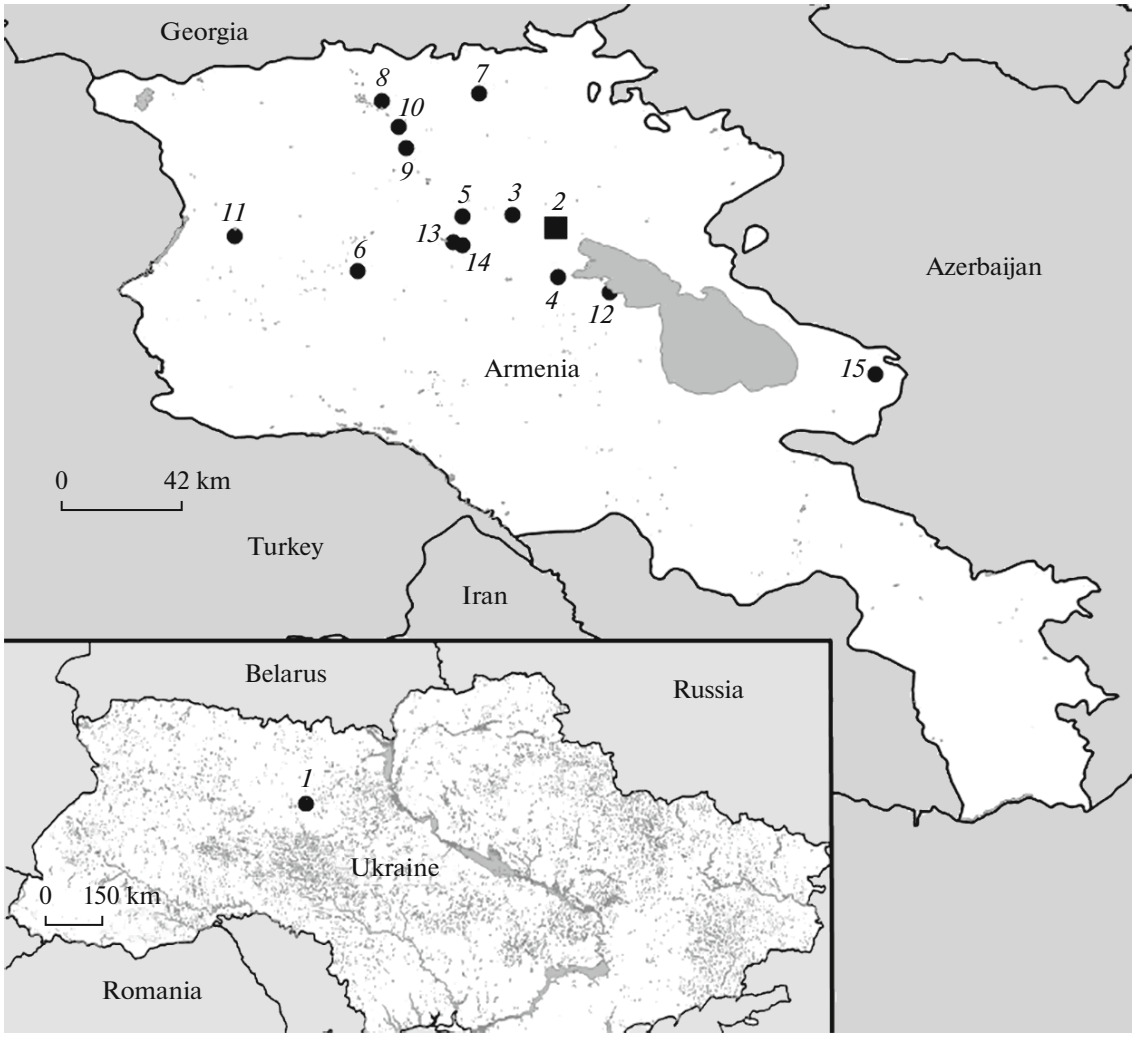

Fig. 1. Location of the populations of *D. armeniaca* in Ukraine and Armenia. (*1*) Ukrainian population; (*2–15*) populations from Armenia: (*2*) Semenovskiy Pass (shown as black square); (*3*) Papanino; (*4*) Lchashen; (*5*) Tezh; (*6*) Kuchak; (*7*) Alaverdi; (*8*) Stepanavan; (*9*) Pushkinskiy Pass; (*10*) Medved-gora; (*11*) Artik; (*12*) Lchap; (*13*) Takyarlu; (*14*) Megradzor; (*15*) Sotk [30].

RESULTS AND DISCUSSION

Molecular Nature of the Allelic Polymorphism of Microsatellite Loci in Parthenogenetic Species D. armeniaca

The data on the molecular structure and variability of Du215, Du281, Du323, and Du47G microsatellite loci in the representatives of parthenogenetic reptile species *Darevskia armeniaca* were for the first time obtained and characterized by using monolocus PCR. Locus-specific PCR analysis showed that all studied individuals of *D. armeniaca* were heterozygous for microsatellite loci Du215, Du281, and Du323. The exception was locus Du47G, for which seven homozygous individuals were found. The examined populations of parthenogenetic species were found to contain from three to seven alleles depending on the chosen locus. Table 1 shows structural features of these alleles in which they differ. It can be seen that the differences are associated with the structure of microsatellite cluster and single nucleotide variations in flanking DNA regions. Earlier, in the analysis of the microsatellite loci polymorphism in parthenogenetic species *D. dahli* and *D. unisexualis*, a similar type of interallelic variation was described [15, 16]. It should be noted that these allelic variations in the DNA structure reflect the hybrid origin of parthenogenetic species and the level of heterozygosity of their genomes. Obviously, the more divergent the individual genomes involved in the interspecific hybridization, the higher the genome heterozygosity level will be in a hybrid individual, the founder of parthenogenetic species, which can be reflected in the number of single nucleotide allelic variations and structural variability of microsatellite DNAs associated with them. However, according to the balance hypothesis [17, 18], the appearance in hybrid individuals (founders of parthenogenetic species) of the oocytes with unreduced chromosome

Table 1. Structural differences in the alleles of Du215, Du281, Du323, and Du47G microsatellite loci in parthenogenetic species *D. armeniaca*

Allele	Length, bp	Sequence of microsatellite cluster	Fixed nucleotide substitutions at flanking sequences*
Du215(arm)1	236	5' $(GACA)(GATA)_8(GACA)_5(GATA)(GCAA)$ 3'	T (–61), G (–41), C (–22)
Du215(arm)2	232	5' $(GACA)(GATA)_7(GACA)_5(GATA)(GCAA)$ 3'	T (–61), G (–41), C (–22)
Du215(arm)3	192	5' $(GATA)_5$ 3'	A (–61), C (–41), T (–22)
Du281(arm)1	229	5' $(GGTA)(GATA)_{10}(GAT)(GATA)(GGTA)_2(GAT)(GATA)_4$ 3'	T (+15)
Du281(arm)2	225	5' $(GGTA)(GATA)_9(GAT)(GATA)(GGTA)_2(GAT)(GATA)_4$ 3'	T (+15)
Du281(arm)3	187	5' $(GATA)_9$ 3'	C (+15)
Du281(arm)4	183	5' $(GATA)_8$ 3'	C (+15)
Du323(arm)1	215	5' $(AC)_6$...$(GATA)_{11}(GAT)(GATA)_2TAT(GA)_4$ 3'	C (–23), T (+28)
Du323(arm)2	211	5' $(AC)_6$...$(GATA)_{10}(GAT)(GATA)_2TAT(GA)_4$ 3'	C (–23), T (+28)
Du323(arm)3	184	5' $(AC)_5$...$(GATA)(GGT)(GATA)_3(GAT)(GATA)TAT(GA)_4$ 3'	A (–23), C (+28)
Du47G(arm)1	188	5' $(GATA)_{10}(GACA)_4(GATA)_2GAT(GATA)_2$ 3'	T (+7), A (+21), G (+52), T (+56)
Du47G(arm)2	184	5' $(GATA)_9(GACA)_4(GATA)_2GAT(GATA)_2$ 3'	T (+7), A (+21), G (+52), T (+56)
Du47G(arm)3	176	5' $(GATA)_{11}(GACA)(GATA)GAT(GATA)_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(arm)4	172	5' $(GATA)_{10}(GACA)(GATA)GAT(GATA)_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(arm)5	168	5' $(GATA)_9(GACA)(GATA)GAT(GATA)_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(arm)6	164	5' $(GATA)_8(GACA)(GATA)GAT(GATA)_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(arm)7	152	5' $(GATA)_2(GACA)(GATA)_4GAT(GATA)_2$ 3'	A (+7), A (+21), G (+52), A (+56)

* In brackets are the distances in base pairs; (–) and (+), upstream and downstream of microsatellite cluster.

number and the transition to clonal reproduction depend on the level of overall heterozygosity, which does not exceed certain limits. In the studied population sample of *D. armeniaca*, three Du215 alleles, four Du281 alleles, three Du323 alleles, and seven Du47G alleles were identified. With respect to the structural features of DNA, alleles of microsatellite loci of *D. armeniaca* can be divided into three groups according to their origin from the parental species or subsequent mutational processes.

The data in Table 1 show that all three Du215 alleles have complexly organized microsatellites containing GATA and GACA repeats. In the microsatellite flanking regions, alleles of this locus contained single nucleotide substitutions, the combination of which formed allele-specific haplotypes, T-G-C (alleles 1 and 2) and A-C-T (allele 3). Accordingly, the Du215 alleles can be divided into two groups. Moreover, each of the groups is characterized by association with microsatellites of specific structure.

The Du281 alleles differ both in the structure of microsatellite cluster and in single nucleotide variations at fixed positions outside the cluster, according to which they can also be divided into two groups. One group consists of two alleles with a single nucleotide substitution (T). These alleles differ in the structure of associated complexly arranged $(GATA)_n$ microsatellites. The third and fourth alleles form a second group, which has another single nucleotide variation (C) and the associated simple $(GATA)_n$ microsatellite.

The Du323 alleles differ in two independent microsatellite clusters, $(AC)_n$ and $(GATA)_n$, as well as in a combination of single nucleotide variations at fixed positions outside microsatellites, forming two groups of haplotypes (C-T and A-C). Moreover, alleles with haplotype C-T contain the $(AC)_6$ microsatellite cluster, while the allele with haplotype A-C contains the $(AC)_5$ cluster. In this case, there is also the association of certain haplotypes and microsatellites.

The Du47G alleles are divided into three groups. The first group contains two alleles with identical sets of single nucleotide variations, the combination of which forms haplotype T-A-G-T. The second group consists of four alleles having a different set of single nucleotide variations (haplotype T-T-C-A). The third group is formed by a single allele with haplotype A-A-G-A. Interestingly, all these alleles carry microsatellite clusters with similar structural organization, and the variations are associated with the number of repeats in the individual units.

It can generally be concluded that the combination of certain microsatellites and single nucleotide variations at the fixed distances in the flanking DNA can be considered as a single haplotype. This haplotype is

Table 2. Structural differences in the alleles of Du215, Du281, Du323, and Du47G microsatellite loci in bisexual species *D. mixta* and *D. valentini*

Allele	Length, bp	Sequence of microsatellite cluster	Fixed nucleotide substitutions at flanking sequences*
Du215(mix)1	232	5' (GACA)(GATA)$_7$(GACA)$_5$(GATA)(GCAA) 3'	T (–61), G (–41), C (–22)
Du215(val)1	192	5' (GATA)$_5$ 3'	A (–61), C (–41), T (–22)
Du281(mix)1	225	5' (GGTA)(GATA)$_9$(GAT)(GATA)(GGTA)$_2$(GAT)(GATA)$_4$ 3'	T (+15)
Du281(mix)2	221	5' (GGTA)(GATA)$_8$(GAT)(GATA)(GGTA)$_2$(GAT)(GATA)$_4$ 3'	T (+15)
Du281(val)1	199	5' (GATA)$_{12}$ 3'	C (+15)
Du281(val)2	195	5' (GATA)$_{11}$ 3'	C (+15)
Du281(val)3	191	5' (GATA)$_{10}$ 3'	C (+15)
Du281(val)4	187	5' (GATA)$_9$ 3'	C (+15)
Du281(val)5	183	5' (GATA)$_8$ 3'	A (+15)
Du323(mix)1	184	5' (AC)$_5$...(GATA)(GGT)(GATA)$_3$GAT(GATA)TAT(GA)$_4$ 3'	A (–23), C (+28)
Du323(val)1	220	5' (AC)$_6$...(GATA)$_{15}$GAT(GA)$_4$ 3'	C (–23), T (+28)
Du323(val)2	216	5' (AC)$_6$...(GATA)$_{14}$GAT(GA)$_4$ 3'	C (–23), T (+28)
Du323(val)3	211	5' (AC)$_6$...(GATA)$_{10}$GAT(GATA)$_2$TAT(GA)$_4$ 3'	C (–23), T (+28)
Du323(val)4	211	5' (AC)$_6$...(GATA)$_5$(GACA)(GATA)$_4$GAT(GATA)$_2$TAT(GA)$_4$ 3'	C (–23), T (+28)
Du323(val)5	164	5' (AC)$_6$...(GATA)$_5$GAT(GATA)$_2$TAT(GA)$_4$ 3'	C (–23), T (+28)
Du323(val)6	188	5' (AC)$_6$...(GATA)$_4$GAT(GATA)$_2$TAT (GA)$_4$ 3'	C (–23), T (+28)
Du47G(mix)1	168	5' (GATA)$_9$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(mix)2	152	5' (GATA)$_2$(GACA)(GATA)$_4$GAT(GATA)$_2$ 3'	A (+7), A (+21), G (+52), A (+56)
Du47G(val)1	212	5' (GATA)$_5$(GAT)(GATA)$_{14}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(val)2	181	5' (GATA)$_{16}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(val)3	177	5' (GATA)$_{15}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(val)4	173	5' (GATA)$_{14}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(val)5	169	5' (GATA)$_{13}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(val)6	165	5' (GATA)$_{12}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)
Du47G(val)7	161	5' (GATA)$_{11}$(GACA)(GATA)GAT(GATA)$_2$ 3'	T (+7), T (+21), C (+52), A (+56)

* Designations are as in Table 1.

specific to each allele and depends on its origin (maternal or paternal bisexual species), and the additional allele variability is associated with microsatellite mutations that may occur under clonal reproduction.

Most microsatellite DNAs were found to contain imperfect inserts, like GAT, GGT, and GGTA. These inserts could be the result of a reading frame shift for an incomplete monomer unit, or recombination events, or mutations like transitions and transversions [19, 20]. Allelic differences associated with the changes in the number of repeats in a microsatellite cluster can be explained by the effect of DNA polymerase slippage during the process of DNA replication [21–23]. Most of these changes in *D. armeniaca* match the stepwise mutation model of microsatellite DNA [24] or its modified version [25]. Obviously, the analysis of microsatellite DNA interallelic differences in parthenogenetic species should take into account not only their inheritance from different bisexual species but also possible mutations under clonal reproduction. In our earlier studies, microsatellite mutations were identified in the first generation progeny of parthenogenetic lizards *D. unisexualis* [26]. These mutations occurred at the early stages of embryogenesis as either microsatellite deletions or insertions and were detected in one or both alleles of the locus. No single nucleotide mutations were found outside the microsatellite.

Determination of Parental Alleles in Parthenogenetic Species D. armeniaca

As noted above, parthenogenetic species *D. armeniaca* originated from interspecific hybridization

Table 3. Frequency of allelic variants of Du215, Du281, Du323, and Du47G loci in the populations of parthenogenetic species *D. armeniaca*

Allele	Populations															Number of individuals
	Alaverdi $n = 3$	Artik $n = 18$	Kuchak $n = 7$	Lchap $n = 1$	Lchashen $n = 1$	Megradzor $n = 9$	Medved-gora $n = 12$	Papanino $n = 4$	Pushkinskiy Pass $n = 7$	Semenovskiy Pass $n = 8$	Sotk $n = 3$	Stepananavan $n = 9$	Takyarlu $n = 21$	Tezh $n = 8$	Ukrainian population $n = 16$	
Du215 (arm)1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	3	4
Du215 (arm)2	2	18	7	1	1	9	12	4	7	8	3	9	21	8	13	123
Du215 (arm)3	3	18	7	1	1	9	12	4	7	8	3	9	21	8	16	127
Du281 (arm)1	–	–	1	–	–	–	–	–	–	–	–	1	–	–	–	2
Du281 (arm)2	3	18	6	1	1	9	12	4	7	8	3	8	21	8	16	125
Du281 (arm)3	–	–	–	–	–	–	–	–	–	7	–	–	–	–	12	19
Du281 (arm)4	3	18	7	1	1	9	12	4	7	1	3	9	21	8	4	108
Du323 (arm)1	–	–	–	–	–	7	–	–	–	–	–	–	–	–	2	9
Du323 (arm)2	3	18	7	1	1	2	12	4	7	8	3	9	21	8	14	118
Du323 (arm)3	3	18	7	1	1	9	12	4	7	8	3	9	21	8	16	127
Du47G (arm)1	2	3	–	–	–	–	2	–	5	–	2	2	–	–	1	17
Du47G (arm)2	–	14	7	1	1	9	10	4	2	6	1	6	21	8	16	100
Du47G (arm)3	–	2	–	–	–	–	–	1	–	–	–	–	–	–	–	3
Du47G (arm)4	–	14	–	–	–	–	–	–	–	–	2	2	–	–	–	18
Du47G (arm)5	3	1	7	1	1	9	12	3	7	8	1	8	21	8	13	103
Du47G (arm)6	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Du47G (arm)7	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Number of allelic variants (17)	9	13	9	8	8	9	9	9	9	9	10	11	8	8	12	

Table 4. Heterozygosity of Du215, Du281, Du323, and Du47G loci in the populations of parthenogenetic species *D. armeniaca*

		Populations														
		Alaverdi	Artik	Kuchak	Lchap	Lchashen	Megradzor	Medved-gora	Papanino	Pushkinskiy Pass	Semenovsskiy Pass	Sotk	Stepanavan	Takyarlu	Tezh	Ukrainian population
Loci	Du215	0.733	0.514	0.538	1.000	1.000	0.529	0.522	0.571	0.538	0.533	0.600	0.529	0.512	0.533	0.595
	Du281	0.600	0.514	0.604	1.000	1.000	0.529	0.522	0.571	0.538	0.592	0.600	0.582	0.512	0.533	0.613
	Du323	0.600	0.514	0.538	1.000	1.000	0.621	0.522	0.571	0.538	0.533	0.600	0.529	0.512	0.533	0.573
	Du47G	0.533	0.705	0.538	1.000	1.000	0.529	0.594	0.679	0.648	0.500	0.867	0.706	0.512	0.533	0.534
Mean		0.617	0.562	0.555	1.000	1.000	0.552	0.540	0.598	0.566	0.540	0.667	0.587	0.512	0.533	0.579
SE		0.042	0.048	0.016	0	0	0.023	0.018	0.027	0.027	0.019	0.067	0.042	0	0	0.017

between bisexual species *D. mixta* (maternal species) and *D. valentini* (paternal species) [3]. Hence, allelic variants of the studied loci in *D. armeniaca* were inherited from the corresponding parental species. To determine which of these alleles has either paternal or maternal origin, we isolated and sequenced alleles of the homeologous loci from a number of individuals of parental species.

Table 2 shows the nucleotide sequences of differing allele regions of four homeologous loci from parental species. It can be seen that alleles of bisexual species are quite diverse in structure of microsatellites and contain single nucleotide variations outside microsatellites. By a combination of these variations and specific microsatellite structure, it can be determined from which parental species a particular allele of each locus was inherited by parthenogenetic species. For instance, in the Du215 locus, alleles with haplotype T-G-C are inherited from maternal species *D. mixta*, and the allele with haplotype A-C-T was transferred from paternal species *D. valentini*. Alleles with variable nucleotide T of the Du281 locus of parthenogenetic species were inherited from maternal species, and alleles with nucleotide C were inherited from paternal species. At the Du323 locus of parthenogenetic species, alleles with haplotype C-T were inherited from paternal species *D. valentini*, and the allele with haplotype A-C was inherited from maternal species *D. mixta*. At the Du47G locus, alleles with haplotype A-A-G-A were inherited from maternal species, while those with haplotype T-T-C-A were inherited from paternal species. Parental alleles with haplotype T-A-G-T were not identified, which can be indicative of a divergence that occurred at this locus in parthenogenetic species or an insufficient number of investigated parental animals.

Genetic Characterization of the Populations of D. armeniaca

Table 3 shows the allele distribution of four microsatellite loci in the populations of *D. armeniaca*. It can be seen that the populations differ in the allele frequencies and the combinations of certain alleles. Alleles Du215/2 and Du215/3 are uniformly distributed in all populations. Allele Du215/1 was found to be rare and was detected only in two populations. Among the alleles of the Du281 locus, two alleles were widely represented in all or most of the populations (Du281/2,4) and were found in many individuals. Two other alleles Du281/1,3) were rare and found only in some populations. At the same time, Du281/3 was the main allele in the population from Ukraine and was found almost in all individuals from the Semyonov Pass population. The Du323 locus in all populations was represented by two alleles, Du323/3 and Du323/2. Allele Du323/1 was rare and it was present in most of the individuals from the Megradzor population and only in two individuals from the Ukrainian population. Alleles Du47G/2,5 were found in many individuals from all or most of the populations. Other allelic variants of the locus were rare. Alleles Du47G/6,7 were found in single individuals only in a polymorphic population of Artik. The analysis showed that the highest number of alleles and their combinations was detected in the individuals from Artik population and the Ukrainian population. At the same time, the Tezh and Takyarlu populations were the most genetically homogeneous.

The heterozygosity levels of the loci in the examined populations are demonstrated in Table 4. It can be seen that the mean heterozygosity in the populations of *D. armeniaca* was 0.627 ($\pm$ 0.023). It is important to note that the heterozygosity of microsatellite-containing loci in the populations of various species of lizards shows a wide range of variation. For example, in the populations of Reeves' butterfly lizard (*Leiolepis reevesii*) distributed in Southeast Asia, the observed

Table 5. Nei's genetic distance D_A (figures below the diagonal) and the divergence time (t, years) (figures above the diagonal) between the populations of parthenogenetic species *D. armeniaca*

Populations	Alaverdi	Artik	Kuchak	Lchap	Lchashen	Megradzor	Medved-gora	Papanino	Pushkinskiy Pass	Semenovskiy Pass	Sotk	Stepanavan	Takyarlu	Tezh	Ukrainian population
Alaverdi	0	241	169	158	158	239	107	181	51	235	130	118	158	158	–
Artik	0.197	0	147	135	135	216	121	114	145	248	47	77	135	135	–
Kuchak	0.138	0.120	0	11	11	92	25	32	82	113	141	37	11	11	–
Lchap	0.129	0.110	0.009	0	0	81	13	21	71	102	130	45	0	0	–
Lchashen	0.129	0.110	0.009	0	0	81	13	21	71	102	130	45	0	0	–
Megradzor	0.195	0.176	0.075	0.066	0.066	0	94	102	152	183	211	126	81	81	–
Medved-gora	0.087	0.099	0.020	0.011	0.011	0.077	0	34	26	113	86	27	13	13	–
Papanino	0.148	0.093	0.026	0.017	0.017	0.083	0.028	0	92	125	141	65	21	21	–
Pushkinskiy Pass	0.042	0.118	0.067	0.058	0.058	0.124	0.021	0.075	0	163	65	43	71	71	–
Semenovskiy Pass	0.192	0.202	0.092	0.083	0.083	0.149	0.092	0.102	0.133	0	230	145	102	102	38
Sotk	0.106	0.038	0.115	0.106	0.106	0.172	0.070	0.115	0.053	0.188	0	42	130	130	–
Stepanavan	0.096	0.063	0.030	0.037	0.037	0.103	0.022	0.053	0.035	0.118	0.034	0	45	45	–
Takyarlu	0.129	0.110	0.009	0	0	0.066	0.011	0.017	0.058	0.083	0.106	0.037	0	0	–
Tezh	0.129	0.110	0.009	0	0	0.066	0.011	0.017	0.058	0.083	0.106	0.037	0	0	–
Ukrainian population	0.167	0.177	0.097	0.088	0.088	0.110	0.086	0.103	0.123	0.031	0.166	0.111	0.088	0.088	0

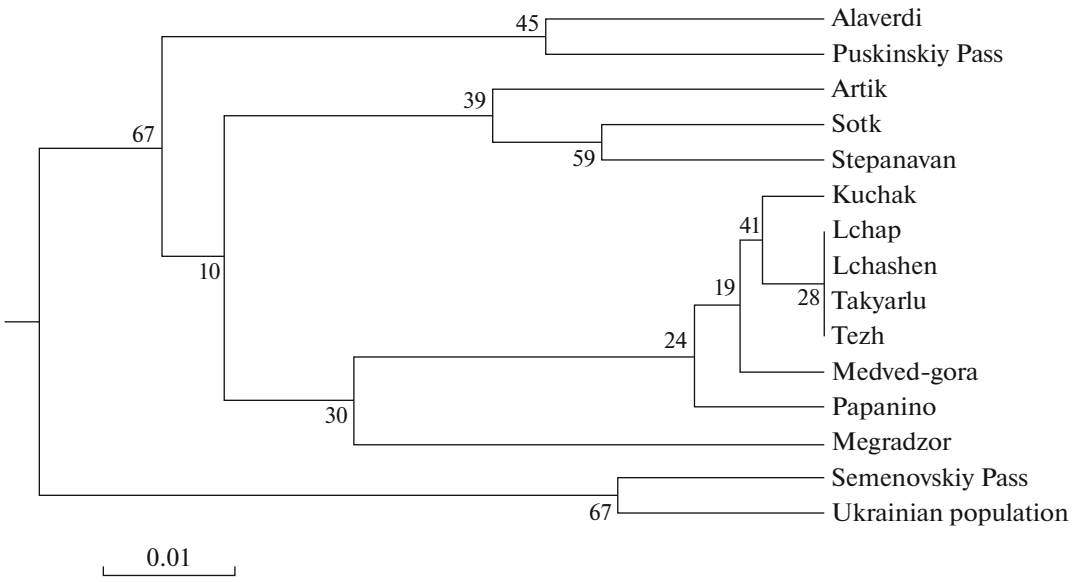

Fig. 2. Phylogenetic tree of the populations of *D. armeniaca* constructed on the basis of Nei's D_A using the UPGMA clustering algorithm with ~1000 bootstrap iterations.

heterozygosity ranges from 0.160 to 0.954 [27], and in the population of South American lizard *Liolaemus fitzingerii*, it varies from 0.311 to 0.956 [28]. It should be noted that, at a low number of alleles per locus, the level of observed heterozygosity in the population decreases. For instance, in Mongolian racerunner (*Eremias argus*), with the mean number of alleles per locus of 2.463, the heterozygosity is 0.398. In the present study, the number of alleles per locus is 2.380 (± 0.293), and the heterozygosity value is quite high. Analysis of the genetic population data from the literature shows that the rarity of lizard species, as well as the small number of samples in general, does not affect the level of the population heterozygosity and the range of its scattering. For example, in legless lizards of the genus *Anniella*, a rather high level of heterozygosity, ranging from 0.438 to 0.938, was observed [29]. Most likely, the heterozygosity level of the populations of *D. armeniaca* presented in this study is determined by the hybrid nature of the genome and other hereditary phylogenetic factors. For instance, the evolutionary parental (paternal) species of *D. valentini*, examined with the recruitment of homeologous genomic loci, shows a similar level of heterozygosity, averaging 0.525 (± 0.052) [30].

The Ukrainian population of *D. armeniaca* deserves special interest, since it originated from the introduction in 1963 of 129 individuals from the Armenian population of Semenovskiy Pass in Zhytomyr oblast of Ukraine into the canyon of the Teterev River [7]. Despite the death of most of the immigrants during the first wintering, by 1998 the population was successfully restored in number [31]. Indeed, the data in Table 3 show that all alleles of the Semenovskiy Pass population from Armenia are detected in the Ukrainian population. In particular, this concerns the rare allele Du281/3, which is unique, i.e., being detected only in the Semenovskiy Pass population and the Ukrainian population.

Given the time of the introduction of the population of Semyonovskiy Pass to Ukraine, it is possible to establish the divergence time between these populations. The total time of the isolated existence of these populations is 38 years. Using the classical functional relationship between genetic distance and time of divergence [14], it is possible to calculate the mutation rate at the examined loci, which is

$$\mu = D_A/2t = 0.031/(2 \times 38) = 4.08 \times 10^{-4}$$
[mutations/generation].

The above data show that, in the genome of *D. armeniaca*, the mutations affect on average one unit per every 2500 units of tetranucleotide microsatellite cluster per one generation (these lizards reproduce once a year). The mutation rate calculated in the present study generally corresponds to that of the microsatellite-containing loci of other vertebrate species. For example, for swine di-, tri-, and tetranucleotide microsatellite loci, the average mutation rate per generation equals 7.52×10^{-5}, and its values within the 95% confidence interval vary from 1.78×10^{-4} to 2.97×10^{-5} [32]. In the study analyzing the mutation rate at dinucleotide microsatellites in fishes, the values of this parameter are slightly higher, averaging 5.56×10^{-4} [33]. The data on Nei's genetic distances and divergence time calculated on their basis between the populations are presented in Table 5, and the phylogenetic tree of the populations of *D. armeniaca* is shown in Fig. 2. On the basis of the above model, it is not possi-

ble to correctly establish the divergence time between the Ukrainian population and Armenian populations of *D. armeniaca*. This model does not take the anthropogenic influence into account (artificial introduction of the species in a new ecosystem). The divergence time between the Ukrainian population and the Semyonovskiy Pass population was established empirically, as described above. This time was inserted in the corresponding field of Table 5, while the other cells of this column were marked by dashes. The data in Table 5 and Fig. 2 indicated that the minimum divergence time, equal to 11 years, was observed between the Kuchak population and Lchap, Lchashen, Takyarlu, Tezh populations. The maximum divergence time, equal to 248 years, was observed between the Semenovskiy Pass and Artik populations. It should be noted that the correlation between geographical distances and genetic distances was weak. The Pearson correlation coefficient was $r = 0.2606$ at $P = 0.0126$. The regression equation $F_{st}/(1 - F_{st}) = a + b\ln$ (geographical distance), assessed using the Mantel test on the basis of 1000 bootstrap iterations, showed the values of coefficients $a = -0.512914$, $b = 0.0203981$. Moreover, the probability that the calculated correlation could be higher was equal to zero. The lack of the correlation between geographical distances and genetic distances may be associated with the high mutation rate at the marker loci, given that the changes in these genomic regions occur much faster than the changes in population ranges, as well as with environmental and genetic characteristics of these territorial parthenogenetic animals.

Thus, in the present study, for the first time, allelic variants of four genomic microsatellite loci of parthenogenetic species *D. armeniaca* were identified, cloned, and sequenced. The molecular nature of allelic polymorphism of the examined loci was determined, as well as the allele distribution patterns in the populations of *D. armeniaca*, and the population genetic parameters were estimated. The data obtained can be used in determining the clonal structure of *D. armeniaca*, in studying the phylogenetic relationships between parthenogenetic species and the other members of the genus *Darevskia*, and in evaluation of the divergence level of these species.

ACKNOWLEDGMENTS

We thank F.D. Danielyan and his colleagues for their help in preparing the biological samples that were used to create a DNA collection of the *Darevskia* lizards.

This study was supported by the Russian Science Foundation (grant no. 14-14-00832). Calculations on the heterozygosity of the loci and assessment of the population genetic parameters were supported by a Grant of the President of the Russian Federation (grant no. MK-6509.2015.4) and the Program of the Presidium of the Russian Academy of Sciences "Molecular and Cellular Biology."

The experiments were conducted using the equipment of the Center for Collective Use of the Institute of Gene Biology, Russian Academy of Sciences.

REFERENCES

1. Darevsky, I.S., Evolution and ecology of parthenogenetic reproduction in reptiles, in *Sovremennye problemy teorii evolyutsii* (Modern Problems of Evolution Theory), Moscow: Nauka, 1993, pp. 89—109.
2. Darevsky, I.S., Kupriyanova, L.A., and Uzzel, T., Parthenogenesis in reptiles, *Biology of the Reptiles*, Gans, C. and Billett, F., Eds., vol. 15: *Development B*, Wiley: New York, 1985, pp. 412—526.
3. Moritz, C., Uzzel, T., Spolsky, C., et al., The maternal ancestry and approximate age of parthenogenetic species of Caucasian rock lizards (*Lacerta:* Lacertidae), *Genetica*, 1992, vol. 87, pp. 53—62.
4. Murphy, R.W., Fu, J., MacCulloch, R.D., et al., A fine line between sex and unisexuality: the phylogenetic constraints on parthenogenesis in lacertid lizards, *J. Linn. Soc. London, Zool.*, 2000, vol. 130, pp. 527—549. doi 10.1006/zjls.1999.0241
5. Kearney, M., Blacket, M., Strasburg, J., and Moritz, C., FAST-TRACK: waves of parthenogenesis in the desert: evidence for the parallel loss of sex in a grasshopper and a gecko from Australia, *Mol. Ecol.*, 2006, vol. 15, pp. 1743—1748. doi 10.1111/j.1365-294X.2006.02898.x
6. Manrquez-Moran, N.L., Mundez-de la Cruz, F.R., and Murphy, R.W., Genetic variation and origin of parthenogenesis in the *Aspidoscelis cozumela* complex: evidence from mitochondrial genes, *Zool. Sci.*, 2014, vol. 31, pp. 14—19.
7. Darevskii, I.S. and Shcherbak, N.N., Acclimatization of parthenogenetic lizards in Ukraine, *Priroda* (Moscow), 1968, no. 5, pp. 93—94.
8. MacCulloch, R.D., Murphy, R.W., Kupriyanova, L.A., and Darevsky, I.S., Clonal variation in the parthenogenetic rock lizard *Lacerta armeniaca, Genome*, 1995, vol. 38, pp. 1057—1060.
9. Murphy, R.W., Darevsky, I.S., MacCulloch, R.D., et al., Old age, multiple formations or genetic plasticity? Clonal diversity in the uniparental Caucasian rock lizard *Lacerta dahli, Genetica*, 1997, vol. 101, pp. 125—130.
10. Fu, J., MacCulloch, R.D., Murphy, R.W., and Darevsky, I.S., Divergence of the cytohrome *b* gene in the *Lacerta raddei* complex and its parthenogenetic daughter species: evidence for recent multiple origins, *Copeia*, 2000, vol. 2, pp. 432—440.
11. Korchagin, V.I., Badaeva, T.N., Tokarskaya, O.N., et al., Molecular characterization of allelic variants of $(GATA)_n$ microsatellite loci in parthenogenetic lizards *Darevskia unisexualis* (Lacertidae), *Gene*, 2007, vol. 392, pp. 126—133. doi 10.1016/j.gene.2006.11.020
12. Tamura, K., Stecher, G., Peterson, D., et al., MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0, *Mol. Biol. Evol.*, 2013, vol. 30, pp. 2725—2729. doi 10.1093/molbev/mst197

13. Takezaki, N. and Nei, M., Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA, *Genetics*, 1996, vol. 144, no. 1, pp. 389—399.
14. Weir, B.S., *Genetic Data Analysis*, Sunderland, MA: Sinauer Associates, 1990.
15. Ryskov, A.P., Genetically unstable microsatellite-containing loci and genome diversity in clonally reproduced unisexual vertebrates, *Int. Rev. Cell Mol. Biol.*, 2008, vol. 270, pp. 319—349. doi 10.1016/S1937-6448(08)01407-X
16. Vergun, A.A., Martirosyan, I.A., Semyenova, S.K., et al., Clonal diversity and clone formation in the parthenogenetic Caucasian rock lizard *Darevskia dahli, PLoS One*, 2014, vol. 9, no. 3. e91674. doi 10.1371/journal.pone.0091674
17. Moritz, C., Brown, W.M., Densmore, L.D., et al., Genetic diversity and the dynamics of hybrid parthenogenesis in *Cnemidophorus* (Teiidae) and *Heteronotia* (Gekkonidae), *Bull. N.Y. State Mus.*, 1989, vol. 466, pp. 87—112.
18. Moritz, C., Wright, J.W., and Brown, C.M., Mitochondrial DNA analysis and the origin and relative age of parthenogenetic *Cnemidophorus*: phylogenetic constraints on hybrid origins, *Evolution*, 1992, vol. 46, pp. 184—192.
19. Strand, M., Prolla, T.A., Liskay, R.M., and Petes, T.D., Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair, *Nature*, 1993, vol. 365, pp. 274—276.
20. Pearson, C.E. and Sinden, R.R., Alternative structures in duplex DNA formed within the trinucleotide repeats of the myotonic dystrophy and fragile X loci, *Biochemistry*, 1996, vol. 35, pp. 5041—5053.
21. Schlötterer, C. and Tautz, D., Slippage synthesis of simple sequence DNA, *Nucleic Acids Res.*, 1992, vol. 20, pp. 211—215.
22. Weber, J.L. and Wong, C., Mutation of human short tandem repeats, *Hum. Mol. Genet.*, 1993, vol. 2, pp. 1123—1128.
23. Wells, R.D., Molecular basis of genetic instability of triplet repeats, *J. Biol. Chem.*, 1996, vol. 271, no. 6, pp. 2875—2878.
24. Ohta, T. and Kimura, M., The model of mutation appropriate to calculate the number of electrophoretically detectable alleles in a genetic population, *Genet. Res.*, 1973, vol. 22, pp. 201—204.
25. Di Rienzo, A., Peterson, A.C., Garza, J.C., et al., Mutational processes of simple sequence repeat loci in human populations, *Proc. Natl. Acad. Sci. U.S.A.*, 1994, vol. 91, pp. 3166—3170.
26. Badaeva, T.N., Malysheva, D.N., Korchagin, V.I., and Ryskov, A.P., Genetic variation and *de novo* mutations in the parthenogenetic Caucasian rock lizard *Darevskia unisexualis, PLoS One*, 2008, vol. 3, no. 7. e2730. doi 10.1371/journal.pone.0002730
27. Hua, L., Mao, L.-X., Chen, C., et al., Isolation and characterization of microsatellite loci in the Reevese's butterfly lizard *Leiolepis reevesii* (Agamidae), *Conserv. Genetics*, 2012, vol. 4, no. 3, pp. 791—794.
28. Hanna, N., Brown, D., Avilaet, L.J., et al., Characterization of 10 polymorphic microsatellite loci in the South American lizard *Liolaemus fitzingerii* with cross-amplification in *L. chehuachekenk*, *Conserv. Genetics*, 2012, vol. 4, no. 1, pp. 105—107.
29. Wogan, G.O.U., Kapelke, J., Feldheimc, K.A., et al., Isolation and characterization of nine tetranucleoide microsatellite loci for the secretive limbless lizards of the genus *Anniella* (Anguidae), *Biochem. Syst. Ecol.*, 2015, vol. 62, pp. 155—158. doi 10.1016/j.bse.2015.07.039
30. Omelchenko, A.V., Girnyk, A.E., Osipov, F.A., et al., Detection of genotypic changes in the parthenogenetic lizards of *Darevskia armeniaca* (Mehely) introduced from Armenia to Ukraine, *Ross. Zh. Biol. Invazii*, 2016, no. 2, pp. 102—115.
31. Dotsenko, I.B., The state of experimental population of the Caucasian rock lizards (*Darevskia*) in the Zhytomyr oblast of Ukraine, *Nauk. Visn. Uzhgorod. Univ., Ser. Biol.*, 2007, no. 21, pp. 14—19.
32. Yue, G.H., Beeckmann, R., and Geldermann, H., Mutation rate at swine microsatellite loci, *Genetica*, 2002, vol. 114, pp. 113—119.
33. Yue, G.H., David, L., and Orban, L., Mutation rate and pattern of microsatellites in common carp (*Cyprinus carpio* L.), *Genetica*, 2007, vol. 129, pp. 329—331.

Translated by N. Maleeva