

## Phylogeography of *Lacerta media* Lantz et Cyrén, 1920 (Lacertidae: Sauria) Based on the Analysis of Mitochondrial Cytochrome *b* Gene

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Received May 24, 2021; revised August 24, 2021; accepted September 7, 2021

**Abstract**—The mitochondrial DNA *cytb* sequences (1143 bp) were examined in the representatives of *Lacerta media*. A total of 34 lizards from 19 localities were analyzed. Special attention was paid to previously unexplored populations on the northern border of the range within the North Caucasus and Western Transcaucasia. Two types of dendrograms of phylogenetic relationships, Bayesian (BA) and maximum likelihood (ML), were reconstructed, showing identical topology. Phylogenetic reconstruction identifies four supported clades. Similarly, on the median-joining haplotype network, four haplogroups are distinguished. At earlier stages of the phylogeny of *L. m. media*, the populations of the eastern part of the Armenian Highlands and the Northern Zagros are isolated. Next, the populations of the central part of the Armenian Highlands and the Greater Caucasus become isolated. At the final stage, these are the populations of the Central Zagros and the eastern part of the Asia Minor Highlands. The data obtained do not support a scenario on the nominative subspecies dispersal from the west to the east and northeast, suggested previously by F. Ahmadzadeh et al. [4]. Instead, we consider the opposite direction, i.e., from the east to the north, south, and west. Two geographical regions, the Armenian Highlands and the Zagros Mountains, inhabited by the representatives of several clades/haplogroups are identified.

**Keywords:** *Lacerta media*, mitochondrial DNA, phylogeny, phylogeography, cytochrome *b*

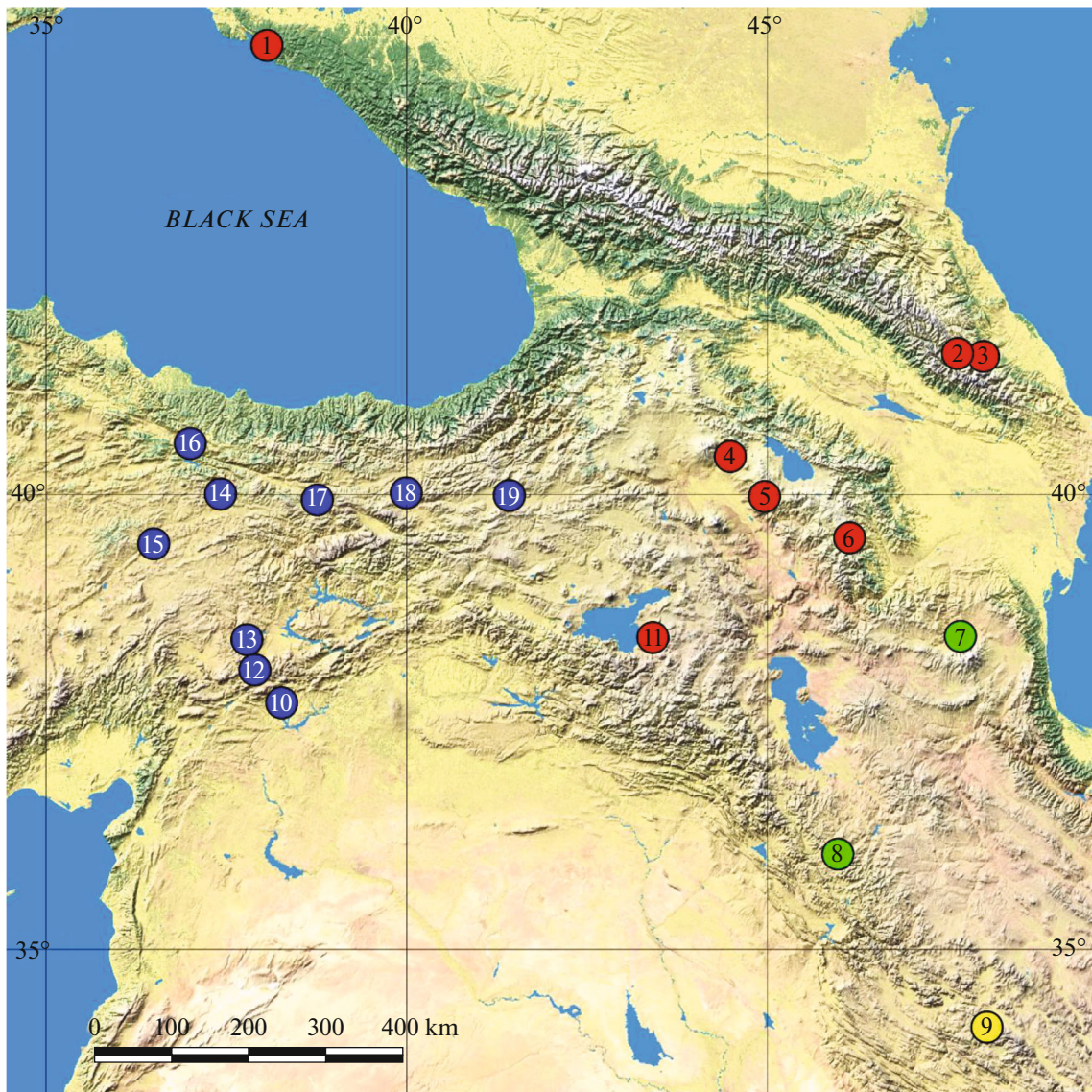
DOI: 10.1134/S102279542202003X

### INTRODUCTION

The *Lacerta media* Lantz et Cyrén, 1920, is a representative of the superspecies complex *Lacerta (trilineata)*, which is the objective of phylogenetic and phylogeographic studies of the genus *Lacerta* Linnaeus, 1758 [1]. In a number of recent studies [2–6], populations from the territory of the Caucasus inhabited by the nominative subspecies (the lectotype sampling locality, i.e., the valley of the Kvabiskhevi River, near the town of Borjomi, Georgia [7]) were represented by a sample from only five localities of the Armenian Highlands. However, lizards from the North Caucasus and Western Transcaucasia (Dagestan and Krasnodar krai), i.e., from the northern border of the species range, were not examined [8, 9]. In a number of studies, this territory is completely absent on *L. media* distribution maps [2, 3]. The indicated Dagestan and Black Sea peripheral populations are isolated from the Transcaucasian ones and are of interest from the standpoint of the study of microevolutionary processes.

At present, timing of the appearance and dispersal of representatives of the *Lacerta (trilineata)* complex is unclear. The discovery of fossil bone fragments of *L. trilineata* in the North Caucasian Late Miocene “Solnechnodolsk” locality [10] casts doubt on the existing scenarios of the complex phylogenesis, considering the Asia Minor Highlands (Anatolia) and the Aegean Sea region as the regions of divergence of the main phylogenetic clades in the Pliocene–Early Pleistocene [3, 6] or in the Late Miocene–Early Pliocene [5]. There are also conflicting data on *L. media*. In particular, on the basis of paleogeography of the region, the time of the three-lined lizard invasion into mountainous Dagestan is attributed to the Sarmatian (Late Miocene) [11], and its invasion into South Ossetia is attributed to the end of the Miocene–middle of the Pliocene [12]. At the same time, according to molecular genetic dating, the species appeared about 3.5 Ma in the Late Pliocene [4, 6] or about 7.12 Ma in the Late Miocene [5].

The aforementioned indicates the need for a more detailed study of the phylogeny and phylogeography of this lizard. In the Caucasus, the discovery of a refu-



**Fig. 1.** Sampling locations of *Lacerta media* individuals used for molecular genetic analysis. Clade designation: A, yellow; B, blue; C, red; D, green. The numbering and letter designation correspond to those in the Table 1.

gium that testifies to ancient colonization of the region or the confirmation of relatively recent dispersal of the species from the territory of Anatolia (the presumptive center of its origin and radiation) can be expected. It is noteworthy that the paleontological evidence that can serve as reference points in the reconstruction of the species phylogeny is limited to the discovery of the three-lined lizard in the Mousterian layers (Upper Pleistocene) of the Shvalieti Cave, Georgia [13].

An important fact is that *L. m. media* is included in the regional [14, 15] and national [16, 17] lists of protected animal taxa (Red Data Books). All this indicates the relevance of the present study from the standpoint of the investigation of genetic diversity and conservation.

## MATERIALS AND METHODS

DNA was extracted from regenerated tail tissues or phalanges of the forelimbs fixed in 96% ethanol. The specimens were obtained from five individuals collected in three localities of the Greater Caucasus and one locality of the Zagros Mountains (Fig. 1). Voucher individuals are kept in the collection of the Zoological Institute of the Russian Academy of Sciences (ZISP) and the Zoological Museum of Moscow State University (ZMMU). The phylogenetic analysis was carried out using 29 sequences of *L. m. media* [2, 3] taken from the GenBank (<http://www.ncbi.nlm.nih.gov/gene/>) (Table 1).

Total DNA was extracted using standard salt protocol with proteinase K lysis [18]. The *cytb* gene was amplified using a T100TM Thermal Cycler PCR

**Table 1.** List of samples of *Lacerta media* used in the molecular analysis

Locality number in Fig. 1	Localities	Specimen codes	GenBank accession number	Clade	Sampling dates	Geographical coordinates	Height, m above sea level	Collector(s)	Source
1	Russia, Krasnodar krai, the vicinity of Gelendzhik, the southern slope of the Markotkh Ridge	ZISP 23571.1-2	MZ065557– MZ065558	C	28.04.2006	38.0624, 44.5992	160	S.V. Ostrovskikh, K.D. Milto	Present study
2	Russia, Dagestan, Akhtynsky raion, the settlement of Kaka	ZISP TS 2979	MZ065555	C	07.07.2020	47.6328, 41.4790	1130	Z.S. Ismailova	“
3	Russia, Dagestan, Akhtynsky raion, the vicinity of the settlement of Akhty, slope of the southern exposure, the Mount of Ukhindag	ZMMU 13972	MZ065556	C	03–04.05.2013	47.6916, 41.4478	1450	A.D. Askenderov	“
4	Armenia, Aragatsothn Province, Mount Ara (=Araler, Ara ler, Arailer)	–	U88603	C	–	44.4833, 40.4000	2150	–	[2]
5	Armenia, Ararat Province, Khosrov Forest State reserve	–	KC896976	C	–	44.9579, 39.9763	1500	–	[4]
6	Armenia, Syunik Province, highway M-2 between the village of Shaki and the city of Goris	–	KC896977	C	–	46.1359, 39.5358	2010	–	“
7	Iran, Ardabil, the village of Arablu	–	KC896978– KC896983	D	–	47.6720, 38.4753	1110	–	“
8	Iran, Kurdistan, 35 km southwest of the city of Sekkes, near the Sekkes–Bane highway	ZISP 29894	MZ065554	D	08.08.2018	45.9746, 36.0727	2070	A.N. Barabanov	Present study
9	Iran, Lorestan, the vicinity of the city of Nurabad	–	KC896984– KC896988	A	–	48.0397, 34.1097	1800	–	[4]

Table 1. (Contd.)

Locality number in Fig. 1	Localities	Specimen codes	GenBank accession number	Clade	Sampling dates	Geographical coordinates	Height, m above sea level	Collector(s)	Source
10	Turkey, Adiyaman, the city of Adiyaman	–	KC897002 – KC897003	B	–	38.2667, 37.7500	640	–	[4]
11	Turkey, Van, the vicinity of the city of Van	–	KC896993, KC896995	C	–	43.4107, 38.4624	1810	–	“
12	Turkey, Malatya, northeast of the city of Doganshehir	–	KC896989	B	–	37.8947, 38.1093	1180	–	“
13	Turkey, Malatya, Karakhan Pass	–	KC896990 – KC896991	B	–	37.7855, 38.3393	1870	–	“
14	Turkey, Sivas, Doganshar–Hafik road	–	KC897000	B	–	37.4197, 40.0029	1660	–	“
15	Turkey, Sivas, the vicinity of the village of Kazandzhik	–	KC897001	B	–	36.4942, 39.4717	1630	–	“
16	Turkey, Tokat, between the city of Nksar and the city of Bashchiftlik	–	KC896994	B	–	37.0004, 40.5374	640	–	“
17	Turkey, Erzincan, the village of Kyurelik	–	KC896996 – KC896998	B	–	38.7608, 39.9397	1730	–	“
18	Turkey, Erzincan, the vicinity of the village of Kyucyukotlukbeli	–	KC896999	B	–	39.9939, 40.0147	2070	–	“
19	Turkey, Erzurum, 20 km northeast of the city of Erzurum	–	KC896992	B	–	41.4220, 39.9864	2020	–	“

(Bio-Rad) and primers 5'-GCC CCA AAA TAA GGA GAT GG-3' and 5'-TAG TGA TGG GGG ATT AAA GC-3' [19, 20]. PCR was performed at 95°C (3 min), followed by 32 cycles (95°C, 30 s; 56°C, 30 s; 72°C, 90 s) and final extension (72°C, 5 min). The PCR reaction mixture (25 µL) contained 50–100 ng of DNA, 0.5 µM of each primer, 0.2 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 2.5 µL of 10× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), and 2 units of Taq polymerases (Thermo Scientific). Sequencing was performed on an ABI PRIZM 3500xL genetic analyzer (Applied Biosystems). The final length of the amplified DNA fragment was 1143 bp. The obtained sequences (MZ065554–MZ065558) were deposited in the GenBank NCBI.

Sequence alignment was performed using the Geneious Prime 2021.0.1 (<https://www.geneious.com>) and Ali View 1.6 software programs [21]. Phylogenetic relationships were reconstructed using the Bayesian approach (BA) in the MrBayes 3.1.2 software program [22–24] and the Maximum Likelihood (ML) method in the MEGA X software package [25]. For the BA, 5 million generations were used; statistical node support of the ML trees was assessed by bootstrap analysis (1500 pseudoreplicates). The choice of an evolutionary model for nucleotide sequences was carried out in the MrModeltest 2.4 software program [26] using the Akaike information criterion (AIC). The GTR (General Time Reversible) model with the parameters I = 0.3909 and G = 0.7440 was chosen. Graphical images of the trees were obtained using the FigTree 1.4.4 software program (<http://tree.bio.ed.ac.uk/software/figtree>). The haplotype network was constructed using the TCS method in the PopART software program [27].

Intraspecific structure of *L. media* was examined, including in the analysis sequences from the study of F. Ahmadzadeh et al. [3] for *L. m. ciliciensis* Schmidtler, 1975 (KC896967–KC896974), *L. m. israelica* Peters, 1964 (KC896975), and *L. m. wolterstorffi* Mertens, 1922 (KC897005–KC897007). To root the dendrograms of phylogenetic relationships, sequences of *L. strigata* Eichwald, 1831 (LN835024), *L. agilis* Linnaeus 1758 (AY616285, AY616398), and *Eremias stummeri* Wettstein, 1940 (NC029878.1) from the GenBank database were used as the outgroup. Genetic variation was analyzed using the DnaSP v.5.10.01 software program [28], and the calculated parameters included the total number of polymorphic positions (*S*), the total number of substitutions ( $\eta$ ), the number of haplotypes (*H*), the haplotype diversity (*h*), the nucleotide diversity per site ( $\pi$ ), the mean number of nucleotide substitutions (*K*), the value of Tajima's *D* test, and Fu's *F<sub>s</sub>* test. Genetic distances (*p*-distances) were calculated using the MEGA X software program.

To analyze taxonomic boundaries based on molecular genetic traits, the ASAP (automated simultaneous analysis phylogenetics) [29] and ABGD (automatic barcode gap discovery) [30] algorithms were used.

This approach makes it possible to identify conditional molecular operational taxonomic units (MOTU), which are monophyletic clades of unspecified rank. The analysis was carried out using the parameters as follows:  $P_{\min}$  (minimum posterior distance) = 0.01,  $P_{\max}$  (maximum posterior distance) = 0.1, *X* (relative interval width) = 0.5; a matrix of pairwise K80 distances was used.

## RESULTS

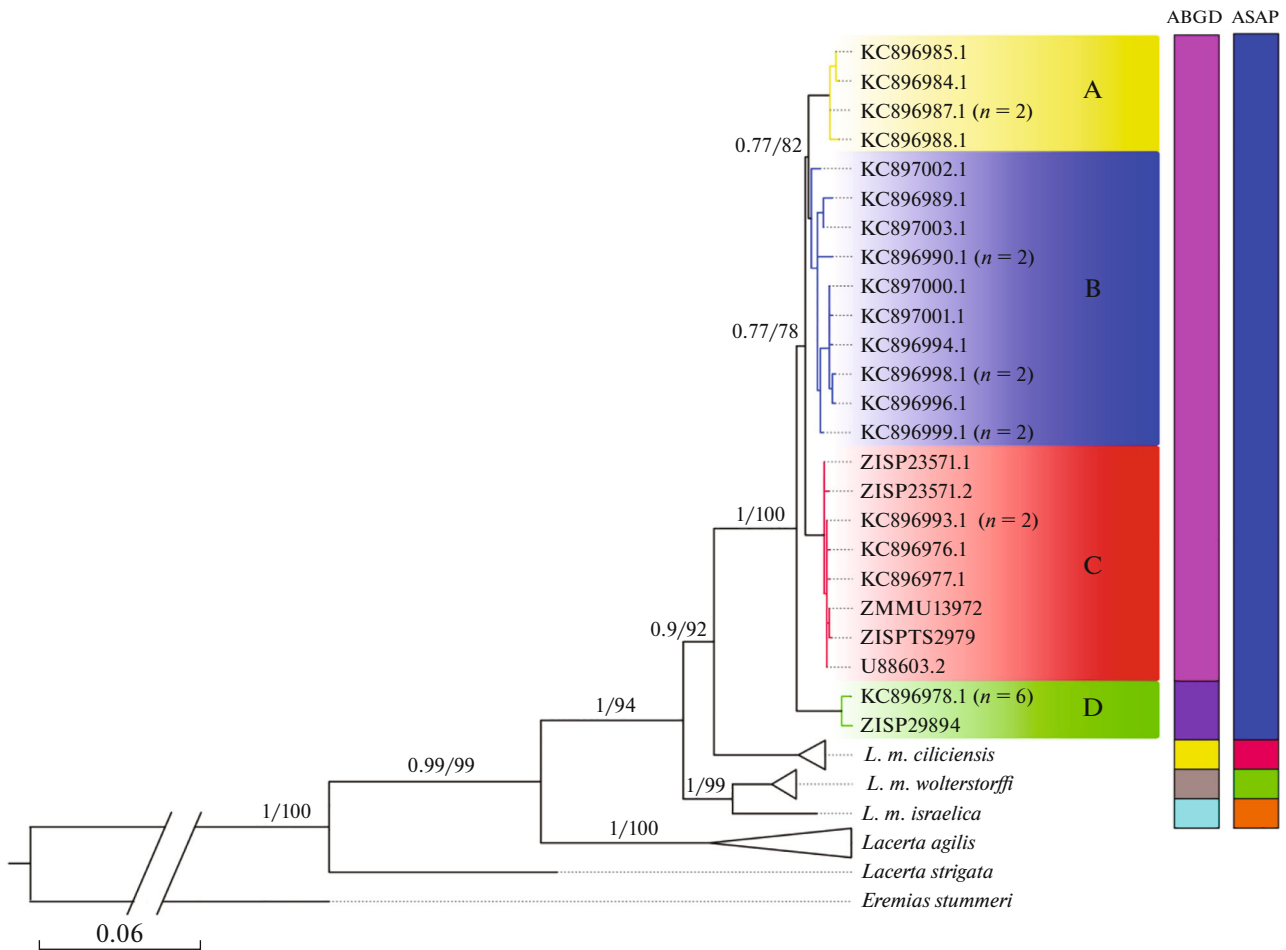
Thirty-four *cytb* gene sequences of *L. m. media* formed 24 haplotypes. Fifty-four (4.7% of the total length) variable positions were identified, of which 44 (3.8%) were parsimony informative. The ratio of transition to transversion was 4.84. The nucleotide composition of the fragment on the L-chain was shifted toward a decrease in the proportion of guanine (A, 26.7; T, 31.3; C, 30; and G, 12%). The genetic diversity indices in the sample were as follows: *S*, 54;  $\eta$ , 57; *h*, 0.964 ( $\pm 0.021$ );  $\pi$ , 0.0160 ( $\pm 0.0010$ ); *K*, 14.01; Tajima's *D*, 0.02 ( $p > 0.1$ ); Fu's *F<sub>s</sub>*,  $-1.80$  ( $p > 0.1$ ). The *F<sub>s</sub>* test was not statistically significant. The insufficiency of statistically significant negative *D* values suggests the absence of sudden population growth (expansion) and/or positive selection in the past, while the insufficiency of positive *D* values suggests the absence of recent population decline (the so-called bottleneck) and fragmentation and/or balancing selection.

Reconstruction of phylogenetic relationships of the representatives of *L. m. media* yielded dendrograms with identical topology, on which four mitochondrial clades could be distinguished (Fig. 2). The sequences from the territory of the Greater Caucasus were grouped into clade C, within which there was no considerable genetic heterogeneity (*h*, 0.889; *K*, 1.67). For comparison, the highest structuredness was observed in clade B (*h*, 0.961; *K*, 6.08). Similarly, on the median-joining haplotype network, four haplogroups (I–IV) are distinguished, the most distant of which is haplogroup IV (Fig. 3). It is distant from haplogroup II by 25 nucleotide substitutions, from haplogroup III by 22 substitutions, and from haplogroup I by 29 substitutions.

Calculation of *p* distances yielded the maximum value upon the comparison of the A/D and B/D clades and the minimum value upon the comparison of the B/C clades (Table 2).

The ASAP/ABGD analysis showed a distinct gap between the population and subspecific variation values (distance threshold = 3.27%, barcoding gap = 2.20%). Application of these algorithms made it possible to identify in *L. media* of four and five monophyletic MOTU, respectively (Fig. 2). In the first case, the molecular operational taxonomic units strictly corresponded to the recognized subspecies of three-lined





**Fig. 2.** Dendrogram of phylogenetic relationships of the *Lacerta media* representatives based on the analysis of the *cytb* gene (1143 bp). At the nodes, Bayesian a posteriori probabilities and bootstrap support values (BA/BS) are shown. Letter designations of the clades correspond to those in Tables 1 and 2. The molecular operating taxonomic units (MOTU) are shown in two columns in different colors.

lizard, while in the second case, clade D was additionally delimited.

**DISCUSSION**

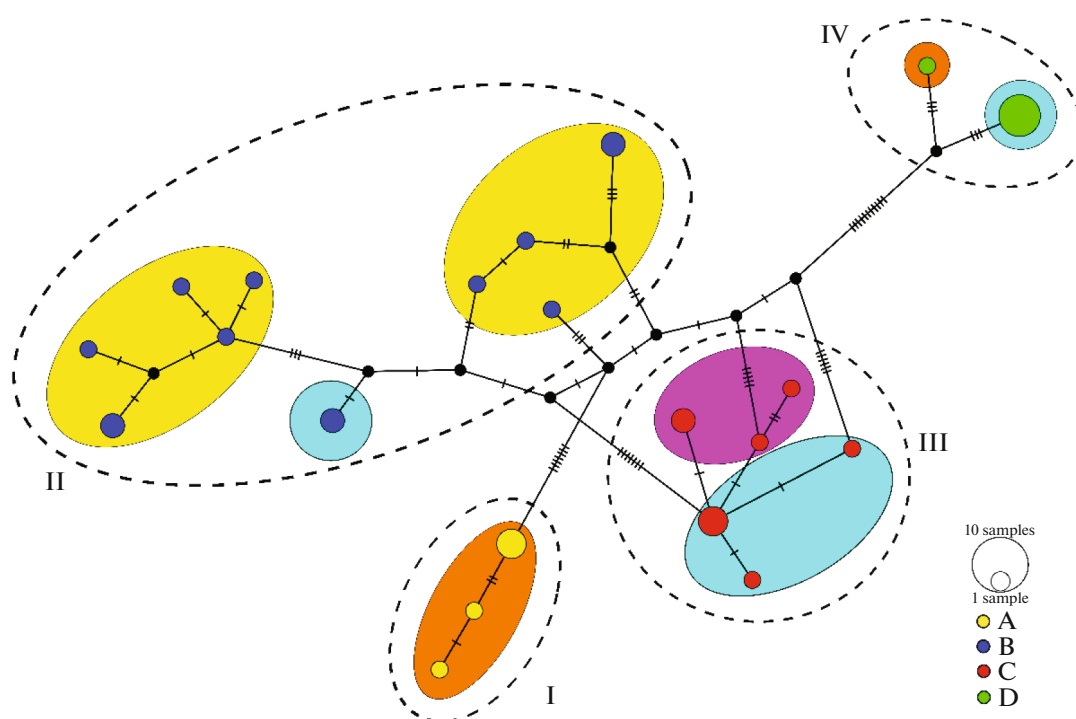
According to the topology of the obtained dendrogram, the pair of *L. m. israelica* and *L. m. wolterstorffi* was the first to be isolated at the subspecies level,

**Table 2.** Average genetic *p*-distances (%) (below the diagonal) and their standard deviations (above the diagonal) between the *Lacerta media* clades based on sequence analysis of the *cytb* gene

Clades	A	B	C	D
A		0.35	0.38	0.51
B	1.58		0.33	0.48
C	1.63	1.39		0.49
D	2.79	2.59	2.45	

Letter designations of the clades correspond to those in Table 1 and Fig. 2.

which was followed by *L. m. ciliciensis* and *L. m. media*. According to previous publications [3, 4], as far as the populations of Anatolia are characterized by the highest genetic diversity within the nominative subspecies, the Asia Minor Highlands can be considered as the initial territory from where multiple independent dispersals of the three-lined lizard to the Armenian Highlands (including the easternmost Turkish populations in the Lake Van region), to the Elburz Mountains in northwestern Iran, and to the Zagros Mountains in the west of Iran took place. In the cited study [3], the Arablú locality in northwestern Iran, situated on the northern slope of the extinct Sabalan volcano (=Savalan, Savelan, or Kuhe-Sebelan), was attributed to the “Elburz” refugium. We cannot agree with this, since the Savelan-Dag mountain range, which includes the volcano, is the eastern end of the Armenian Volcanic Highland [31]. In addition, *L. media* is traditionally included in the Eastern Mediterranean zoogeographic group of reptile species, while Elburz is a refugium of the Hyrcanian herpetofauna



**Fig. 3.** Median-joining *cytb* (1143 bp) haplotype network for the representatives of *Lacerta m. media*. Each colored circle represents a unique haplotype, and the circle size is proportional to the haplotype occurrence in the sample; the circle color corresponds to that of the clade in Fig. 2: A, yellow; B, blue; C, red; D, green. Connecting lines are probable evolutionary links; notches are nucleotide substitutions; black circles at the line nodes are predicted haplotypes. Geographic regions are painted as follows: orange, the Iranian Highlands; yellow, the Asia Minor Highlands; turquoise, the Armenian Highlands; pink, the Greater Caucasus. Dashed lines border the haplotype groups.

[32]. Representatives of the latter do not inhabit the Sabalan region [33, 34].

Obviously, the ancestral form of *L. m. media* initially penetrated into the indicated region from the west, where the main radiation of the species took place [3, 4]. However, according to the dendrogram obtained in the present study (Fig. 2), at earlier stages of the phylogeny of *L. m. media*, populations of the eastern part of the Armenian Highlands and the Northern Zagros (clade D) are distinguished. Next, the populations of the central part of the Armenian Highlands and the Greater Caucasus (C) become isolated, and at the final stage, these are the populations of Central Zagros (A) and the eastern part of the Asia Minor Highlands (B). Instead of the scenario of the nominative subspecies dispersal from the west to the east and northeast, we suggest the opposite direction, i.e., from the east to the north, south, and west. Our conclusions on the formation of the *L. m. media* range are consistent with the scheme proposed in 1924 by O. Cyrén [35]. It is noteworthy that I.S. Darevsky [36] and T.A. Muskhelishvili [37] considered the Armenian Highlands “the center of origin” of the lizard species.

In the Armenian Highlands, representatives of three clades (B, C, D) and three haplogroups (II, III, IV) were found, which is clearly demonstrated on the

haplotype network (Fig. 3). Another region where several clades (A and D) and haplogroups (I and IV) were identified was the Zagros Mountains. In the sample of *L. m. media*, these clades/haplogroups are genetically the most distant from each other. According to the dating of F. Ahmadzadeh et al. [3], isolation of populations constituting clades A, B, and C, happened about 650 000 years ago, and of those constituting clade D, about 1 million years ago. This was probably caused by climatic fluctuations in the Pleistocene, which influenced the landscapes of this mountainous area [38] and the chorology. Clade A is genetically the closest to the haplotypes from Adiyaman included in clade B. This reflects close geographical links between the southeast of Asia Minor (Eastern Taurus) and the northwest of the Iranian (Zagros) highlands [39]. Note that populations from this territory (Ostan Kermanshah) were designated by J.F. Schmidtler [40] as an independent “*L. m. media*–Zagrosform” form. The obtained data on the determination of the boundaries between taxa (according to the ABGD algorithm) point to relatively high taxonomic status of the populations the sequences of which formed clade D.

The absence of considerable genetic distance in the sample from the extremities of the Greater Caucasus (points 1–3 in Fig. 1, the straight-line distance between which is about 850 km) deserves special inter-

est. This can be interpreted as evidence of rapid dispersal of the species from the south in two directions, bypassing the Main Caucasian Ridge. However, the existence of a single Circum-Caucasian species range cannot be excluded. It could have been fragmented during the last glacial maximum (about 22000 years ago) or formed (for the second time?) in the xerothermal period of the Holocene (about 4500–2500 years ago) and fragmented owing to subsequent climate borealization. This scenario is supported by the presence of isolated North Caucasian populations of another representative of the Mediterranean zoogeographic group of reptiles, *Platycephalus najadum* (Eichwald, 1831), the shape of the distribution range of which in the region is extremely similar to *L. media* [41]. Reduction of the probable distribution area (habitat suitability) of this lizard in the northwest of the Greater Caucasus during this period was demonstrated in GIS modeling [42].

In the future, it will be necessary to include in the analysis lizards from the territories bordering between the clades, which will make it possible to detail the history of the species dispersal. For instance, new findings of *L. media* in the Talysh Mountains [43] may be indicative of either the existence here of small relict populations belonging to clade D or recent penetration of the representatives of clade C into the mountain forest belt of Talysh; the distribution area of clade B may extend to the northwestern slope of the Lesser Caucasus within Georgia. Finally, comprehensive analysis requires the study of the species phylogeography in the Zagros, a still poorly explored territory that, on one hand, acts as a barrier between Mesopotamia and the highlands and, on the other hand, acts as a corridor for the distribution of “northern” herpetofauna species to the south of the region [33].

#### ACKNOWLEDGMENTS

We thank N.B. Ananyeva, O.A. Ermakov, V.F. Orlova, A.A. Kidov, B.S. Tuniyev, and N.A. Shapoval for valuable advice and assistance to the study.

#### FUNDING

The study was supported by the Ministry of Science and Higher Education of the Russian Federation (grant no. 075-15-2021-1069) and the Russian Science Foundation (project no. 22-24-00079).

#### COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interest.* The authors declare that they have no conflicts of interest.

*Statement on the welfare of animals.* All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

#### REFERENCES

- Kornilios, P., Thanou, E., Lymberakis, P., et al., A phylogenomic resolution for the taxonomy of Aegean green lizards, *Zool. Scripta*, 2019, vol. 49, no. 1, pp. 14–27. <https://doi.org/10.1111/zsc.12385>
- Godinho, R., Crespo, E.G., Ferrand, N., and Harris, D.J., Phylogeny and evolution of the green lizards, *Lacerta* spp. (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences, *Amphibia—Reptilia*, 2005, vol. 26, no. 3, pp. 271–285. <https://doi.org/10.1163/156853805774408667>
- Ahmadzadeh, F., Flecks, M., Carretero, M.A., et al., Rapid lizard radiation lacking niche conservatism: ecological diversification within a complex landscape, *J. Biogeogr.*, 2013, vol. 40, no. 9, pp. 1807–1818. <https://doi.org/10.1111/jbi.12121>
- Ahmadzadeh, F., Flecks, M., Rödder, D., et al., Multiple dispersal out of Anatolia: biogeography and evolution of oriental green lizards, *Biol. J. Linn. Soc.*, 2013, vol. 110, no. 2, pp. 398–408. <https://doi.org/10.1111/bij.12129>
- Sagonas, K., Poulakakis, N., Lymberakis, P., et al., Molecular systematics and historical biogeography of the green lizards (*Lacerta*) in Greece: insights from mitochondrial and nuclear DNA, *Mol. Phylogenet. Evol.*, 2014, vol. 76, pp. 144–154. <https://doi.org/10.1016/j.ympev.2014.03.013>
- Kornilios, P., Thanou, E., Lymberakis, P., et al., Genome-wide markers untangle the green-lizard radiation in the Aegean Sea and support a rare biogeographical pattern, *J. Biogeogr.*, 2019, vol. 46, pp. 552–567. <https://doi.org/10.1111/jbi.13524>
- Doronin, I.V. and Doronina, M.A., Review of type specimens of *Lacerta media* Lantz et Cyrén, 1920 (Sauria: Lacertidae), *Proceedings of the Zoological Institute RAS*, 2019, vol. 323, no. 2, pp. 85–92. <https://doi.org/10.31610/trudyzin/2019.323.2.85>
- Nettmann, H.-K. and Rykena, S., *Lacerta trilineata* Bedriaga 1886—Riesensmaragdeidechse, *Handbuch der Reptilien und Amphibien Europas*, vol. 2/1: *Echsen (Sauria): II. Lacertidae II: Lacerta*, Böhme, W., Ed., Wiesbaden: Aula, 1984, pp. 100–128.
- Agasyan, A., Tuniyev, B., Cogalniceanu, D., et al., *Lacerta media*, *The IUCN Red List of Threatened Species*, 2009. <https://doi.org/10.2305/IUCN.UK.2009.RLTS.T164-749A5922769>
- Čerňanský, A. and Syromyatnikova, E.V., The first Miocene fossils of *Lacerta* cf. *trilineata* (Squamata, Lacertidae) with a comparative study of the main cranial osteological differences in green lizards and their relatives, *PLoS One*, 2019, vol. 14, no. 8. <https://doi.org/10.1371/journal.pone.0216191>
- Mazanaeva, L.F. and Tuniyev, B.S., Zoogeographical analysis of the Dagestan herpetofauna, *Curr. Studies in Herpetol.*, 2011, vol. 11, nos. 1–2, pp. 55–76.
- Lotiev K.Yu. and Tuniyev, B.S., Possible pathways and stages of the herpetofauna formation in South Ossetia, *Gornye ekosistemy i ikh komponenty* (Mountain Ecosys-



- tems and Their Components) (Proc VI All-Russian Conf. with Int. Participation, Dedicated to the Year of Ecology in Russia and the 100th Anniversary of Wildlife Management in Russia), Nal'chik: Alef, 2017, pp. 159—160.
13. Vekua, A.K., Animal history—vertebrates, in *Gruziya v antropogene: razvitie komponentov landshafta i paleogeograficheskie rekonstruktsii* (Georgia in Anthropogene: Development of Components and Paleogeographic Reconstructions), Tbilisi: Sakartvelo, 1991, pp. 308—381.
  14. Tuniyev, B.S. and Ostrovskikh, S.V., *Lacerta media* (Lantz et Cyrén, 1920), in *Krasnaya kniga Krasnodarskogo kraia: zhivotnye* (The Red Data Book of Krasnodar krai: Animals), Krasnodar: Admin. Krasnodar krai, 2017, 3rd ed., pp. 492—493.
  15. Mazanaeva, L.F. and Askenderov, A.D., *Lacerta media* (Lantz et Cyrén, 1920), in *Krasnaya kniga Respubliki Dagestan* (The Red Data Book of the Republic of Dagestan), *Makhachkala: Dzhamaludinov*, 2020, pp. 532—533.
  16. Tuniyev, B.S. and Lotiev K.Yu., *Lacerta media* Lantz et Cyrén, 1920, in *Krasnaya kniga Respubliki Yuzhnaya Osetiya* (The Red Data Book of the Republic of South Ossetia), Nal'chik: Poligrafservis i T, 2017, pp. 226—227.
  17. *Ob utverzhdenii Perechnya ob'ektov zhivotnogo mira, zanesennykh v Krasnuyu knigu Rossiiskoi Federatsii* (On the Approval of the List of Wildlife Resources Included in the Red Book of the Russian Federation), Order of the Ministry of Natural Resources of the Russian Federation of March 24, 2020, no. 162. Registered with the Ministry of Justice of the Russian Federation on April 2, 2020, no. 57940. <https://minjust.consultant.ru/documents/45937>.
  18. Aljanabi, S.M. and Martinez, I., Universal and rapid salt-extraction of high genomic DNA for PCR-based techniques, *Nucleic Acids Res.*, 1997, vol. 25, no. 22, pp. 4692—4693. <https://doi.org/10.1093/nar/25.22.4692>
  19. Marzahn, E., Mayer, W., Joger, U., et al., Phylogeography of the *Lacerta viridis* complex: mitochondrial and nuclear markers provide taxonomic insights, *J. Zool. Syst. Evol. Res.*, 2016, vol. 54, no. 2, pp. 85—105. <https://doi.org/10.1111/jzs.12115>
  20. Kukushkin, O.V., Ermakov, O.A., Ivanov, A.Yu., et al., Cytochrome *b* mitochondrial gene analysis-based phylogeography of a Sand lizard in the Crimea: ancient refugium at the peninsula, late expansion from the North, and first evidence of *Lacerta agilis tauridica* and *L. a. exigua* (Lacertidae: Sauria) hybridization, *Proceedings of the Zoological Institute RAS*, 2020, vol. 324, no. 1, pp. 56—99. <https://doi.org/10.31610/trudyzin/2020.324.1.56>
  21. Larsson, A., AliView: a fast and lightweight alignment viewer and editor for large data sets, *Bioinformatics*, 2014, vol. 30, no. 22, pp. 3276—3278. <https://doi.org/10.1093/bioinformatics/btu531>
  22. Huelsenbeck, J.P. and Ronquist, F., MRBAYES: Bayesian inference of phylogenetic trees, *Bioinformatics*, 2001, vol. 17, pp. 754—755. <https://doi.org/10.1093/bioinformatics/17.8.754>
  23. Ronquist, F. and Huelsenbeck, J.P., MrBayes 3: Bayesian phylogenetic inference under mixed models, *Bioinformatics*, 2003, vol. 19, no. 12, pp. 1572—1574. <https://doi.org/10.1093/bioinformatics/btg180>
  24. Altekar, G., Dwarkadas, S., and Huelsenbeck, J.P., and Ronquist, F., Parallel metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference, *Bioinformatics*, 2004, vol. 20, no. 3, pp. 407—415. <https://doi.org/10.1093/bioinformatics/btg427>
  25. Kumar, S., Stecher, G., Li, M., et al., MEGA X: molecular evolutionary genetics analysis across computing platforms, *Mol. Biol. Evol.*, 2018, vol. 35, no. 6, pp. 1547—1549. <https://doi.org/10.1093/molbev/msy096>
  26. Nylander, J.A.A., MrModeltest v2, Program distributed by the author, Uppsala: Evolutionary Biology Centre, Uppsala University, 2004.
  27. Leigh, J.W. and Bryant, D., PopART: full-feature software for haplotype network construction, *Methods Ecol. Evol.*, 2015, vol. 6, no. 9, pp. 1110—1116. <https://doi.org/10.1111/2041-210X.12410>
  28. Librado, P. and Rozas, J., DnaSP v5: a software for comprehensive analysis of DNA polymorphism data, *Bioinformatics*, 2009, vol. 25, pp. 1451—1452. <https://doi.org/10.1093/bioinformatics/btp187>
  29. Sarkar, I.N., Egan, M.G., Coruzzi, G., et al., Automated simultaneous analysis phylogenetics (ASAP): an enabling tool for phylogenomic, *BMC Bioinf.*, 2008, vol. 9, no. 103, pp. 1—5. <https://doi.org/10.1186/1471-2105-9-103>
  30. Puillandre, N., Lambert, A., Brouillet, S., and Achaz, G., ABGD, Automatic Barcode Gap Discovery for primary species delimitation, *Mol. Ecol.*, 2012, vol. 21, no. 8, pp. 1864—1877. <https://doi.org/10.1111/j.1365-294X.2011.05239.x>
  31. Sebelan, in *Bol'shaya Sovetskaya Entsiklopediya v 30 tomakh* (The Great Soviet Encyclopedia in 30 Volumes), Prokhorov, A.M., Ed., Moscow: Bol'shaya Sovetskaya Entsiklopediya, 1976, vol. 23, 3rd ed., p. 101.
  32. Tuniyev, B.S., Orlov, N.L., Ananjeva, N.B., and Aghasyan, A.L., *Snakes of the Caucasus: Taxonomic Diversity, Distribution, Conservation*, St. Petersburg: KMK, 2019.
  33. Anderson, S.C., *The Lizards of Iran*, Oxford, Ohio: Society for the Study of Amphibians and Reptiles, 1999, vol. 15.
  34. Kidov, A.A. and Litvinchuk, S.N., Distribution and conservation status of the Hyrcanian wood frog (*Rana pseudodalmatina*) in Azerbaijan, *Russ. J. Herpetol.*, 2021, vol. 28, no. 2, pp. 97—107. <https://doi.org/10.30906/1026-2296-2021-28-2-97-107>
  35. Cyrén, O., Klima und Eidechsenverbreitung. Eine Studie der geographischen Variation und Entwicklung einiger Lacerten, insbesondere unter Berücksichtigung

- der klimatischen Faktoren, *Medd. Göteborgs Mus. Zool. Avd.*, 1924, vol. 27, no. 3, pp. 1–82.
36. Darevsky, I.S., The fauna of reptiles of Armenia and its zoogeographical analysis, *Extended Abstract of Cand. Sci. Dissertation*, Erevan, 1957.
37. Muskhelishvili, T.A., On the distribution of the *Lacerta strigata* Eichwald and *Lacerta trilineata media* Lantz et Cyrén in Eastern Georgia, *Soobshch. Akad. Nauk Gruz. SSR*, 1966, vol. XLIII, no. 3, pp. 753–756.
38. Kehl, M., Quaternary climate change in Iran—the state of knowledge, *Erdkunde*, 2009, vol. 63, no. 1, pp. 1–17. <https://doi.org/10.3112/ERDKUNDE.2009.01.01>
39. Atalay, I., Efe, R., and Soykan, A., Mediterranean Ecosystems of Turkey: Ecology of Taurus Mountains, in *Environment and Culture in the Mediterranean Region*, Efe, R., Cravins, G., Öztürk, M., and Atalay, I., Eds., Newcastle, UK: Cambr. Scholars Publ., 2008, pp. 3–37. <https://doi.org/10.5848/csp.1087.00001>
40. Schmidtler, J.F., Orientalische Smaragdeidechsen: 2. Über Systematik und Synökologie von *Lacerta trilineata*, *L. media* und *L. pamphylica*, *Salamandra*, 1986, vol. 22, nos. 2–3, pp. 126–146.
41. Tuniyev, B.S., On the Mediterranean influence on the formation of herpetofauna of the Caucasian isthmus and its main xerophylous refugia, *Russ. J. Herpetol.*, 1995, vol. 2, no. 2, pp. 95–119. <https://doi.org/10.30906/1026-2296-1995-2-2-95-119>
42. Doronin, I.V., Mazanaeva, L.F., and Doronina, M.A., The use of GIS-modeling for the analysis of the distribution of three-lined lizard, *Lacerta media* Lantz et Cyrén, 1920, in the territory of Dagestan (Russia), *Proceedings of the Zoological Institute RAS*, 2018, vol. 322, no. 4, pp. 463–480. <https://doi.org/10.31610/trudyzin/2018.322.4.463>
43. Kidov, A.A., Doronin, I.V., Pykhov, S.G., and Doronina, M.A., A new record of *Lacerta media* Lantz et Cyrén, 1920 (Reptilia, Lacertidae) in Talysh, *Curr. Studies in Herpetol.*, 2019, vol. 19, nos. 1–2, pp. 68–73. <https://doi.org/10.18500/1814-6090-2019-19-1-2-68-73>

*Translated by N. Maleeva*