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Electrophoretic comparison of blood-serum proteins of *Lacerta trilineata, Lacerta media* and *Lacerta pamphylica* (Sauria, Lacertidae) from Turkey

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Blood-serum proteins of the Anatolian populations of *Lacerta trilineata*, *L. media* and *L. pamphylica* were studied comparatively by polyacrylamide disc electrophoresis. In order to obtain useful biochemical data for classification, differences between the electrophoreograms of the samples collected from different regions were distinguished qualitatively and quantitatively. These comparisons indicate that the Anatolian populations of *L. trilineata* should be subdivided into three species.

Key words: Lacertidae, blood serum proteins, classification, Turkey.

Introduction

The Anatolian populations of the polytipic lacertid species *Lacerta trilineata* Bedriaga, 1886 was classified in different subspecific divisions by BODENHEIMER (1944), MERTENS (1952), PETERS (1964), BARAN (1969) and SCHMIDTLER (1975). However; according to the morphological observations and morphometric analyses, SCHMIDLER (1986) suggested that lacertid populations in Anatolia belonged to three species: *Lacerta trilineata* Bedrıaga, 1886 ranged in W Anatolia, *L. media* Lantz et Cyrién, 1920 in E Anatolia and *L. pamphylica* Schmidtler, 1975 in S Anatolia.

In order to confirm the above conclusion, it is necessary to obtain further information and the purpose of the present study is to identify the similarities and differences of the patterns of blood serum proteins in these lacertids. This was done on samples collected from different areas of Anatolia, that were examined by electrophoretic methods and the results were compared in details.

Material and methods

Twenty-nine adult specimens (17 ♂♂, 12 ♀♀) collected from the western, eastern and southern regions of Anatolia were brought alive to the laboratory and color slides of all the specimens were taken. Blood samples were taken from the postorbital sinuses with heparinized hematocrite tubes and the blood serums were centrifuged at 600 g and kept at −20 °C. The etherized lizards were properly fixed and kept in 70% ethanol. All samples were deposited at the Museum of Zool-

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ogy Department, Ege University (ZDEU). The data of these materials are as follows:

Lacerta trilineata (n = 11): ZDEU 24/1991, 1-4 (2 ♂♂; 2 ♀♀), Karagöl-İzmir (38°33′ N, 27°10′ E), 10.IV.1991, leg. M. Tosunoğlu; ZDEU 87/1991, 1 (1 ♂), Bornova (38°28′ N, 27°13′ E), 12.VI.1991, leg. M. Tuzcu; ZDEU 6/1993, 1-2 (2 ♂♂), Karagöl-İzmir, 11.III.1993, leg. E. Çevik; ZDEU 10/1993, 1-4 (2 ♂♂; 2 ♀♀), Selçuk-İzmir (37°56′ N, 27°21′ E), 26.III.1993, leg. B. Keskin.

Lacerta media (n = 8): ZDEU 98/1992, 1-6 (2 ♂♂; 4 ♀♀), Kale-Gümüşhane (40°23′ N, 39°40′ E), 21.VII.1992, leg. S. Üçüncü; ZDEU 99/1992, 1 (1 ♂), Aşkale-Erzurum (39°55 N, 40°42′ E), 22.VII.1992, leg. S. Üçüncü; ZDEU 100/1992, 1 (1 ♂), Aşkale-Erzurum, 23.VII.1992, leg. S. Üçüncü.

Lacerta pamphylica (n = 10): ZDEU 14/1992, 1-8 (5 ♂♂; 3 ♀♀), Cevizli-Antalya (37°12′ N, 31°46′ E), 13.V.1992, leg. S. Üçüncü et C.V. Tok; ZDEU 50/1992, 1-2 (1 ♂; 1 ♀), Beşkonak-Antalya (37°09′ N, 31°12′ E), 31.V.1992, leg. S. Üçüncü et M. Tosunoğlu.

The separation of blood-serum proteins was carried out by the methods of Özeti & Atatür (1979) and Arikan (1983), who slightly modified the polyacrylamide disc-electrophoresis method of Davis (1964). The separations (of 4 μl serum samples) were conducted at room temperature with a Canalco Model 1200 electrophoresis chamber. After electrophoresis, gels were stained with a 0.5% Amido Black (Naphthol Blue Black 10-B) solution and gels were passively destained in 7% acetic acid. The quantitative and qualitative evaluations of the separations were done on densitometric tracing curves, obtained using a Gelman ACD model 39430 densitometer scanning at 500 nm. The electrophoreograms were also visually compared.

In order to compare the different populations, one-way analysis of variance was carried out using IBM Statgraphic (Version 6.0).

Results

All specimens examined were sexually mature. Males and females were evaluated together, and discernible sex-related diffeqrences were observed neither on the serum protein electrophoreograms nor on the densitometric curves. The protein distribution patterns of three specimens are given in Fig. 1. Serum protein fractions were divided into regions (albumin and globulin) (Chen, 1967).

Blood-serum proteins of 11 specimens of *L. trilineta* consisted of a total of 14–15 electrophoretic fraction groups; 13–14 fractions were found in the globulin and one fraction in the albumin zone. All of the *L. trilineata* specimens had one B_1 fraction in the B region, while the B_4 zone had one (45.45%) or two (54.55%) adjacent fraction. In only four specimens, the A_3 of A region consisted of two fractions (Fig. 1a).

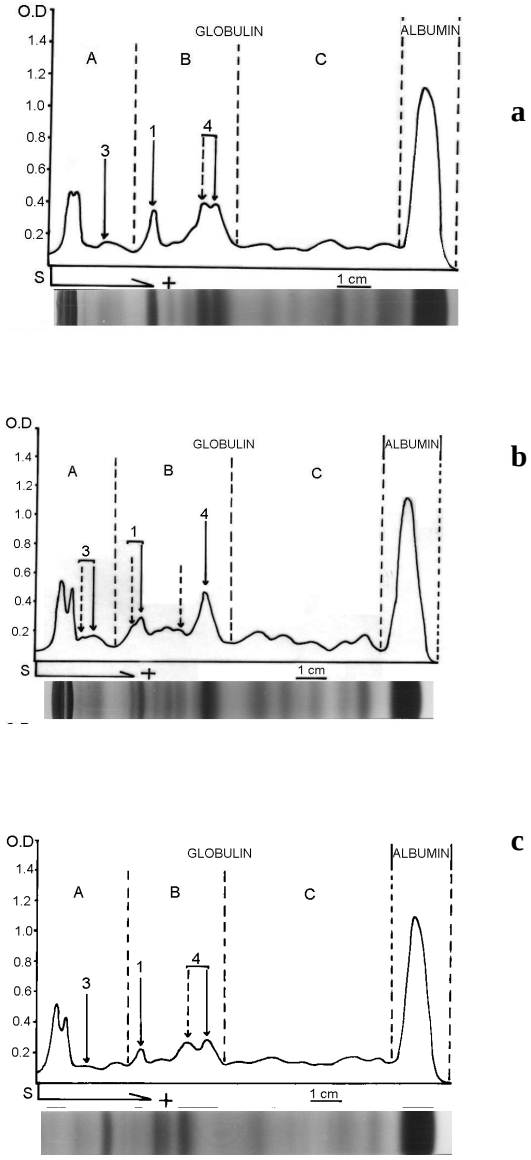

Fig. 1. Electrophoreograms and their densitometric tracing curves of: a – *Lacerta trilineata* from İzmir; b – *Lacerta media* from Erzurum; c – *Lacerta pamphylica* from Antalya. Designations: O.D. – optical density, A to C – arbitrarily chosen globulin zones to facilitate the comparison of the same areas of the different tracing curves.

As can be seen in *L. media*, protein fractions could only be divided into 14–16 fraction groups, one of which was in the albumin region and 13–15 were in the globulin region. While there is only a single fraction in the region B_4 of the globulins of

Table 1. The summarized analysis of various parameters of the specimens of *Lacerta trilineata*, *L. media* and *L. pamphylica*.

	L. trilineata	*L. media*	*L. pamphylica*
Prealbumin fraction	–	–	Present
The total number of fraction (Albumin +Globulin)	14–15	14–16	14–15
TheA_3 fraction of A region of Globulin	One (36%)	Two (100%)	One (100%)
The B_4 fraction of B region of Globulin	One (45%)	♂ One (100%) ♀ Two (75%)	Two (80%)
The B_1 fraction of B region of Globulin	One (100%)	Two (75%)	One (100%)

Table 2. Statistical analysis of albumin, globulin and albumin/globulin ratio (Alb/Glb) of *Lacerta trilineata*, *L. media* and *L. pamphylica* populations.

Characteristics	*L. trilineata* ($N = 11$)			*L. media* ($N = 8$)			*L. pamphylica* ($N = 10$)			ANOVA
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	$F_{1.8}$; P
Albumin	29.19	3.03	23.83–33.41	27.52	2.28	24.11–30.70	29.19	3.58	23.63–35.17	0.86; 43
Globulin	70.73	3.03	66.54–76.10	72.41	2.28	69.24–75.82	71.14	3.95	64.77–76.31	0.65; 0.52
Alb/Glb	0.40	0.05	0.31–0.50	0.37	0.04	0.31–0.44	0.41	0.07	0.30–0.54	0.90; 0.42

Key: N – number of specimens; SD – standard deviation.

male specimens, there are two visible fractions in that of female specimens. Region B of the globulins comprises five dense fractions in *L. media*. Finally, the region A_3 of the globulin of whole samples contains two fractions (Fig. 1b).

The densitometric curves of blood-serum proteins of *L. pamphylica* specimens were observed as a total of 14–15 fraction groups. A discernible pre-albumin fraction was seen before the albumin fraction. In eight of ten specimens, B_1 of B region had one, and B_4 had two fractions. While a single B_1 fraction was evident in eight of ten specimens, the remaining two had two fractions. In all of the specimens two B_4 fractions were seen. It was possible to differentiate one fraction within A_3 of the whole samples (Fig. 1c). The quantitative evaluations of the blood-serum proteins of those different populations are given in Tables 1 and 2.

Discussion

Conventional taxonomic treatment has resulted in uncertainty as to the taxonomic status of the genus *Lacerta*. As previously indicated, Schmidtler (1986) was of the opinion that *L. trilineata* populations of Anatolia should be accepted as three different species. According to his report, western populations had to be classified as *L. trilineata*, while the eastern populations as *L. media*. However, it was difficult to make a definitive description for the populations of *L. pamphylica*, the endemic inhabitants of S Anatolia, because of their variable morphometric features. The author also noted that, when compared with the other green lizard groups (*L. trilineata, L. media, L. viridis-strigata*), the morphometric and pholidotic differences of *L. pamphylica* were relatively higher. Engelman & Schaffner (1981) also determined the differences in *L. pamphylica*, based on the data obtained by immunoserological analyses. As a conclusion, Schmidtler (1986) suggested that the S Anatolia populations of *Lacerta* should be classified into different species.

Several authors (Dessauer & Fox, 1956; Chen, 1967; Ferguson, 1980) have stressed the taxonomical importance of the number, mobility and relative abundance of protein fractions obtained from electrophoretic separation of blood-serum proteins of amphibians and reptiles. According to Ferguson (1980), genetic variation leads to qualitative differences in protein fractions while variation in factors like age, sex, physiological adaptation and environment lead to quantitative differences. Therefore, qualitative differences are taxonomically important.

To this controversy, we added our data on electrophoresis of blood-serum proteins. Qualitative analysis of the samples examined revealed that W Anatolia, E Anatolia and S Anatolia populations of *Lacerta* show slight differences in

both albumin and globulin regions. According to these serological qualitative differences, we distinguished three populations that are taxonomically distinct on the species level. Our results support SCHMIDTLER's (1986) conclusions. As given in Fig. 1, the three different populations of Anatolian *Lacerta* have notable differences. Consequently, Anatolian populations of *L. trilineata* should really be accepted as three different species according to their serological differences distinguished on their blood-serum proteins.

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