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Electrophoretic comparison of blood-serum proteins of *Apathya cappadocica* (Sauria, Lacertidae) subspecies from Anatolia

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Abstract. Blood-serum proteins of the known subspecies of *Apathya cappadocica* (Werner, 1902) were studied comparatively by polyacrylamide disc gel electrophoresis. In order to obtain useful biochemical data for classification, differences between the electrophoreograms of the samples included in the morphologically different subspecies were distinguished qualitatively and quantitatively. These comparisons indicated that electrophoretic results supported morphological discrimination of the known subspecies of *A. cappadocica*.

Keywords. *Apathya cappadocica*, blood-serum proteins, Lacertidae, Anatolia.

INTRODUCTION

Apathya is a small genus of lacertid lizard including 2 species [*Apathya cappadocica* (Werner 1902) and *Apathya yassujica* (Nilson, Rastegar-Pouyani, Rastegar-Pouyani and Andrén 2003)] found in Central, East, South and Southeastern Anatolia, Northern Iraq, and West Iran (Eiselt, 1979; Baran and Atatür, 1998; Nilson et al., 2003; Arnold et al., 2007). *A. cappadocica* was first described as *Lacerta cappadocica* from Erciyes Mountain in Kayseri, Turkey (Werner, 1902). *A. cappodocica* is a polytypic species and includes five subspecies [*A. c. cappadocica* (Werner, 1902) – type locality: Erciyes Mountain, Turkey; *A. c. urmiana* (Lantz-Suchow, 1934) – type locality: 20 km SW of Rezaiyeh, Iran; *A. c. wolteri* (Bird, 1936) – type locality: 16 km W of Gaziantep, Turkey; *A. c. muhtari* (Eiselt, 1979) – type locality: 26 km SW of Bitlis, Turkey and *A. c. schmidtlerorum* (Eiselt, 1979) – type

locality 10 km S of Diyarbakır, Turkey] (Lantz and Suchow, 1934; Bird, 1936; Eiselt, 1979; Baran and Atatür, 1998; Sindaco et al., 2000).

After a detailed study by Eiselt (1979), regional studies on morphology and ecology of *A. cappadocica* in its distribution sites were conducted (Bischoff and Schmidtler, 1994; Schmidtler and Bischoff, 1995; Schmidtler, 1997; Anderson, 1999; Kumlutaş and Olgun, 1999). Schmidtler and Bischoff (1995) examined specimens separated into four groups collected in Gaziantep, Hatay, Adana and Mersin in terms of pholidolial characteristics. They found considerable differences between the specimens dependent on climatic conditions in terms of examined pholidolial characteristics. They finally stated that color-pattern features of the specimens belonging to three known subspecies *A. c. wolteri*, *A. c. muhtari and A. c. schmidtlerorum* show variability dependent on their habitats. The taxonomical status of known subspecies of *A. cappadocica* is doubtful. There was no information on blood serum electrophoretic pattern in the comparison between the known subspecies of *A. cappadocica*. In order to confirm the present taxonomical status of known subspecies of *A. cappadocica*, it is necessary to obtain further information outside of morphological studies and the purpose of the present study is to identify the similarities and differences of the patterns of blood serum proteins in known subspecies of *A. cappadocica*.

MATERIALS AND METHODS

Adult male and female specimens of *A. cappadocica* were collected from known distribution sites of each subspecies in the Southern and Southeastern part of Turkey between 10 June to 15 June 2005 by Y. Kumlutaş, Ç. Ilgaz and A. Avcı. The specimens were transferred to the laboratory to obtain blood samples for electrophoretic analysis. After obtaining blood samples they were fixed with 5% formaldehyde in 70% ethanol and then preserved in 70% ethanol according to the method described by Başoğlu and Baran (1977). Collection numbers (ZDEU, Zoology Department of Ege University) were given to the specimens kept in the Lab. of the Department of Biology at Buca Education Faculty of Dokuz Eylül University. Data on specimens used for electrophoretic analysis is given in Table 1. All blood-serum protein study specimens were of similar length, i.e. they were of similar age.

Table 1. Data on the specimens of *A. cappadocica* used for electrophoretic analysis (N: Number of specimens)

Subspecies	N	Locality	Collection Date	Altitude (m)	Coordinates
A. c. cappadocica	2 (1♂ - 1♀)	Pozantı, Adana, Turkey	10.06.2005	1210	N 3733292 – E 3458453
A. c. wolteri	2 (1♂ - 1♀)	Between Kilis and Hassa 32. km, Kilis, Turkey	11.06.2005	540	N 3650280 – E 3707517
A. c. urmiana	2 (1♂ - 1♀)	Hasankeyf, Batman, Turkey	15.06.2005	479	N 3742742 – E 4124547
A. c. muhtari	2 (1♂ - 1♀)	Küçükalanlı village, Şanlıurfa, Turkey	12.06.2005	799	N 3710524 – E 3838335
A. c. schmidtlerorum	2 (1♂ - 1♀)	Between Diyarbakır and Siverek, Diyarbakır, Turkey	14.06.2006	1058	N 3750897 – E 3942794

For electrophoretic analysis, blood samples were obtained from two specimens (one male and one female) for each subspecies of *A. cappadocica*. Blood samples were obtained from the postorbital sinuses of living specimens via heparinized hematocrit capillary tubes according to the method described by MacLean et al. (1973). Samples were centrifuged for 5 minutes at 600 g and were stored in equal amounts (4 µl) at -20°C for each separation until analysis. Blood-serum samples were separated using polyacrylamide-disc electrophoresis according to Davis (1964), slightly modified by Özeti and Atatur (1979). Electrophoretic separations were carried out at room temperature (20-25°C) with a Canalco Model 1200 electrophoresis apparatus. Separation gels were first stained with 0.5% Amido Black, and then de-stained passively with repeated 7% acetic acid baths. Gels were qualitatively evaluated directly from the electrophoretograms and densitometric tracing curves of the separations were obtained using a Gelman ACD-15 Model 39430 densitometer scanning at 500 nm.

RESULTS

All specimens examined were sexually mature and no obvious difference was recorded in serum protein phenograms (in densitometric curves) between sexes in all subspecies. Consequently, the specimens were pooled by sex for further evaluation. We also pooled the same aliquots of serum together and so this situation is important for the densitometric quantitative analysis.

Significant differences were established among the subspecies from the viewpoints of fraction numbers, electrophoretic mobilities and densities of the blood proteins which suggests that all subspecies of *A. cappadocica* are clearly distinct at the subspecific level (Fig. 1).

Gel electrophoretograms of the blood protein samples of the five subspecies are shown in Fig. 1. The gel electrophoretograms of blood-serum proteins of a specimen from each subspecies, together with their densitometric tracing curves, are shown in Figs 2, 3, 4, 5 and respectively.

In *A. c. cappadocica*, *A. c. wolteri* and *A. c. urmiana* the blood protein fractions were divided into 14 fractions or fraction groups (1 albumin-like fraction and 1 postalbumin-like fraction at albumin region and 12 globulin-like fractions at globulin region) while the total number of fractions or fraction groups were found to be 12 (2 albumin-like fractions and 1 postalbumin-like fraction at albumin region and 9 globulin-like fractions at globulin region) in *A. c. schmidtlerorum*. Finally the total blood protein fractions were divided into 11 fractions or fraction groups (1 albumin-like fraction and 1 postalbumin-like fraction at albumin region and 9 globulin-like fractions at globulin region) (Figure 2-6).

Although all subspecies except *A. c. schmidtlerorum* show similarity in terms of albumin fractions, the fractions matched to each other at the globulin region show both qualitative and quantitative differences among all subspecies.

DISCUSSION

Many researchers have highlighted the taxonomic importance of the number of fractions, and the mobility and density of blood-serum proteins obtained from electrophoretic separation (Dessauer and Fox, 1956; Chen, 1967; Ferguson, 1980; Arıkan, 1990; Arıkan et

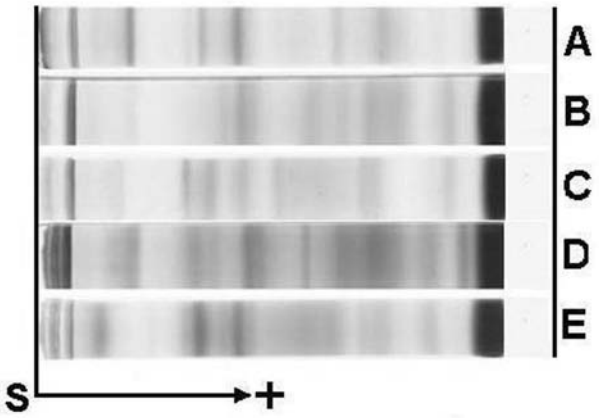

Fig. 1. Blood protein samples electrophoretograms of the five subspecies of *Apathya cappadocica*. **A:** *A. c. cappadocica*, **B:** *A. c. wolteri*, **C:** *A. c. urmiana*, **D:** *A. c. schmidtlerorum*, **E:** *A. c. muhtari*. (S: Start, junction between the stacking and separation gels).

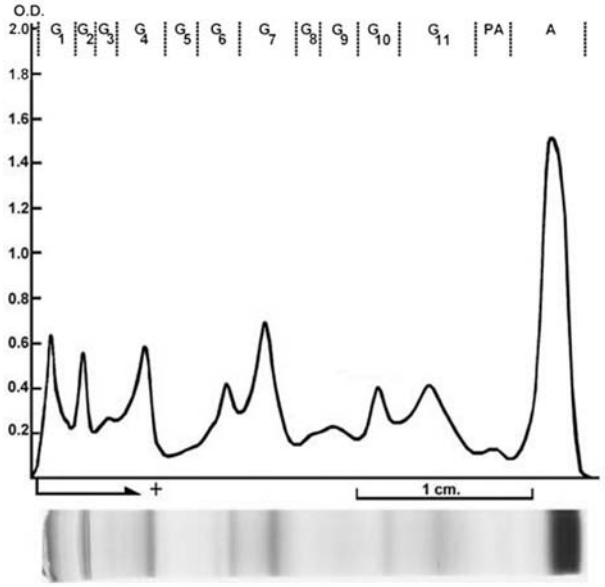

Fig. 2. Gel photograph showing the electrophoretic separation of the blood protein sample obtained from *A. c. cappadocica*, together with its densitometric tracing curve. OD: Optical density, S: Start (junction between the stacking and separation gels), G1-G11: Globulins zone, PA: Postalbumine zone, A: Albumine zone.

al., 1998, 1999; Kumlutaş et al., 2007). Ferguson (1980) stated that while the quantitative difference of fractions could reflect gender, age, environmental and physiological factors, the qualitative differences of fractions could be caused by genetic variations. So, qualitative differences are important for taxonomic evaluations.

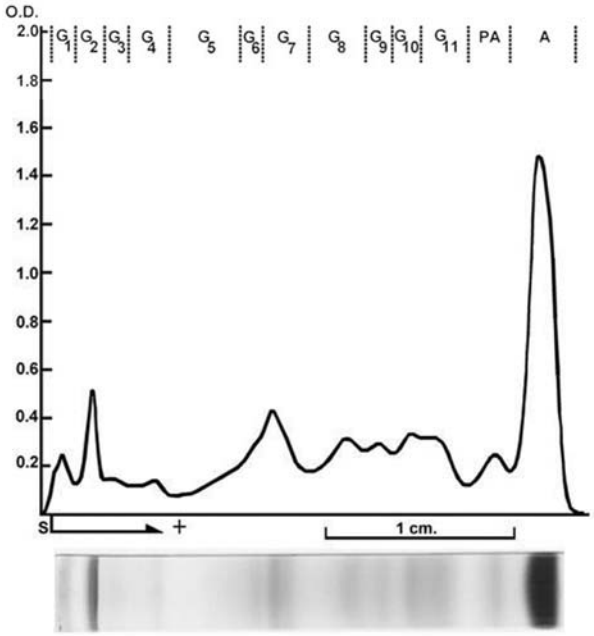

Fig. 3. Gel photograph showing the electrophoretic separation of the blood protein sample obtained from *A. c. wolteri*, together with its densitometric tracing curve. For further explanation, see legend to Figure 2.

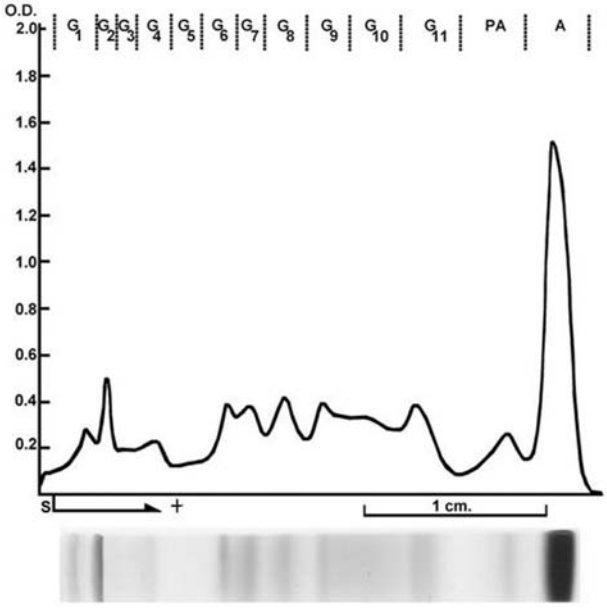

Fig. 4. Gel photograph showing the electrophoretic separation of the blood protein sample obtained from *A. c. urmiana*, together with its densitometric tracing curve. OD: Optical density, S: Start (junction between the stacking and separation gels).

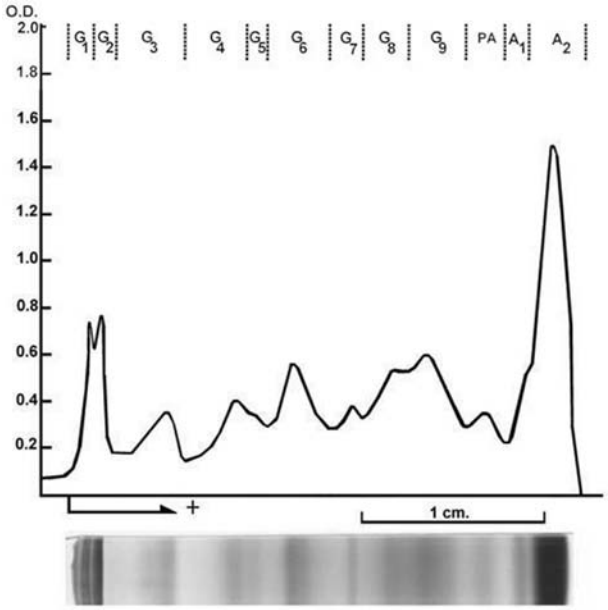

Fig. 5. Gel photograph showing the electrophoretic separation of the blood protein sample obtained from *A. c. schmidtlerorum*, together with its densitometric tracing curve. OD: Optical density, S: Start (junction between the stacking and separation gels).

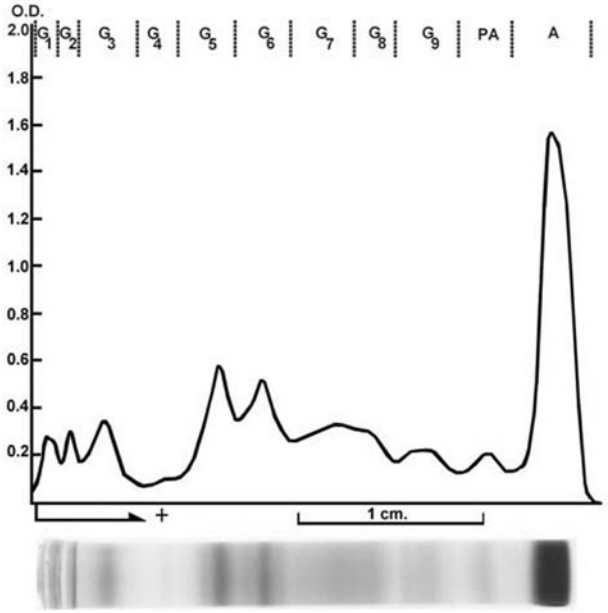

Fig. 6. Gel photograph showing the electrophoretic separation of the blood protein sample obtained from *A. c. muhtari*, together with its densitometric tracing curve. OD: Optical density, S: Start (junction between the stacking and separation gels).

There is no information concerning its serological characterization on known *A. cappadocica* subspecies. The results obtained in the blood-serum electrophoretic separation indicate significant differences among known subspecies of *A. cappadocica* in terms of the number of fractions, and the mobility and density of blood-serum fractions. While *A. c. cappadocica*, *A. c. wolteri* and *A. c. urmiana* show significant differences quantitatively, there is a considerable difference between *A. c. schmidtlerorum* and *A. c. muhtari* qualitatively. Finally, it should be stated that each subspecies has special electrophorenograms.

The taxonomical status of this species, which has formed the subject of the study since 1902, the date when it was first identified, up to this day, has been continuously controversial. The species which was identified as *Lacerta cappadocica* by Werner in 1902 was included in a new genus under the name of *Apathya,* taking these features into consideration: subdigital lamellae being obviously crytopodion, being black in color and having a semi-opal palpebral aperture that is generally composed of 6-8 scales in the lower eyelid; the existence of tiny postnasal plates in the lower part of the nostrils; the first supraocular being multi partial; the existence of many small scales that form a transversal line on the posterior of anal; the occipital being wider than interparietal; and the existence of pterygoid teeth (Mehely, 1907). Mehely's *Apathya* genus was mentioned as the synonym of *Latastia* (Bedriaga, 1884) by Boulenger (1907). Later, this taxon was accepted in the world of science in the following century (Mertens, 1924). The taxon, which was evaluated under the species of *Apathya* until the mid 20th century, was included again in the species of *Lacerta* in the following decades (Mertens, 1952; Clark and Clark, 1973; Böhme, 1971). One genus which is evaluated in the Lacertidae family, but whose taxonomical condition has not been clearly highlighted, is *Apathya* (Mayer and Arribas, 2001). In the study which observed the morphological and molecular evaluation of 19 taxa in total, which are included in the *Lacertini* group in Palearctic – Oriental area; *cappadocica* form was regarded in *Apathya* (Arnold et al., 2007). In the study, as a result of both the evaluation of 64 different morphological characters and the observation of the mitochondrial DNA of taxa that formed the subject of the research with molecular techniques, the result was that *Apathya* has a close relationship with *Hellonolacerta*. In another study carried out recently (Pavlicev and Mayer, 2009), the result was contrary to the results obtained by Arnold *et al.* (2007); a clear interpretation cannot be achieved regarding the taxonomical status of *A. cappadocica* and close relative forms of it (e.g., *Timon lepidus* and *Lacerta agilis*). As can be understood from the explanations given above, more studies need to be carried out at the molecular level together with morphological data in order to clarify the current taxonomical situation and relative relationship of *Apathya* more clearly.

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