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Serological characterization and confirmation of the taxonomic status of *Montivipera albizona* (Serpentes, Viperidae) with an additional new locality record and some phylogenetical comments

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Abstract
This is the first record of the presence of *Montivipera albizona* (Nilson, Andrén & Flärdh 1990), in Kahramanmaraş province, Mediterranean Region of Turkey. Here, one young male specimen was collected and is described. The present record of *M. albizona* extends its known distribution (Kulmac Mountain Range, Sivas) some 250 km to the south-west, where the Anatolian Diagonal exhibits a bifurcation. Our data based on the electropheoretic analysis of blood-sera, indicate that the *M. xanthina* populations from the western Anatolia and *M. albizona* distributed along the Anatolian Diagonal show significant differences, qualitatively and quantitatively. Accordingly, it is concluded that *M. albizona* should not be included within the polymorphic species *M. xanthina* which lives in western Anatolia and therefore, it should be accepted as a valid species. Moreover, the actual distributions of the related taxa in Anatolia and the geographic structure of the Anatolian Diagonal were discussed.

Keywords
Montivipera albizona; *Montivipera xanthina;* Viperidae; mountain vipers; blood-serum proteins; Anatolia

Introduction

Montivipera albizona is a mountain viper endemic to Anatolia, firstly described by Nilson et al. (1990; as *Vipera albizona*) on the basis of two specimens from Kulmac Mountain Range, Sivas (Central Anatolian Region) close to the "Anatolian Diagonal". This species is parapatric with *M. xanthina* (Gray, 1849) and a group of opponents led by Schätti (Schätti et al. 1991; 1992; Schätti & Baran 1988) soon argued that *M. albizona*, *M. wagneri* (Nilson & Andrén 1984), *M. bulgardaghica* (Nilson & Andrén 1985)

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were likely conspecific, belonging to the polymorphic species *M. xanthina*. After the original description of Nilson et al. (1990), additional records were reported by different authors (Teynié 1991; Bettex 1993; Mulder 1994; 1995b). Bettex (1993) stated that it was difficult to distinguish *M. albizona* from *M. wagneri* based on color and pattern alone. However, Mulder (1994) asserted that the color and pattern of *M. albizona* is actually quite distinct from that of *M. wagneri*, comparing and revising all specimens collected by him and Bettex (1993) plus the holotype and paratype (totally 15 specimens). During an expedition in 2006 to Kahramanmaraş province in the Mediterranean Region of Turkey, we collected *M. albizona,* a species previously unknown from that geographic area. To avoid collecting by commercially interested Turkish and European snake catchers, the detailed location will not be given. Since no information is available on the presence of this mountain viper in the Mediterranean Region of Turkey and the absence of a useful blood serum electrophoretic pattern comparison with closely related species *M. xanthina*, a recent record is briefly presented here. In this context, the main aim of this paper is to clarify and confirm the status of *M. albizona*: a valid species.

Material and methods

A young male *M. albizona* was collected from Balik Mountain Range, Kahramanmaras Province, Mediterranean Region of Turkey, where the "Anatolian Diagonal" (Figure 1) shows a bifurcation, on 2 July 2006 by B. GÖÇMEN, M. Z. YILDIZ and E. A. YAGMUR. The specimen was placed in a terrarium alive, but after obtaining a blood sample for electrophoretic analysis, it died. The specimen was then preserved and deposited in the collection of the ZDEU Museum, Bornova-Izmir (ZDEU 188/2006). For the comparison of the blood serum protein patterns, additional blood samples

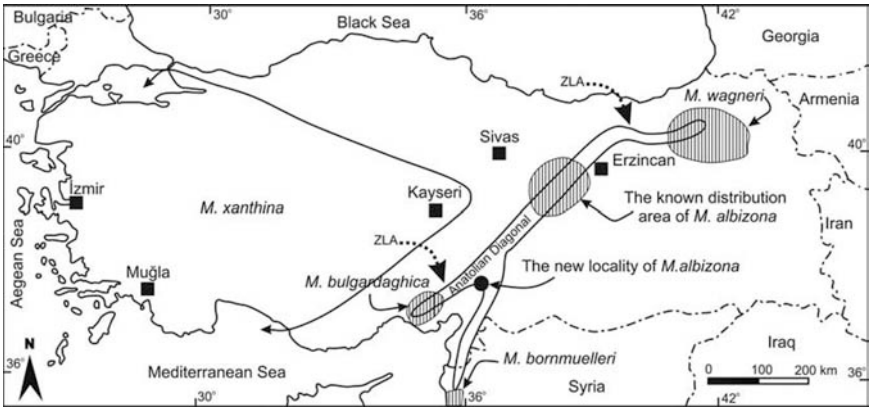

Figure 1. Map showing the Anatolian Diagonal extends from the northeast to Mediterranean region and the known distributions of the four mountain viper species and *Montivipera xanthina* in Turkey. Solid circle indicates the new locality record of *M. albizona*. ZLA: Zone of lower altitude.

were obtained from three *M. xanthina* males collected from different locations of its known distribution area (the provinces of Izmir, Mugla and Kayseri). All blood-serum protein study specimens were of similar length, i.e. they were of similar ages and all were collected in the same month (July).

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 the sera 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 Atatür (1979). Electrophoretic separations were carried out at room temperature (20-25°C) with a Canalco Model 1200 electrophoresis apparatus. Separation gels were firstly stained with 0.5% Amido Black, and then de-stained passively with repeated 7% acetic acid baths. Gels were qualitatively evaluated directly from the electropherograms, and densitometric tracing curves of the separations were obtained using a Gelman ACD-15 Model 39430 densitometer scanning at 500 nm. Since no variation amongst all *M. xanthina* samples in the electropherograms were found, their data were pooled.

The colour and pattern characteristics of the specimen were recorded while it was still alive; colour photos were also taken. The terminology used in describing the specimen conforms to Nilson & Andrén (1986) and Nilson et al. (1990). The characters of all known specimens of the species were taken from the descriptions in related papers (Nilson et al. 1990; Bettex 1993; Mulder 1994). Morphometric measurements were taken using a dial caliper with an accuracy of 0.01 mm.

Results

The specimen was found at an elevation of approx. 1300 m, on moist ground with sparsely distributed various grasses under a partly sun-exposed red pine (*Pinus brutia*) forest. It was day time, around 16.00 hr; air temperature was 20°C.

The specimen is a young male. Snout-vent length 199 mm, tail length 22 mm (total length 221 mm), tail length being equal to 9.9% of total length. Length of head (from posterior border of lower chin to tip of snout) 14.10 mm, breadth of head (at position of eyes) 5.42 mm, width of head 9.84 mm, size of eye horizontally 2.58 mm and vertically 2.30 mm, distance between eye and border of mouth 1.73 mm. On top of head, except for two enlarged supraoculars small scales are present. Supraoculars are separated by a row of nine interocular scales. 12 intercanthals and 44 intersupraocular scales on upper surface of head. One canthal between supraocular and supranasal on each side and two apicals in contact with rostral. Supraoculars are not raised and in broad contact with eye. The lower half of the nasal is fused with the prenasal on each side of the head. There are two subocular rows. First (inner) and second distal (outer) circumocular rings contain 11 and 14 scales on both sides of the head, respectively. There are nine supralabials and 12 sublabials on each side of the head. Upper preocular is enlarged and separated from nasal by a loreal scale on both

sides of the head. There are 12 sublabials on each side. First pair of sublabials is in contact with posterior of the mental. Chin shields twice as long as broad and bordered behind by four smaller plates. Dorsal scales except only 7 scales of lowermost row close to cloaca are keeled on body and tail. Two preventrals followed by 155 ventrals and a single anal plate. Subcaudals are 28 and 28+1 on the right and left side of the tail, respectively. There are 23 transverse scale rows on body at one head length posterior of the head; 23 on mid-body, 17 at one head length anterior to the anal plate.

Dorsal pattern consist of brick-red brown slightly irregular zonal blotches two to four scales wide which especially at anterior and posterior ends are surrounded by a blackish oblique narrow border (Figure 2). There are 34/30 transverse and distinct white bands separated by brick-red brown zonal blotches. Number of dorsal blotches in pattern estimated to about 40 on body. Lateral ground colour is gray, except the two head length posterior of the head, dark (black with a dash of brick-red colour) transverse blotches run along the body. There are dark lateral spots of two head length posterior of the head on the flanks. Neck pattern consist of two blackish oblique blotches separated from each other and from the dorsal pattern. Two small black (with a dash of brick-red colour) oblique blotches on the head anterior to blackish oblique blotches are present. A broad blackish band extends from the posterior border of the eye to the corner of the mouth. There is one small dark dot on supralabials below the eye, and two on sublabials. In addition to these, a dark spot above the supralabials

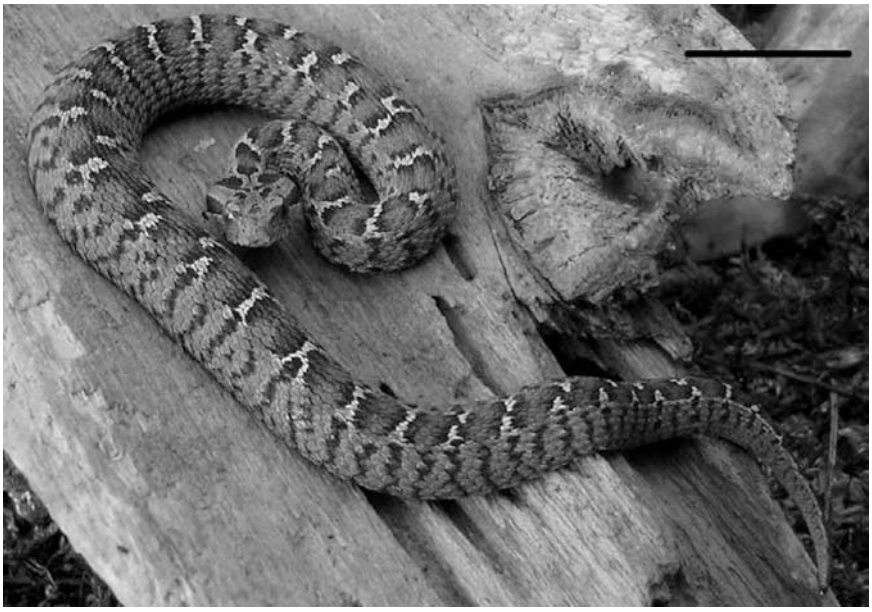

Figure 2. General view of the young male specimen of *Montivipera albizona* collected from Kahramanmaras province, Mediterranean Region of Turkey (bar = 19 mm).

present on each side of the head. Belly and throat grayish white with weak numerous small blotches, dots and less distinct small spots laterally.

Since no information concerning its serological characterization is available on this rare mountain viper, we also compared the electophoretic pattern of its blood-serum protein with the electrophoretic patterns of the similarly sized (similar age) and same sex *M. xanthina* specimens which were collected in the same month from different Anatolian localities. The electrophoretic patterns of the examined blood-serum proteins from Izmir, Mugla and Kayseri (the closest locality to *M. albizona* amongst all mountain viper species in Anatolia, Figure 3) specimens (n = 3) of *M. xanthina* were found to be quite similar. Therefore, they were evaluated together.

The gel electropherograms of blood-serum proteins of a specimen from each species, together with their densitometric tracing curves, are given in Figures 6 and 7 for *M. xanthina* and *M. albizona*, respectively. In three young males of *M. xanthina* collected from different localities of its known distribution area there was only one albumin fraction and according to its densitometric tracing curve it was possible to differentiate nine fraction or fraction groups within the globulin zones (Figure 4). In the specimen of young male *M. albizona*, a single albumin band, but with a higher optical density than *M. xanthina* was also seen (Figure 5). However, densitometrically, eleven fractions or fraction groups were observed in the corresponding globulin zones in *M. albizona*.

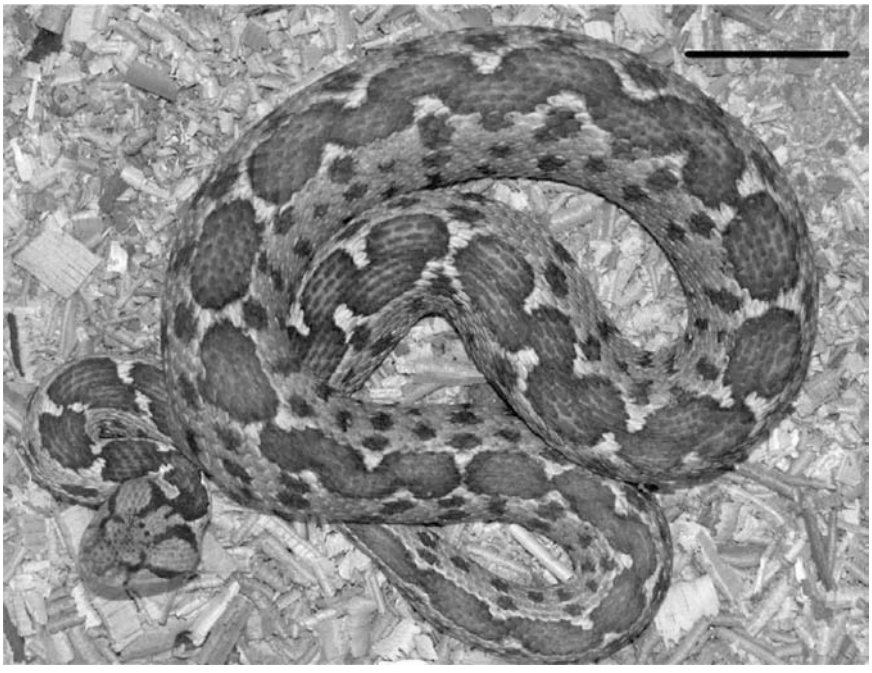

Figure 3. Dorsal view of a young male specimen of *Montivipera xanthina* collected from Erciyes Mountain, Kayseri Province the closest locality to *M. albizona* (bar = 11 mm).

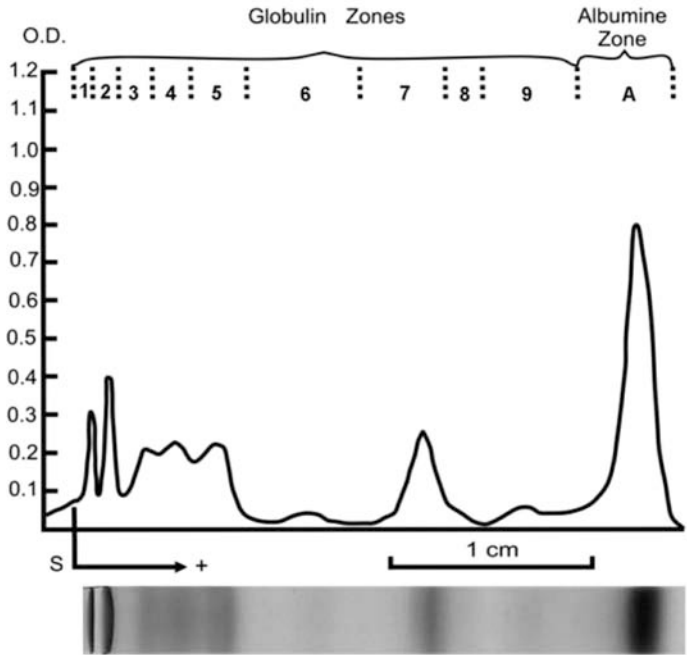

Figure 4. Representative electropherogram and its densitometric tracing curve of a young male of *Montivipera xanthina*. A: Albumin; 1 to 9: Globulins; O.D.: Optical density, S: Start, junction between the stacking and separation gels.

Discusion

The *Vipera xanthina* complex can be defined as an assemblage taxa formerly included in *V. xanthina* sensu lato, i.e. *V. xanthina* (Gray 1849), *V. raddei* Boettger 1890 with its all known subspecies, *V. bornmuelleri* Werner 1898, *V. latifi* Mertens Darevsky & Klemmer 1967, *V. wagneri* Nilson & Andrén 1984, *V. albicornuta* Nilson & Andrén 1985, *V. bulgardagica* Nilson & Andrén 1985 and *V. albizona* Nilson, Andrén & Flärdh 1990. These mountain vipers of the genus *Vipera* has been subjected to a revision (except *V. albizona*) by Nilson & Andrén (1986), and Nilson et al. (1999). They described a new subgenus, *Montivipera*, which differs morphologically, genetically, serologically and ecologically from the other related taxa. Finally, Joger (2005) has changed its taxonomic status to generic level. Of these mountain vipers belonging to the genus *Montivipera*, four allopatric species inhabit in only Anatolia (Fig. 1).

M. albizona has been described from a young female (holotype) and an adult male (paratype) from Kulmac Mountain Range (Vilayet Sivas) related with Anatolian Diagonal, central Turkey (Nilson & Andrén 1990). After the original description of the species, Bettex (1993), Schätti & Baran (1988) and Schätti et al. (1992) considered the three species (*M. wagneri, M. bulgardaghica* and *M. albizona*) described by Nilson et al. (1984, 1985, 1990) to be conspecific and belonging to the polymorphic species *M. xanthina*. Later, Mulder (1994; 1995b) has conducted a revisionist study with some

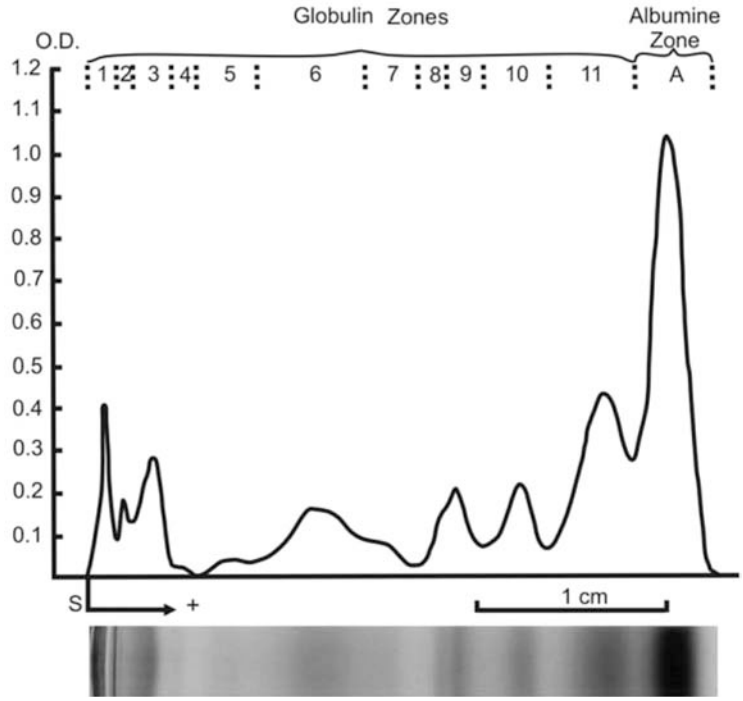

Figure 5. The electropherogram and its densitometric tracing curve of the young male of *Montivipera albizona* collected from Kahramanmaras Province. A: Albumin; 1 to 11: Globulins; O.D.: Optical density; S: Start, junction between the stacking and separation gels.

additional new material of *M. albizona* collected from its known distribution area (Sivas and Erzincan provinces). His aim was to reach a better insight in the intraspecific variation of these mountain vipers, irrespective of the status of *M. albizona*: as a valid species (sensu Nilson et al. 1990) or included within the polymorphic species *M. xanthina* (sensu Schätti et al. 1992). However, Mulder (1994) has detected some additional diagnostic criteria concerning the colour-pattern (the transverse wideness of the orange-brown dorsal blotches and the coloration of head and body).

The new, Kahramanmaras specimen agrees almost completely in pholidosis and colour-pattern, except for the lowermost row of keeled (instead unkeeled) dorsal scales on body and tail, with all the descriptions given by Nilson et al. (1990) and Mulder (1994). Distinction between *M. albizona* (Fig 2) and *M. xanthina* (Fig. 3) based on the dorsal colour-pattern is very easy since we observed and compared the specimens of both species in that time during our trip. This distinction was also suggested by Mulder (1994). The total length of our specimen collected from Kahramanmaraş is the smallest size (221 mm) amongst the given values up to date by different authors (Nilson et al. 1990; Bettex 1993; Mulder 1994). Although Bettex (1993) shows a hatchling which has the head and body pattern connected, this is not valid for our specimen. It supports Mulder's (1994, 1995a) idea that the dorsal colour-pattern would not be regular in captive-bred vipers. The other remarkable difference is on the relative tail

length. According to Mulder (1994) this value varies around 8% (7.5-8.8%) in *M. albizona*, while mean values in *M. xanthina* are above 9% (9.2-9.8%), depending on sex and population. In our specimen, the relative tail length was found as 9.9% of total length. This deviation may depend on age, since it is the smallest of all available *M. albizona* specimens.

Blood-serum electrophoresis has been utilized to obtain data which can be added to the available data sets and lead to more stable classifications in herpetology by many researchers (Ferguson 1980; Joger 1986; Herrmann et al. 1992; Arikan et al. 1988). The qualitative differences of fractions could be caused by genetic variations (infections, parasites or various bodily mutilations may also cause qualitative differences in blood globulins, but no such factors were evident in our specimens), and the quantitative differences could reflect age, gender, environmental and physiological factors (Ferguson 1980; Arikan et al. 1988); therefore, qualitative differences are important for taxonomic investigations. Our data indicate that *M. xanthina* populations from the western Anatolia and *M. albizona* distributing along the Anatolian Diagonal show significant difference regarding the blood-serum electrophoretic patterns qualitatively and quantitatively as indicated in Figs. 6 and 7. So, *M. albizona* should not be included within the polymorphic species *M. xanthina* which lives in the western Anatolia, as previously suggested by Bettex (1993), Schätti & Baran (1988) and Schätti et al. (1992). Therefore, it is a valid species.

Our specimen is from a locality out of the human settlement, in a mountain forest, so that a recent, anthropochore transportation from the previous known distribution is highly unlikely. It is more likely that it hatched at the site where it was found, forming part of a reproductive population of *M. albizona* on Balik Mountain, Kahramanmaras (Mediterranean Region of Turkey). Our record of *M. albizona* extends from its known distribution (Kulmac and Tecer Mountain Ranges, Sivas & Mercan Mountain, Erzincan) some 250 km to the south-west, where the Anatolian Diagonal exhibits a bifurcation.

Anatolia is located at an important transitional zoogeographical region between Asia and Europe. In eastern parts, there are no natural boundaries with the neighboring countries and therefore, little endemism is seen (Göçmen et al. 2002). However, according to Davis (1971), Nilson et al. (1990) and Sindaco et al. (2000), the Anatolian Diagonal forms an important barrier for both faunal and floral species. Therefore, the composition of fauna and flora on the two sides of the Anatolian Diagonal is different. This barrier is a mountain range that extends from Erzurum province in the northeast towards Kahramanmaraş in the southwest (Fig. 1). From there it bifurcates and one branch continues further southwest towards Bolkar Mountain. The other branch runs to southeast toward the direction of Lebanon Mountains. The elevation of the mountain range on average changes between 3000-4000 m. The Kızılırmak and Yeşilırmak (rivers) break through the mountain barrier in the zones of lower altitude where summits are at 650 m, dividing the eastern sections. A similar zone of lower altitude is present along the Seyhan river between Bolkar (Adana Province) and Balık mountains (Kahramanmaraş Province). Herrmann et al. (1987) have proposed a time scale based on immunological distance between *M. wagneri* and *M. bornmuelleri*. They suggest a

separation between these two taxa in early Pliocene, less than 5 million years ago. Based on this assumption, we can say that the vicariance events along the Anatolian Diagonal took place in early Pliocene. Furthermore, based on their present distributions, we can propose that *M. albizona* was mainly restricted along the Diagonal between zones of lower altitude, while *M. bulgardaghica* and *M. wagneri* were isolated in the tips of the Diagonal. This isolation would lead to the speciation of these closely related mountain vipers.

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