Turk J Zool
30 (2006) 225-234

Morphological, Hemipenial and Venom Electrophoresis Comparisons of the Levantine Viper, *Macrovipera lebetina* (Linnaeus, 1758), from Cyprus and Southern Anatolia

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Received: 30.11.2005

Abstract: Levantine vipers, *Macrovipera lebetina* (Linnaeus), from Cyprus and southern Anatolia were investigated comparatively regarding morphology, hemipenes and electrophoresis patterns of venom proteins. There were discernible differences between these compared features of the 2 populations. In the light of these differences, it is concluded that the southern Anatolian population should not be identified as the nominate subspecies *M. l. lebetina* (Linnaeus), which lives in Cyprus. The study material from northeastern Anatolia did not allow us to make a good comparison between *M. lebetina* from southern and NE Anatolia.

Key Words: *Macrovipera lebetina*, hemipenes, venom electrophoresis, Cyprus, southern Anatolia

Kıbrıs ve Güney Anadolu'da Dağılış Gösteren Koca Engerek, *Macrovipera lebetina* (Linnaeus, 1758)'nın Morfoloji, Hemipenis ve Venom Elektroforezi Karşılaştırılması

Özet: Kıbrıs ve Güney Anadolu'da yaşayan Koca Engerek yılanları, *Macrovipera lebetina* morfoloji, hemipenis ve venom proteinlerinin elektroforetik modelleri açılarından karşılaştırmalı olarak araştırılmıştır. Karşılaştırılan karakterler açısından her iki populasyon arasında dikkate değer farklılıklar saptanmıştır. Bu farklılıklar ışığında, türe ait Güney Anadolu populasyonunun Kıbrıs'ta yaşayan nominat alttür, *M. l. lebetina* (Linnaeus) olarak ele alınamayacağı sonucuna varılmıştır. Kuzeydoğu Anadolu'dan çalışma materyalinin yetersiz sayıda oluşu, Güney ve Kuzeydoğu Anadolu populasyonları arasında uygun bir karşılaştırmalı çalışma yapmamıza olanak vermemiştir.

Anahtar Sözcükler: *Macrovipera lebetina*, hemipenis, venom elektroforezi, Kıbrıs, Güney Anadolu

Introduction

The Levantine viper, *Macrovipera lebetina*, has a rather extensive geographical range in Central Asia, the Middle East and northern Africa (Nilson and Andren, 1988; Nilson et al., 1988; David and Ineich, 1999; David et al., 1999; Tok et al., 2002).

Hermann et al. (1992) examined the phylogenetic relationships of Palaearctic vipers by means of immunological comparisons, and found that the *lebetina* group consists of 4 species, namely *lebetina* (Linnaeus, 1758) of Asia and northern Africa, *schweizeri* (Werner, 1935) of Europe, and *mauritanica* (Gray, 1849) and *deserti* (Anderson, 1892) of North Africa. Furthermore, in this study the name *Macrovipera* Reuss, 1927 was resurrected to accommodate species of the *Vipera lebetina* group and then the name *Vipera* Schwarz, 1936 was accepted as a synonym. Various herpetologists later accepted this nomenclature (Rage and Schätti, 1993; David et al., 1999; Sindaco et al., 2000; Tok et al., 2002).

There has been considerable confusion and disagreement among authors with regard to the taxonomic status of *M. lebetina*. After the initial species description of *M. lebetina* by Linnaeus, several subspecies were described, some of which are still valid (*M. l. lebetina*, *M. l. obtusa*, *M. l. transmediterranea*, *M. l.*

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schweizeri, *M. l. turanica* and *M. l. chernovi*) while others are currently invalid (*euphratica, peilei*, etc.). The subspecies *lebetina* seems to be endemic to Cyprus. Nilson and Andrén (1988) referred to the North African populations as *M. l. transmediterranea* and also raised to specific status the Milos viper, *M. l. schweizeri* (Werner, 1935). Asiatic and Russian *lebetina* were attributed to the subspecies *obtusa* for a long time by several authors (Chernov, 1944, 1959; Terentjev and Chernov, 1949; Bannikov et al., 1977; Basoglu and Baran, 1980; Joger, 1984; Bruno, 1985; Nilson and Andrén, 1988; Chickin and Szczerbak, 1992; Leviton et al., 1992; Rage and Schätti, 1993; Baran and Atatür, 1998; David and Ineich, 1999; Tok et al., 2002), but there was no general consensus about its definition. The populations from the easternmost parts of the range of *M. lebetina*, previously regarded as belonging to the subspecies *turanica* (Chernov in Terentjev and Chernov, 1949), were described as *chernovi* by Chikin and Szczerbak (1992). Different authors argued over the validity of the other subspecies *turanica, euphratica, peilei* and *chernovi* from Asia, and, for example, Joger (1984) considered only 2 valid subspecies: *lebetina* of Cyprus and *obtusa* of Asia (except Cyprus).

Adding to the confusion, Werner (1935) described the Anatolian specimens as *V. l. mauritanica*, and Bird (1936) as *V. l. xanthina*. However, Mertens (1952) included them in the subspecies *obtusa*, which was subsequently accepted by Eiselt and Baran (1970), Baran (1976), and the other authors mentioned above. However, Billing and Schätti (1984), Broodmann (1987) and Mulder (1995) stated that the specimens from the southern parts of Anatolia closely resemble those of Cyprus (*M. l. lebetina*) regarding head shape, color pattern, and number of ventrals. Thus, they considered the southern population of Anatolia as *M. l. lebetina*.

In this study, we examined the morphology and biometry, the venom electrophoresis patterns and the hemipenial features of several Cypriot and Turkish specimens of *M. lebetina* by using vouchers in the ZDEU Museum (Zoology Department, Ege University, Bornova, İzmir) and freshly collected specimens. Then we attempted to clarify the subspecific status of the southern Anatolian populations of this taxonomically problematic species.

Material and Methods

Study area

Figure 1 shows the localities of the specimens used for this study, including the specimens deposited in ZDEU and in the private collection of Bayram Göçmen (PCBG) and freshly collected specimens. We examined 7 ZDEU vouchers (3 from Cyprus and 4 from Turkey), and 1 PCBG voucher from Cyprus, and used 4 additional unregistered specimens for venom electrophoresis analysis (a male-female pair from Cyprus and a male-female pair from Turkey). All 4 venom study specimens were of similar length, i.e. they were of similar ages and all were collected in September. Catalogue reference numbers of the museum specimens are as follows:

Cypriot specimens ZDEU 123/1992 (♂), Koruçam-Kyrenia, leg. B. Göçmen & M. Kofali; ZDEU 38/1995 (♂), Geçitköy-Kyrenia, leg. B. Göçmen; ZDEU 187/1994 (♂, semi-adult), Famagusta, Leg. B. Göçmen & M. Kofali; PCBG 01/2004 (♂), Dikmen-Kyrenia, leg. B. Göçmen. In terrarium an additional live adult male specimen is kept from Dikmen (Nicosia).

Turkish specimens from Southern Anatolia ZDEU 102/1957 (♀), Hüseyni-Siirt, leg. M. Başoğlu; ZDEU 84/1969 (♂), Birecik-Şanlıurfa, leg. I. Baran; ZDEU 261/1957 (♂), Düziçi-Adana, leg. M. Başoğlu; ZDEU 12/2001 (♂, semi-adult), Nusaybin-Mardin, leg. D. Cihan. In terrarium an additional live adult male specimen is kept from Ceylanpınar (Şanlıurfa).

Biometry

Morphological parameters used in most snake taxonomy studies were taken into consideration (Eiselt and Baran, 1970; Baran, 1976; Billing and Schätti, 1984; Nilson and Andrén, 1988, 2001; Nilson et al., 1988; Böhme and Wiedl, 1994). For each specimen we recorded total length (TL), snout-vent length (SVL), tail length (TaL), head width (HW), head length (HL), distance between nostrils (DBN) in mm; and the number of ventrals (VS, excluding anal), number of subcaudals (SCS), number of dorsal scale rows at mid-body (DS), number of supralabials (SpL), number of sublabials (SbL), number of canthals (C) and apicals (A), number of supraoculars (SpO), number of scales between supraocular rows (SSR), number of scales arranged in a ring around the eyes (circumoculars, CO, including

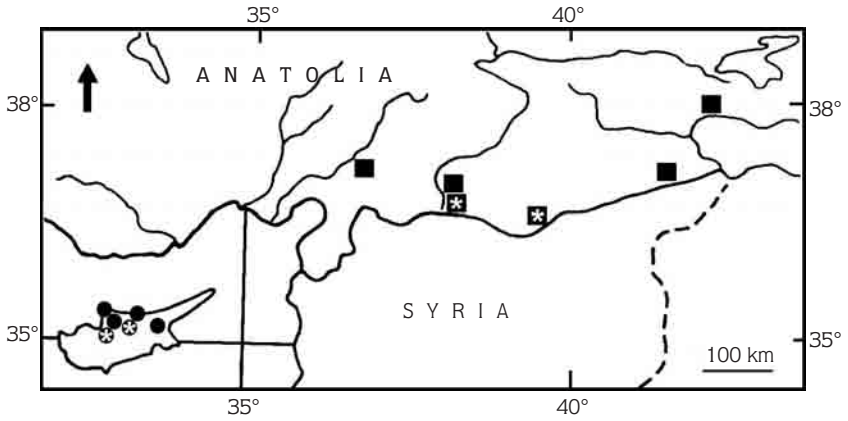

Figure 1. Map showing the examined *Macrovipera lebetina* specimens' localities. Black circles and squares indicate the Cypriot and Turkish localities of the examined specimens, respectively. Circles and squares with asterisks show the localities of the unregistered specimens used for venom electrophoresis.

supraoculars), and number of scales arranged in a ring around the nostrils (circumnasals, CN). Additional data on morphology and biometry, e.g., color-pattern, head shape [the ratio of the head width to the head length (HL/HW ratio) and the ratio of the head width to the distance between nostrils (HW/DBN ratio)] were taken.

Hemipenial morphology

For hemipenial comparisons, 2 male specimens from Cyprus (ZDEU 123/1992, ZDEU 38/1995) and 2 males from the southern Turkey (ZDEU 261/1957, ZDEU 84/1969) were used. The terminology used in the description of hemipenial morphology follows Dowling and Savage (1960), Nilson and Andrén (1988), Joger et al. (1997) and Rasmussen (1997). Pesantes' (1994) method, including the softening of hemipenes in a KOH solution (of 1%), was used to extrude the male organs completely, which had been fixed with a formaldehyde-alcohol solution for some time. Hemipenis length was measured as the corresponding number of subcaudals (CNSC) (Rasmussen, 1997). Furthermore, we recorded the lobe length (LL), lobe width (LW), basis length (BL), basis width (BW), total hemipenis length (THL), calyculate area length of the sulcate side (CALSS), distance from the bifurcation of the sperm groove to the lobe apex (BSG-LA) (accuracy ± 0.01 mm), and the total number of basal spines (TNBS) for each hemipenis.

Electrophoresis and densitometry

Two adult males from Şanlıurfa (Ceylanpinar and Birecik) and 2 adult males from Cyprus (Geçitköy and Dikmen, Kyrenia) were used. Live specimens were taken to the laboratory and their venoms were extracted without applying any pressure on their venom glands, as described by Tare et al. (1986). Due to the fact that the venom extracts contained some dead cells, they were centrifuged for 5 min at 600 x g and stored at –20 °C until electrophoretic separation. For electrophoretic study, 5 µl of venom extract was used for each separation. The venom proteins were separated according to Özeti and Atatür (1979), who applied polyacrylamide gel disk electrophoresis with some modifications to the method described by Davis (1964). Electrophoretic separations were performed at room temperature (approx. 20-25 °C) using a Canalco Model 1200 disk electrophoresis apparatus. Gels containing separated proteins were stained with 0.5% Amido Black (Naphthol Blue Black 10-B) and excess stain was removed passively in 7% acetic acid baths. Then photographs of the gels were taken. Qualitative evaluations of the gels were done directly from the electropherograms and the densitometric curves of the separations were created by means of a Gelman ACD-15 Model 39430 densitometer scanning at 500 nm.

Results

Morphological data (biometry, pattern and pholidosis) obtained from 4 specimens collected from Cyprus and 4 specimens collected from southern Turkey (Adana, Şanlıurfa, Mardin, Siirt) are summarized in Table 1. Generally, the data on morphology (e.g., head size and scalation, body size) fall within the ranges of variability in the literature (Eiselt and Baran, 1970; Baran, 1976; Billing and Schätti, 1984; Osenegg, 1989; Schätti and Sigg, 1989; Böhme and Wiedl, 1994; Göçmen et al., 1996; Joger et al., 1997; Nilson et al., 1988; Nilson and Andrén, 1988, 2001; Atatür and Göçmen, 2001; Tok et al., 2002). However, the Cypriot and southern Anatolian specimens were distinctly different in terms of SpO, CO and VS. According to most authors (Mertens, 1952; Eiselt and Baran, 1970; Baran, 1976; Nilson and Andrén, 1988, 2001; Nilson et al., 1988; Joger et al., 1997; Atatür and Göçmen, 2001; Tok et al., 2002) the nominate subspecies (restricted to Cyprus) is distinguished from *M. l. obtusa* (which lives in Anatolia and in Trans Caucasus), by a lower number of ventral plates and a smaller body size (however, in our material a male specimen from Koruçam, Cyprus is larger than our Anatolian specimens).

We also found that SpO and CO numbers are lower for the Cypriote specimens than for those of southern Turkey. According to Billing and Schätti (1984) and Osenegg (1989), however, considerable differences occur within the Anatolian populations. These authors (*l.c.*) stated that the specimens from northeastern Turkey have a longitudinally narrow head and a less distinctly marked pattern, but the specimens from southern Turkey and Iraq have a heart-shaped pointed head and often a well-marked pattern as in those from Cyprus. Thus, Billing and Schätti (1984) allocated the southern Turkish and Iraqi populations to *M. l. lebetina*. Our specimens from northeastern Anatolia (which have been referred to in the literature as the subspecies *obtusa*) showed the typical

Table 1. Morphological data obtained from the 2 populations of *Macrovipera lebetina* [*Right and left side, sa: semi-adult; abbreviation explanations are given in Materials and Methods].

	Cyprus Specimens				Southern Turkey Specimens			
Catalogue number	ZDEU 38/1995	ZDEU 123/1992	ZDEU 187/1994	PCBG 01/2004	ZDEU 102/1957	ZDEU 84/1969	ZDEU 261/1957	ZDEU 12/2001
Gender	♂	♂	♂sa	♂	♀	♂	♂	♂sa
Locality	Geçitköy Kyrenia	Koruçam Kyrenia	Dörtyol Famagusta	Dikmen Nicosia	Hüseyni Siirt	Birecik Şanlıurfa	Düziçi Adana	Nüsaybin Mardin
TL	877	969	216	577	883	945	966	285
SVL	770	889	186	501	778	835	846	247
TaL	107	80	30	76	105	119	120	38
HL	47.2	45.9	16.1	31.4	42.1	35.4	41.4	19.7
HW	36.5	31.2	9.1	19.6	30.1	27.6	25	11.6
DBN	8.3	8.4	3	4.8	8.2	8.4	8.4	3.2
HL/HW	1.29	1.47	1.76	1.6	1.39	1.28	1.65	1.69
HW/DBN	4.39	3.71	3.03	4.08	3.67	3.28	2.97	3.62
SpO*	4/4	3/3	4/4	4/3	6/6	5/5	5/5	5/5
SSR	9	9	9	9	9	9	9	10
SpL*	10/11	10/10	10/10	10/10	11/10	10/10	11/10	10/9
SbL*	14/14	13/13	14/14	13/13	13/13	13/13	14/14	13/13
CO*	17/17	13/13	13/14	16/14	16/16	17/16	17/17	18/18
CN*	5/5	5/6	4/5	4/5	5/4	5/5	5/5	5/5
C*	3/3	3/3	3/3	3/3	3/2	3/3	3/3	3/3
A	2	2	2	3	2	3	2	2
VS	155	151	154	146	161	170	164	171
SCS	43	--	43	45	42	44	45	43
DS	25	25	25	25	26	25	25	25

HW/DBN values noted in many previous studies (Baran, 1976; Billing and Schätti, 1984; Osenegg, 1989, etc): the values were 2.44-3.19 for the specimens from NE Anatolia. We also obtained considerably higher values for the specimens from Cyprus (3.03-4.39) and southern Anatolia (2.97-3.67) as indicated by Billing and Schätti (1984). However, as shown in Figure 2, this characteristic shows a clinal variation, with an increasing gradient from the northeast to the southwest. Therefore, it is not a diagnostic feature of the southern Anatolian specimens to allocate them to the nominate subspecies. Moreover, our Cypriot specimens had higher values in HL/HW and HW/DBN ratios than specimens from the southern Anatolia, which means that the Cypriot specimens had a shorter and wider head.

Regarding the dorsal pattern, our data mirrored those published by Billing and Schätti (1984). Indeed, the dorsal patterns of our 2 specimens from northeastern Anatolia (ZDEU 257/1957♂, Tuzluca-Iğdır and ZDEU 159/2002♂, Kağızman-Kars) showed a less marked dorsal pattern than those from Cyprus and southern Anatolia (Figure 3).

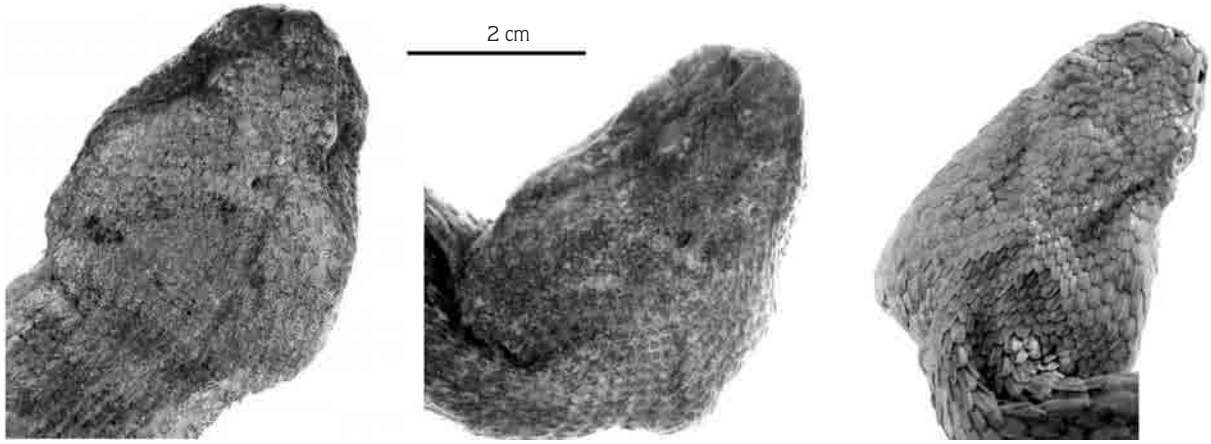

Figure 2. Dorsal aspects of the heads of 3 adult specimens collected from: Geçitköy-Kyrenia/Cyprus (a), Birecik-Şanlıurfa/southern Turkey (b) and Kağızman-Kars/ northeastern Turkey (ZDEU 159/2002 ♂) (c).

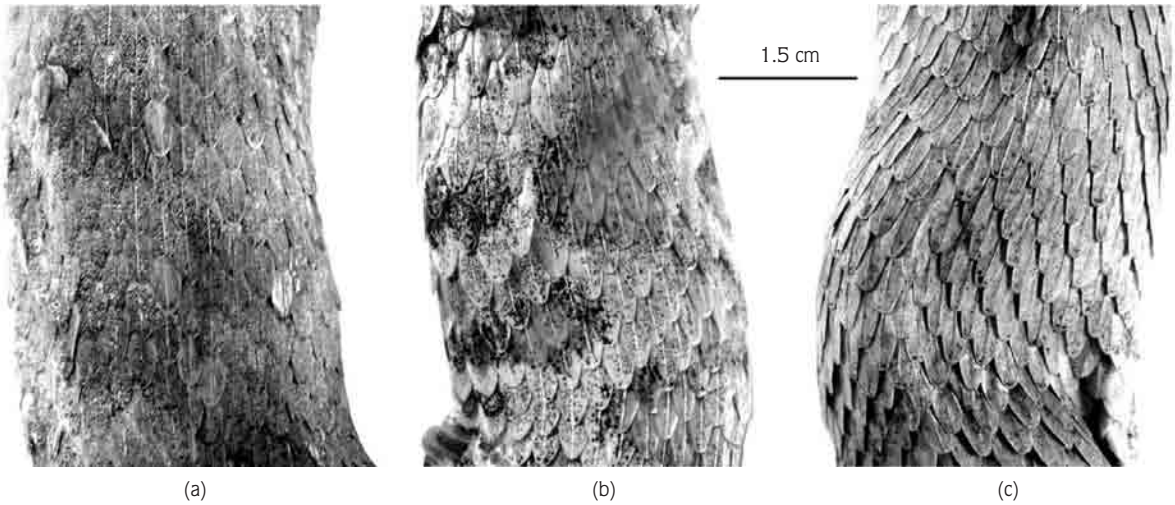

Figure 3. Dorsal aspects at mid-body of 3 adult specimens collected from: Geçitköy-Kyrenia/Cyprus (a), Birecik-Şanlıurfa/southern Turkey (b) and Kağızman-Kars/northeastern Turkey (ZDEU 159/2002 ♂) (c).

The hemipenial morphology of *M. lebetina* is described for the first time in this study. The hemipenes of *M. lebetina* were generally strong and elongate, with a deep sperm groove, and bi-lobed. The right hemipenes were longer than the left ones in all vouchers examined (Table 2). The sperm groove was bordered by well-marked sulcal lips. Hemipenes showed well separated lobes and they were relatively longer than the basal segment in the specimens from southern Anatolia (LL/BL ratio: 1.002-1.298, Table 2). However, in Cypriot specimens the value of LL/BL ratio is lower (LL/BL ratio: 0.894-0.963) than those of southern Anatolia, i.e. the Cypriot *M. lebetina* has relatively shorter lobes. Hemipenes generally possess large basal spines on the sulcate and the asulcate surface; both lobes have smaller spines that reach the sulcal lips and become more smaller towards the first third level of the lobe length; the remaining portion of the lobes was covered by a marked calyculate area up to the apex (Figure 4); the calyces were better developed on the asulcate surface, and the apex of the lobes terminated in a more or less differentiated pointed tip. The total numbers of the basal spines (TNBS) were remarkable (Table 2): the Cypriot specimens were distinctly different from the southern Anatolian specimens by a lower number of TNBS (10-14), as compared to the higher numbers (19-22) in the southern Anatolian specimens (Figure 4).

When we compared the hemipenial morphology of *M. lebetina* with that of *Vipera aspis, V. barani* and *V. nikolskii* obtained by Zuffi (2002) and Joger et al. (1997), it appeared that the hemipenes of *M. lebetina* closely resembled those of *V. aspis* (apart from *V. aspis*

Table 2. Hemipenial data obtained from 2 southern Anatolian (upper row) and 2 Cypriot (lower row) Levantine viper specimens (measurements are in mm, abbreviation explanations are given in Material and Methods).

Catalogue number	ZDEU 84/1969 Birecik- Şanlıurfa/S Turkey		ZDEU 261/1957 Düziçi- Adana/ S Turkey	
	Right Hemipenis	Left Hemipenis	Right Hemipenis	Left Hemipenis
LL	14.48-13.51	12.45-11.80	16.48-18.42	15.70-15.30
LW	4.22-4.70	4.06-3.99	3.26-3.39	2.34-3.53
BL	14.44	9.59	16.38	14.74
BW	3.68	3.75	4.53	4.3
CALSS	10.54-10.96	9.90-10.41	11.55-12.30	9.70-9.57
TNBS	20	22	21	19
BSG-LA	23.58	17.27	22.78	20.03
THL	28.92	22.04	32.86	30.44
LL/BL	1.002	1.298	1.124	1.065
CNSC	10	10	9	9
Catalogue number	ZDEU 123/1992 Koruçam-Kyrenia/Cyprus		ZDEU 38/1995 Geçitköy-Kyrenia/Cyprus	
	Right Hemipenis	Left Hemipenis	Right Hemipenis	Left Hemipenis
LL	14.06-13.96	13.79-13.10	13.98-13.94	13.49-12.96
LW	3.24-3.76	3.27-3.68	3.18-3.64	3.27-3.56
BL	15.71	14.31	15.41	14.05
BW	4.64	4.15	4.59	4.06
CALSS	10.81-10.91	9.86-9.61	10.58-10.82	9.78-9.52
TNBS	14	11	13	10
BSG-LA	19.63	18.3	19.48	18.16
THL	29.77	28.1	29.39	27.54
LL/BL	0.894	0.963	0.907	0.96
CNSC	9	9	9	9

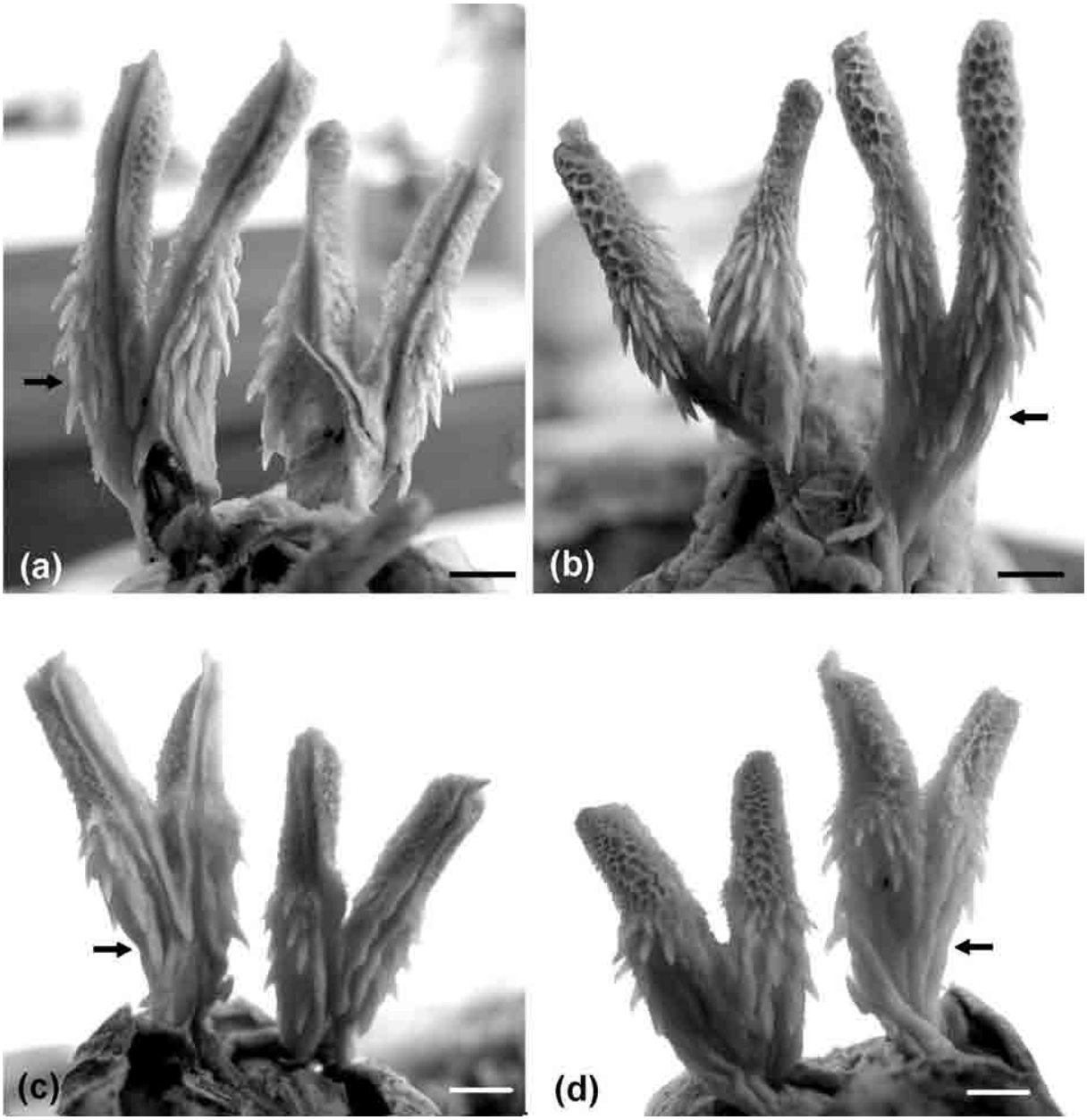

Figure 4. Hemipenial aspects from a southern Anatolian (upper row, ZDEU 84/1969, Birecik-Şanlıurfa) and a Cypriot (lower row, ZDEU 123/1992, Koruçam-Kyrenia) Levantine viper specimen (bars are 4 mm, arrows indicate the right hemipenes, a & c from sulcate side; b & d from asulcate side).

hugyi) by the markedly long calyculate area covering the distal 2/3 portion of the lobe length. On the other hand, the hemipenes of *V. barani* and *V. nikolskii* have a limited calyculate area (Joger et al., 1997). Hemipenial similarities between *V. aspis* and *M. lebetina* indicate a close relationship between the 2 species as compared to *V. barani* and *V. nikolskii*. On the other hand, the differences in hemipenial structure observed between Cypriot and Anatolian *M. lebetina* populations regarding the LL/BL ratio and the TNBS suggest a subspecific differentiation.

No discernible differences were seen in the venom electropherograms of the similarly sized (of similar age)

male and female specimens, which were collected in the same month. The electrophoretic patterns of the venom protein samples from 2 southern Anatolian and 2 Cypriot specimens showed quite a qualitative difference in both albumin-like and globulin-like regions, which suggests that Cypriot and southern Anatolian populations are clearly distinct at the subspecific level (Figure 5).

The main qualitative differences between the 2 groups from the viewpoint of venom proteins were: 1- In Cypriot samples the number of discernible globulin-like fractions or fraction groups was 10, while in samples from southern Anatolia they number 9. 2- While there was a single albumin-like fraction in the samples from Cyprus, 2 were evident in Anatolian samples; preceding the

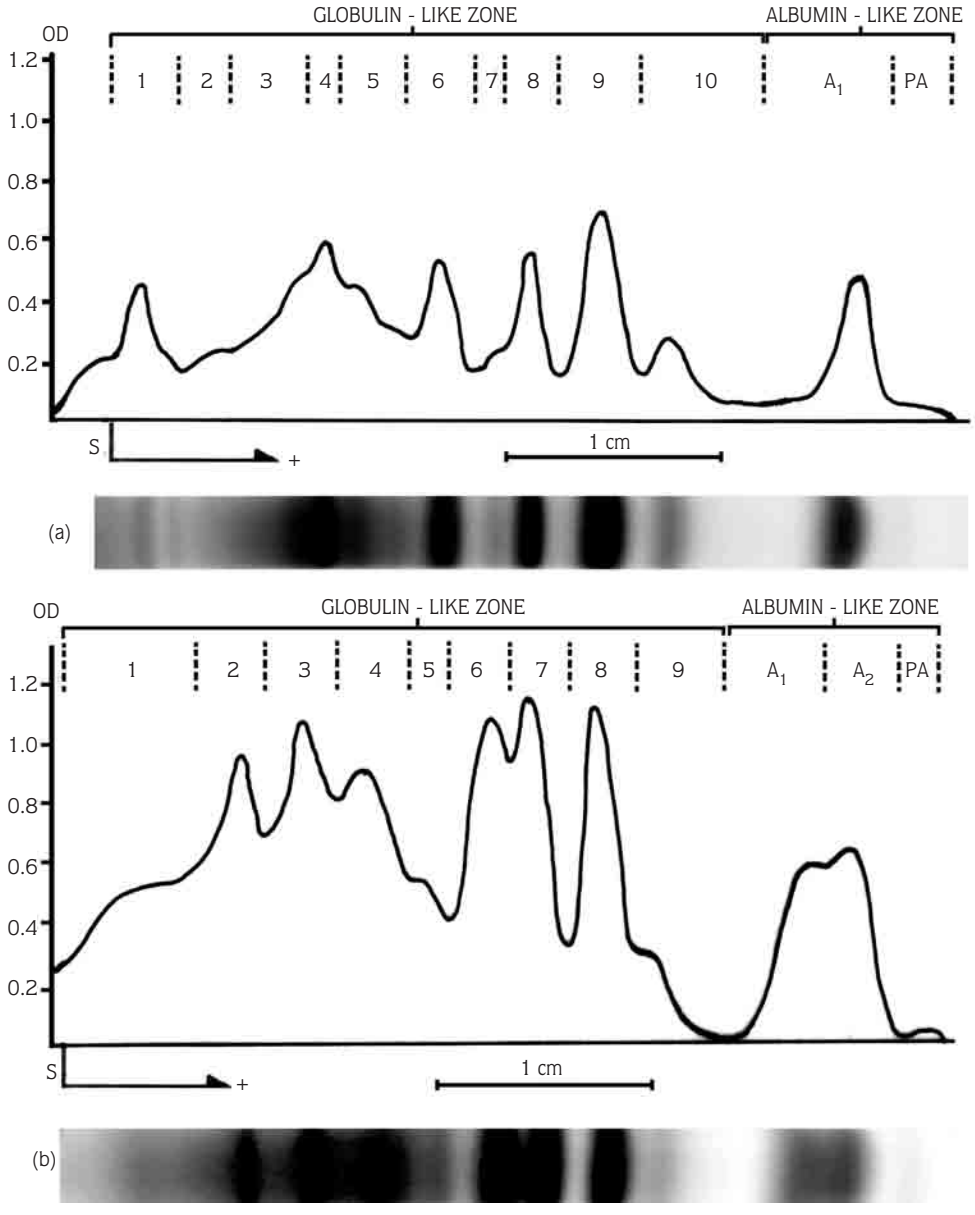

Figure 5. Electropherograms showing the venom protein samples obtained from 1 Cypriot (a) and 1 southern Anatolian (b) *Macrovipera lebetina*, together with their densitometric tracing curves (OD: Optical density; S: Start (Junction between spacer and separation gels).

albumin-like fractions, a prealbumin-like fraction was also present, but was much weaker in Anatolian samples.

Discussion

Our data indicate that *M. lebetina* populations from southern Anatolia and Cyprus show significant differences regarding some aspects of general morphology, hemipenial morphology and venom proteins. Therefore we conclude that these populations are taxonomically distinct. Consequently, the population from southern Anatolia should not be allocated to the nominate subspecies *M. l. lebetina*, which lives in Cyprus, as previously suggested by Billing and Schätti (1984), Broodmann (1987), Ossenegg (1989) and Mulder (1995).

In the relevant literature, the population of *M. lebetina* from NE Anatolia has long been referred to as *M. l. obtusa* (Mertens, 1952; Eiselt and Baran, 1970; Baran, 1976; Nilson et al., 1988; Nilson and Andrén, 1988, 2001; Joger et al., 1997; Atatür and Göçmen 2001; Tok et al., 2002). As suggested by Billing and Schätti (1984), Broodmann (1987), Ossenegg (1989) and Mulder (1995), the population existing in southern Anatolia is distinctly different at subspecies level from the population in NE Anatolia, which should be resurrected as *M. l. euphratica* Martin, 1838 from the synonym list of the species, since its type locality was given as "shores of Euphrates". In summary, our findings indicate that the southern Anatolian population might be quite distinct from the Cypriot population at a subspecific level; and while we were unable to compare (from the viewpoints of hemipenial characteristics and venom proteins) a significant number of specimens from northeastern and southern Anatolian populations, according to the relevant literature, it is possible to suggest that they also belong to distinctly different subspecies. We thus accept, provisionally, considering the limited number of our specimens for comparison, the subspecific status of the southern Anatolian population as *Macrovipera lebetina* ssp.

Acknowledgments

We thank Prof. Emeritus Mehmet Kutsay ATATÜR, Ege University, İzmir, for his advice on and review of an earlier version of the manuscript.

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