

Original Article**Morphometric Study on Male Specimens of *Hyalomma anatolicum* (Acari: Ixodidae) in West of Iran**A Hosseini ¹, *A Dalimi ¹, M Abdigoudarzi ²¹Department of Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran²Razi Vaccine and Serum Research institute, Tehran, Iran

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Abstract

Background: *Hyalomma anatolicum* is the well-known hard tick, which is one of the most important livestock and human pathogens vector, wide range in host and distributed in all over the *Hyalomma* geographic fauna as well as in Iran. Taxonomy of the *Hyalomma* spp. is debatable whereas their identification is a problematic work. The reasons for this claim is time consuming Delpy's researches in Iran also Schulze School, Feldman-Muhsam and the Russian tick workers. We would like to understand morphometric variation in the field collected *H. anatolicum* in Iran also validating some morphologic quantitative and qualitative characters.

Methods: A total 247 field-collected tick specimens from different geographical regions in west of Iran includes Khuzestan and Lorestan Provinces were studied. The morphologic characters of the ticks were measured by the calibrated stereomicroscope armed scaled lens. The measurements were analyzed using SPSS for windows, version 16 on an IBM PC, so varied shapes of species in different geographic regions were drawn by the aid of a drawing tube connected to a light stereomicroscope.

Results: One way ANOVA test revealed significant differences among the quantitative parameters in five zones ($P < 0.001$) also each zone to other zone by Post Hoc Tests e.g. LSD. No significant differences in the lateral grooves length/conscutum length ratio parameter were found.

Conclusion: Morphometric variation in *Hyalomma* spp is poorly studied. The variation in range and quantity of the morphometric parameters of *H. anatolicum* underlies that the correct recognition and key construction for *Hyalomma* species depends on a complement morphometric study on the other species.

Keywords: *Hyalomma anatolicum*, Morphologic characters, Morphometric study, Variation, Iran

Introduction

The small Anatolian hard tick, *Hyalomma anatolicum* Koch, 1844 is a vector of the important pathogen agents, includes animals and human (Hoogstraal 1979, Burkot and Graves 2004). This species is the most abundant *Hyalomma* species in its geographical zone (Kaiser and Hoogstraal 1964). *H. anatolicum* formerly was includes of two subspecies *anatolicum* and *excavatum*, but recently reestablish as full species rank (Apanaskevich and Horak 2005). Identification of *Hyalomma* spp. is not simple (Pomerantzev 1950)

and is very debatable because endless verity in faces which is due to exchange of environmental condition between different populations and genetic instability within their gene pool, hybridation and teratogenic abnormality (Delpy 1936b, Pervomaisky 1950, Hoogstraal 1956). Morphometric study of *H. anatolicum* in its geographical zone is not yet investigated, although some taxonomic characters on museum and field collected specimens was introduced but not measured (Mazlum 1968, Apanaskevich and Horak 2005).

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The medically and veterinary importance of *H. anatolicum* showed in Iran (Hashemi-Fesharki 1997, Telmadarraiy et al. 2010) and is widely distributed throughout in this country (Rahbari et al. 2008). Misidentification of *H. anatolicum* to closely related species e.g. *Hyalomma asiaticum*, *H. excavatum*, *H. marginatum*, generally the other *Hyalomma* species in Iran is a very common manner between tick workers whereas we have not an original and complete *Hyalomma* identification key for Iranian specimens. The traditional morphometric studies started by Delpy in Iran (Delpy 1936a, 1937a, 1937b), he believed the correct identification of *Hyalomma* species might be based on observation of the numerous specimens from any species.

The purpose of the present investigation was to identify valuable discriminating characters for male specimens of *H. anatolicum* also intraspecific variation study of the morphologic characters of this species in the five Iranian population in west of Iran based on Delpy's conception (Delpy 1936a). The present study was designed to obtain information on morphometric variability on one of the most important *Hyalomma* species in the Iranian ixodid fauna. This study was performed on males of *H. anatolicum* since the morphologic characters of the females may be shallow and obscure when the tick engorged.

Materials and Methods

Material examined

A 247 field-collected tick specimens from different geographical regions in west of Iran includes Khuzestan and Lorestan Provinces were studied (Table 1). Ticks were examined for preliminary identification by several *Hyalomma* identification keys including Delpy 1936a, Pomerantzev 1950, Kaiser and Hoogstraal 1964, and other related keys. The morphologic characters of ticks were measured by the calibrated stereo-

microscope armed scaled lens (Stemi SR-Zeiss Germany).

The measurements were analyzed using SPSS for windows, version 16 on an IBM PC. Significances were measured at the 5% level. Afterwards, varied shapes of this species in different geographic regions were drawn by the aid of a drawing tube connected to a light stereomicroscope.

Description of used characters

The list of zones and characters for male specimens include nine quantitative and three qualitative parameters summarized in Table 1.

Table 1. List of parameters used for characterization and zones of study

Quantitative Parameters
Basis capituli depression posteriodorsally
Cervical grooves length/conscutum length ratio
Conscutum length
Conscutum length/width ratio
Conscutum width
Lateral grooves length/conscutum length ratio
Parma length
Parma length/width ratio
Parma width
Qualitative Parameters
Arch
Caudal depression
Pigmented parma
Zones of Study
*I: Ramhormoz, Izeh, Baghmalek and Masjedsolyman
**II: Dashtezadegan, Shadegan and Shush
**III: Aleshtar and Taf
**IV: Kohdasht
**V: Mamolan

*These zones are situated in Khuzestan Province

**These zones are situated in Lorestan Province

Basis capituli depression posterodorsally

The cornua are paired projections from the outer margins of the posterior dorsal surface of the basis capituli. The depression of between cornua is characteristic for identification.

Cervical grooves

These are paired depressions near the central anterior part of the conscutum; they go down from the raised intercervical field into the cervical fields (also known as mesial grooves).

Conscutum: The sclerotized (=hardened) plate which covers most of the dorsal surface of male ixodid ticks (so called scutum in female).

Lateral grooves

In the conscutum of males a groove may be present in the lateral area, starting most clearly near the position of the spiracles and possibly extending forward to the eyes (in some literature these are called marginal grooves or lines).

Parma: Festoons are a regular series of bulges in the posterior margin of many adult ixodid ticks. The central festoon may also be called the parma when it is developed as a distinct structure, separated from the surrounding festoons by grooves.

Arch: In *Hyalomma* males the pair of festoons

next to the central one is paracentral and may be joined anteriorly to form an arch shape.

Caudal depression

In *Hyalomma* males the posterior of the conscutum may be broadly convex (caudal depression is absent) or it may be broadly concave (caudal depression is present).

Pigmented parma

The parma may be pale colored (unpigmented), or dark colored (pigmented).

Results

The result of relevant to the quantitative parameter is summarized in Table 2 to Table 4. One way ANOVA test revealed significant differences among the quantitative parameters in five zones ($P < 0.001$) also any zone to other zone by Post Hoc Tests eg LSD. No significant differences in the lateral grooves length/conscutum length ratio parameter were found (Fig. 4). Variation in some parameters (characters) is illustrated in Fig.1, Fig. 2 and Fig. 3. The observation on qualitative parameters revealed the presence of three characters in under studying specimens which is summarized in Table 5.

Table 2. Conscutum parameters of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces

Parameter	Zone	No	Size (mm)		*LSD		
			Average	SD	Max	Min	
Conscutum length	I	51	2.97	0.26	3.53	2.43	III,IV,V
	II	62	3.03	0.27	3.79	2.29	III,V
	III	45	3.48	0.31	4.04	2.78	I,II,IV,V
	IV	39	3.13	0.27	3.65	2.62	I,III
	V	50	3.14	0.24	3.61	2.52	I,II,III
Conscutum width	I	51	1.83	0.15	2.19	1.40	III
	II	62	1.87	0.18	2.29	1.46	III
	III	45	2.27	0.25	2.83	1.77	I,II,IV,V
	IV	39	1.88	0.15	2.21	1.44	III
	V	50	1.88	0.14	2.23	1.48	III
Conscutum length/width ratio	I	51	1.63	0.08	1.82	1.42	III,V
	II	62	1.63	0.08	1.85	1.44	III,IV,V
	III	45	1.55	0.07	1.69	1.40	I,II,IV,V
	IV	39	1.66	0.09	1.96	1.49	II,III
	V	50	1.67	0.07	1.82	1.39	I,II,III

*Least Significance difference, P value in all parameters is similar ($P < 0.001$)

Table 3. Parma parameters of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces

Parameter	Zone	No	Size (mm)				LSD
			Average	SD	Max	Min	
*Parma length	I	51	0.15	0.03	0.21	0.10	III
	II	62	0.14	0.03	0.27	0.06	III,IV
	III	44	0.19	0.05	0.31	0.10	I,II,IV,V
	IV	39	0.16	0.02	0.21	0.12	II,III
	V	50	0.15	0.02	0.19	0.10	III
*Parma width	I	51	0.90	0.84	2.17	0.10	III
	II	62	0.17	0.04	0.27	0.10	III,IV
	III	44	0.21	0.05	0.39	0.12	I,II,IV,V
	IV	39	0.20	0.03	0.29	0.16	II,III
	V	50	0.19	0.03	0.29	0.12	III
*Parma length/width ratio	I	51	0.50	0.40	1.60	0.06	II,III,IV,V
	II	62	0.83	0.20	1.29	0.55	I,III
	III	44	0.95	0.28	1.67	0.50	I,II,IV,V
	IV	39	0.81	0.17	1.25	0.46	I,III
	V	50	0.78	0.20	1.33	0.46	I,III

*Containing missing data in zone III

P value in all parameters is similar (*P* < 0.001)**Table 4.** Other quantitative parameters of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces

Parameter	Zone	No	Size (mm)				LSD
			Average	SD	Max	Min	
*Basis capituli depression	I	51	0.02	0.01	0.06	0.00	III
	II	62	0.03	0.01	0.08	0.00	IV,V
	III	45	0.03	0.01	0.06	0.02	I,IV,V
	IV	38	0.02	0.00	0.04	0.00	II,III
	V	50	0.02	0.00	0.06	0.00	II,III
**cervical grooves length/consutum length ratio	I	49	0.33	0.06	0.51	0.25	III
	II	62	0.32	0.05	0.45	0.21	III
	III	45	0.42	0.10	0.80	0.26	I,II,IV,V
	IV	39	0.31	0.04	0.45	0.23	III,V
	V	50	0.34	0.07	0.57	0.23	III,IV

*containing missing data in zone IV

**containing missing data in zone I

P value in all parameters is similar (*P* < 0.001)**Table 5.** Qualitative parameters of *H. anatolicum* in two groups of specimen (with and without qualitative parameter)

*Group	Qualitative parameters		
	Arch (n)	Pigmented parma (n)	Caudal depression (n)
I	21	30	57
II	225	216	188

I: specimens with qualitative parameter

II: specimens without qualitative parameter

*containing missing data in group I and II in all qualitative parameters

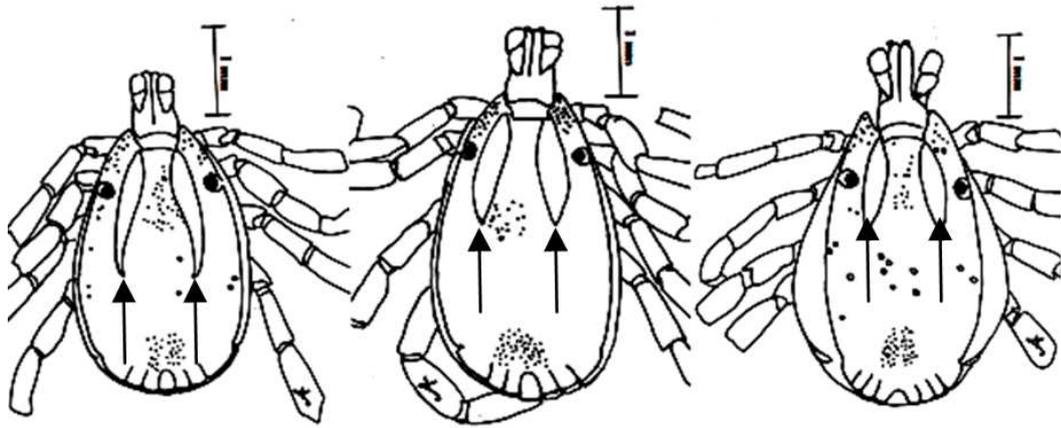


Fig. 1. Variation in cervical grooves length/conscutum length ratio parameter of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces (Original figure).

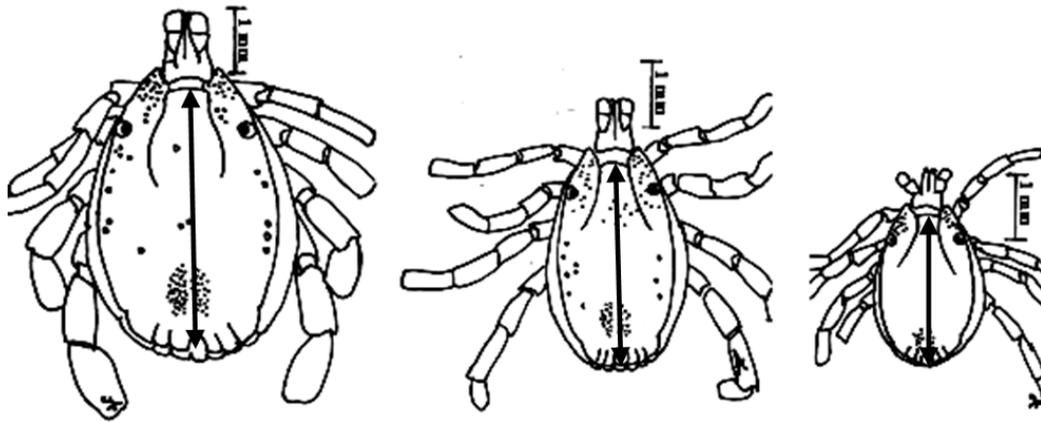


Fig. 2. Variation in conscutum length parameter of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces (Original figure).

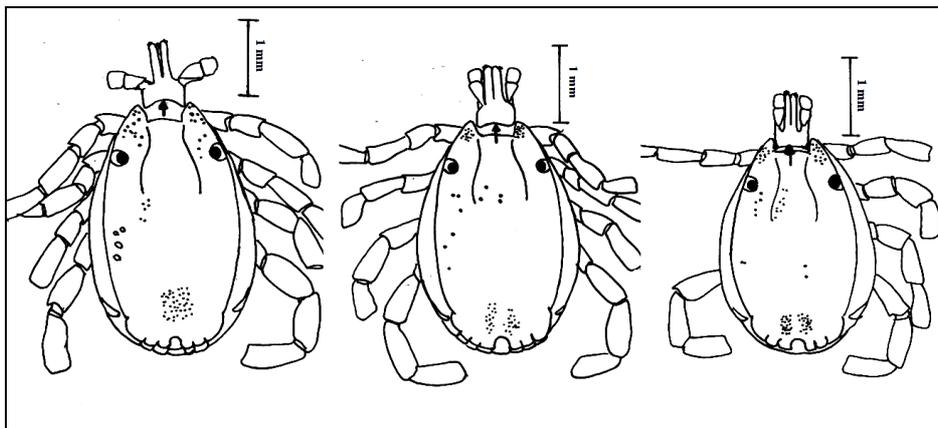


Fig. 3. Variation in basis capituli depression parameter of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces (Original figure).

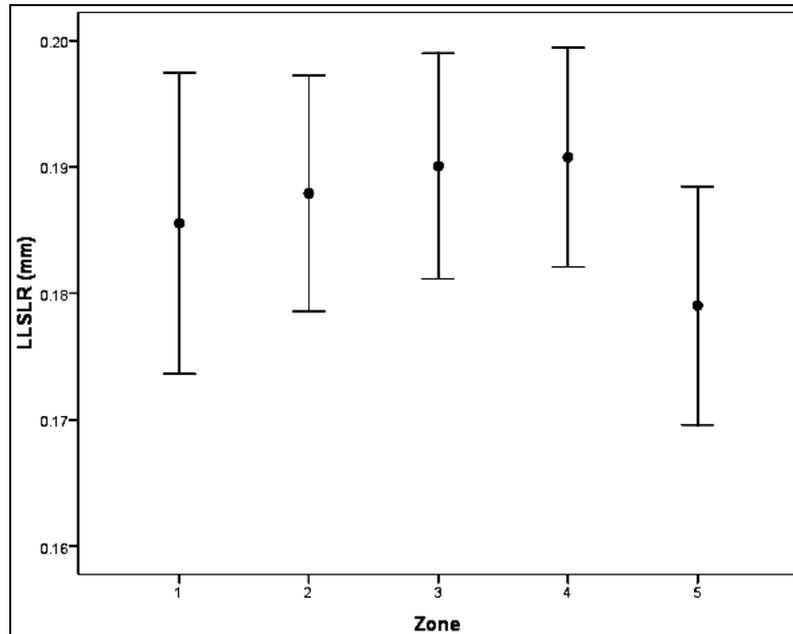


Fig. 4. Graph illustrating variation in lateral grooves length/conscutum length ratio parameter of *H. anatolicum* collected from different zones of Khuzestan and Lorestan Provinces

Discussion

Morphometric analysis in *Hyalomma* spp.

is poorly studied perhaps because morphologic characters in some species are obscure and unconfined. Delpy (1936a), after studying the genus *Hyalomma*, came to conclusion that diagnosis of *Hyalomma* species was almost impossible and suggested that the range of variations among laboratory bred offspring of individual females must be studied, for instance he reported high spectrum of qualitative variation in the adanal shields of *Hyalomma dromedarii*. Hoogstraal (1956) stated that the variation in the morphologic characters of *Hyalomma* species caused to nomination and presentation of the numerous *Hyalomma* species (about eighty species) by Schulze and his collaborators.

Delpy (1936a, 1937a) described all parasitic stages of the camel tick, *H. dromedarii*, he measured the length and width of the scutum, basis capituli, II and III palpi segments, spiracular plate and the many other characters in addition some of the comparative pa-

rameters. *Hyalomma schulzei*, (Olenev, 1931) the robust and rare Iranian *Halomma* species, also studied by Delpy (1937b), the measured characters include corps length and width, corps length to capituli length ratio and IV genua length ratio and the other characters (Delpy 1937b). Mazlum (1978) revealed the taxonomic status and presence of *H. asiaticum* in the Iranian ixodid fauna and focused on scutum length as a taxonomic discriminating character for four closely related *Hyalomma*. Filippova and Musatov (1996) studied morphologic variability in eight population of prostrate tick *Ixodes persulcatus* (Schulze, 1930) to distance 8700 and 2900 km in the Western-Eastern and Northern-Southern populations, respectively, they have shown morphometric variation in the anal ring and gnathosoma length and width, hypostome, II and III palpi also I tarsi length.

Scutum size (body length in many literature) in the genus *Hyalomma* is a good inter-specific discriminating character but no for closely related species as Apanaskevich and

Horak (2005) reported 1.48–2 mm and 1.64–2.05 mm in conscutum size range of males of two similar and debatable species *H. anatolicum* and *H. excavatum* respectively. The statistical significance of size in *H. anatolicum* immature stages were studied by Arthur and Snow (1966), they reported a non-linear relationship between the length of the egg, and the emergent larva.

The parma (central festoon) is a suitable discriminating character for interspecific segregating of *H. marginatum* complex (Pomerantzev 1950) as parma is not seen in the representative specimens. Mazlum (1978) reported which is parma as a multishape character for identification of *excavatum* and *asiaticum* groups and showed oval, oval, subtriangular and subrectangular shapes in *H. anatolicum*, *H. excavatum*, *H. asiaticum* and *H. dromedarii*, respectively. Usually color of parma is dark in *H. marginatum* complex while sometime is pale and may be cause to misidentification of this species with *H. anatolicum*. Adler and Feldman-Muhsam (1948) used from pigmented and unpigmented parma phrase in the Palestin *Hyalomma* identification key, they believed the ticks of *H. marginatum* complex might be separated from the other species using this character (Adler and Feldman-Muhsam 1948). Pomerantzev (1950) remarked the basis capituli depression posteriodorsally as specific character for discriminating of *H. asiaticum* from *H. anatolicum* however stated more concave and slightly concave modal phrases for two mentioned species, respectively (Pomerantzev 1950). Kaiser and Hoogstraal (1964) used from depth of the basis capituli depression in addition to be angular in *H. asiaticum* and *H. anatolicum*, respectively. In the present study we observe several individuals from very deep to less deep range in *H. anatolicum* (0 to 0.08 mm), also its angularity were seen by our observation. Abdigoudarzi (2003) stated that the basis capituli depression posteriodorsally is a specific feature for differentiating of *H. anatolicum* from

H. asiaticum specimens in the Iranian *Hyalomma* fauna (Abdigoudarzi 2003). The arch is a qualitative character which is debatable for identification of two closely related species *H. anatolicum* and *H. excavatum* as the former species this character not seen as connection of pair festoons 2 anteriorly and usually may be seen as wart like projection in the position of posteriodorsal groove to parma connection (Hoogstraal and Kaiser 1959b), or a weak junction similar to arch feature (Abdigoudarzi 2003) nevertheless in the future species the semicircle arch may be seen obviously. Adler and Feldman-Muhsam (1948) believed the arch is very important trait and may be causes to misidentification of the closely related *Hyalomma* species.

The lateral groove length is a reliable character for interspecific identification of *H. anatolicum* as the value of this parameter in our study was not significant statistically. This means which the amplitude of variation in the mentioned character is limited. Our object from the studying of this important character was to obtain numerical value for its which is mentioned as qualitative character in many *Hyalomma* identification keys (Delpy 1936a, Hoogstraal 1956, Kaiser and Hoogstraal 1964, Apanaskevich and Horak 2005) for instance as lateral grooves not extending beyond the posterior third of the scutum (Hoogstraal 1956) or lateral grooves short, not reaching central third of scutum (Kaiser and Hoogstraal 1964), which are the vague definitions. Hoogstraal (1956) believed the lateral grooves length is an important character for discriminating of the genus *Hyalomma*, but Pomerantzev (1950) and Adler and Feldman-Muhsam (1948) was not mentioned this character in their *Hyalomma* identification keys. Abdigoudarzi (2003) after morphologic observation on the Iranian *Hyalomma* specimens reported that the lateral groove is a valuable character in some *Hyalomma* species and emphasis on the lateral grooves based on observation and description, no through statis-

tics, therefore the present study may be complementary work.

The cervical grooves alike the lateral grooves which has never been measured, and described as cervical grooves short, not reaching mid-scutum (Kaiser and Hoogstraal 1964) or cervical grooves are seen as slight depression (Pomerantzev 1950) which are the vague descriptive statements. This study declare the length of the cervical grooves quantitatively, however the length of this character in under studied specimens of *H. anatolicum* was significance statistically, which means the trait from the cervical grooves which is important for discriminating of *H. anatolicum* from *H. asiaticum* may be its depth instead length of this character. Abdigoudarzi (2003) accepted our result on validity of the cervical grooves but stated that it may be a less useful and unstable character in him observed materials (Abdigoudarzi 2003).

Kaiser and Hoogstraal (1964) emphasize on elongation and are more depth of the cervical grooves of *H. asiaticum* from *H. anatolicum* while, Pomerantzev (1950) no stated about length of cervical grooves whereas considered the depth of this character as valuable taxonomic feature. Feldman-Muhsam (1962) shown that the cervical grooves are deep, wide and long in *H. anatolicum* of the Koch's type collection. In the present study, we observed number of specimens of *H. anatolicum* with the deeper and longer cervical grooves. Identification of the *Hyalomma* species not is possible unless to observation of many specimens from species and measure of the taxonomic and morphologic traits, features or characters and data analyzed via morphometric and statistics methods. The variation in range and quantity of the morphometric parameters of *H. anatolicum* underlying that the correct identification and key construction for *Hyalomma* species depended on a complement morphometric study on the other species especially closely related species.

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