INT. J. BIOL. BIOTECH., 8 (2): 179-187, 2011.

RE-DESCRIPTION AND NEW RECORD OF *HYALOMMA* (*EUHYALOMMA*) *MARGINATUM ISSACI* SHARIF, 1928 (ACARI: IXODIDAE) FROM BALOCHISTAN, PAKISTAN

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ABSTRACT

Hyalomma (Euhyalomma) *marginatum isaaci* Sharif, 1928 is recorded and re-described for the first time from Balochistan, Pakistan in detail with special reference to its capitulum, basis capituli, hypostome, palpi, scutum, genital aperture, adanal and plates subanal plates, anus and festoons. Taxonomic structures not discussed and not illustrated before are described and illustrated as additional information to facilitate zoologists and veterinarians in correct identification of female and male of this tick. A key is erected to Acari families and included genera highlighting the relationships. It is hoped that this paper will provide an anatomical base for future morphological studies.

Kew words: Re-description**,** *Hyalomma marginatum issaci*, Ixodidae, Balochistan, Pakistan**.**

INTRODUCTION

The medical and economic importance of ticks has long been recognized due to their ability to transmit diseases to humans and animals. Ticks cause great economic losses to livestock, and adversely affect livestock hosts in several ways (Rajput, *et al.*, 2006). Approximately 10% of the currently known 867 tick species act as vectors of a broad range of pathogens of domestic animals and humans and are also responsible for damage directly due to their feeding behavior (Jongejian and Uilenberg, 2004). This has attracted the attention of a large number of researchers all over the World, but in our country ticks did not receive the attention they deserve. Most of the Pakistani workers dealt with the tick prevalence and control (Hussain and Gyanchandani, 1991; Gyanchandani *et al.*, 1992; Gyanchandani and Hussain, 1993; Hayat *et al.*,1993; Zaman, 1997; Khan, 2001; Wahid-ur-Rahman *et al.*, 2004; Manan *et al.*, 2007), but a few explored the taxonomy of ticks (Kaisar and Hoogstraal, 1964; Kosar, 1965; McCarthy, 1967; Hussain and Gyanchandani, 1983, 1985; Iqbal and Nawaz, 2007; Kakar and Kakarsulemankhel, 2007, 2008) from a very limited area. For one or the other reason, one of the most important subjects in the biology of ticks has not been properly documented. This motivated the author's scientific interest in the performance of taxonomic study on tick fauna. While conducting ecto-parasitic surveys during May to October, 2009, in central, eastern, northern, western and southern Balochistan Province, some 381 ticks were collected from domestic animals including camels. Since there is incomplete and insufficient literature on taxonomy of Pakistani ticks, therefore, to facilitate zoologists and veterinarians in correct identification of ticks, *Hyalomma marginatum issaci* Sharif, 1928, is re-described here in detail for the first time from Balochistan, Pakistan. Taxonomic characters studied here are perhaps never been studied or figured by Pakistani workers before because of the tendency in most publications on ticks to study only the prevalence of ticks. The present report is an attempt to encourage further investigation in this country. It is hoped that this attempt will not only serve as base line study of the taxonomy of Ixodid ticks of livestock but also guide researchers in correct identification of these ticks.

MATERIAL AND METHODS

During routine ecto-parasitic survey in May to October 2009 of domestic animals including camels in central, eastern and southern Balochistan Province, 381 specimens of ticks were collected following the collection techniques of Kakar and Kakarsulemankhel (2008). Animals were observed at random from houses, meat markets and Animal-Sell-Purchase Market. The head, ears, eyelids, neck, back, ventral side, tail area, legs and other external surfaces of the animal were thoroughly examined. Ticks were also collected from cracks and crevices in the walls of animal's sheds.

Collection and Identification of ticks: Sampling was conducted in central, eastern and southern Balochistan Province. Collected samples were preserved in 70% ethanol in glass vials (labeled with the date, locality, host-information) which were brought to the laboratory of Department of Zoology for processing and identification. For making permanent slides, ticks were cleared for few hours in cold KOH solution and were then dehydrated with ascending grades of ethanol till absolute grade, thereafter washed by Xylene. Each tick was picked up with the help of fine brush and placed on glass slide and slowly pressed by an insect pin to remove all internal content through anal opening. Each tick was studied under light compound microscope (CH2, Olympus, Japan). Taxonomic structures have been drawn with the help of camera lucida mounted on microscope. The data of specimens critically examined for the description and measurements are designated under "examined". Specimens were identified according to the criteria presented by Kaiser and Hoogstraal (1963, 1964), McCarthy (1967) and Lloyd (2004). Measurements are given in micrometers unless otherwise indicated. Most of the measurements were taken under X 10X10 and 4X10 magnification. Specimens placed in the vials and permanent slides are deposited with the author's collection of ticks.

RESULTS

***Hyalomma* (*Euhyalomma*) *marginatum issaci*, Sharif, 1928**
(Male Fig. A-1 to A-27; Female Fig. B-1 to B-13)

Synonyms: *Hyalomma aegyptium isaaci* Sharif, 1928; *Hyalomma dromedarii indosinensis* Toumanoff, 1944 *Hyalomma* (Hyalomma) *aegyptium Isaaci* Sharif, 1928; *Hyalomma* (Hyalomma) Marginatum Isaaci Kaiser & Hoogstraal, 1964; *Hyalomma isaaci* Kratz, 1940; *Hyalomma marginatum indosinense* Kolonin, 1992; *Hyalomma sharifi isaaci* Schulze, 1950. See also Camicas *et al.*, (1998).

The genus *Hyalomma* Koch, 1844 characterizes with in-ornate scutum, ♂ with adanal and subanal plates. Palpal segment-II longer than III, festoons may or may not be present, or they may be partially concealed. Ticks of this genus have been arranged in two sub genera. Subgenus *Hyalomma* Koch, 1844 consists of *H. aeygyptium* (Linnaeaus). Its adults parasitize tortoises (Robbins *et al.*,1998; Siroky *et al.*,2006; Tavassoli *et al.*, 2007) with characteristics (Apanaskevich, 2003): ♂: any groves in scutum absent, except short and pit-like cervical ones, punctuation sparse and impressive, adenal shields short and wide, without inner branch, posterior part widened, antero-median margin straight, spurs of coxae-I widely separated, triangular wide, sub-equal in size. ♀: genital orifice as wide arch with straight posterior margin,, vestibular part of vagina funnel like, greatly swollen, setae of alloscutum stick-like, tapering in apical one, II-segment of palps with proximal narrowing, spurs of coxae-I widely separated, triangular, wide, sub-equal in size. Immature stages retain the primary wide diapason of hosts, which are various mammals,, birds and reptiles. The life cycle of this tick is the three-host type that is considered as a primary type in Ixodid ticks. The species of Subgenus *Euhyalomma* Filippova, 1984, has been arranged in two groups. In the first group, the immature stages infest only small mammals and birds, and the adults parasitize large mammals. This sub group includes: *H. albiparmatum* Schulzei, 1919 (three-host tick), *H. asiaticum* Schulzei and Schlottke (three-host tick), *H. excavatum* Koch, 1844 (two-or three-host tick), *H. franchinii* Tonelli-Rondelli, 1932, *H. impeltatum* Schulzei and Schlottke, 1930 (three-host tick), *H. impressum* Koch, 1844 (three-host tick), *H. lusitanicum* Koch, 1844 (three- host tick), *H. marginatum* Koch, 1844 (two-host tick), *H. nitidum* Schulzei, 1919 (three-host tick), *H. schulzei* (two-host species), and *H. truncatum* Koch, 1844 (three-host tick). The second group includes: *H. dromedarii* Koch, 1844 (two or three host tick); *H. anatolicum* Koch, 1844, and *H. scupense* Schulzei, 1918. The immature as well as the adult stage parasitize large mammals.

Key to the sub orders, families and genera

1 With dentate faces of cheliceral digits directed externally (laterally), presence of a well developed hypostome provided ventrally with re-curved teeth ; with lateral stigmatal openings behind coxae-IV or laterally above coxae II-III; prosoma completely fused; no trace of segmentation to the abdominal section; behind the legs size large, possession of true Haller's organ on the I pair of legs……..... ……..sub order Ixodida

- External and internal parasitic mites of a wide variety of vertebrate hosts ……..…….. sub order Gamsida (Mesostigmata)

2 Capitulum situated anteriorly, not concealed from above, dorsal scutum present, Spiracles posterior to coxae IV, well developed sexual dimorphism….. ….Family ……Ixodidae Koch, 1844….3

- Capitulum ventral, partially or completely concealed from above, dorsal scutum absent, spiracles usually anterior to coxae-IV, sexual dimorphism slight …….. Family Argasidae Koch, 1844

Family Ixodidae

3 Anal groove distinct or indistinct but never surrounding the anus anteriorly, festoons often present ……. 4

- Anal grooves distinct, surrounding the anus in front/ anteriorly and usually uniting in a pointed arch, festoons absent. … *Ixodes* Latreille 1795

4 Capitulum short in relation to width, II palpal segment not especially longer than wide …… 5

- Capitulum long in relation to width, II palpal segment longer than wide …… 11

5 II palpal segment not acutely laterally produced at base, eyes present ……. 6

- Short conical palpi, II palpal segment projects laterally extending beyond the basis capituli, about twice as broad as long, eyes absent …….. …….. *Hymophysalis* Koch 1844

6 Festoons absent, anal grooves faint or absent ……. 7

- Festoons present, anal grooves very distinct to indistinct ... …… 8

7. Palpi compressed with acute transverse ridges dorsally and laterally, ♂ with normal legs, adanal and accessory plates but without preanal plate …….. *Boophilus* Curtice 1891

- Palpi not ridged and somewhat more elongate ♂ with massive beady leg segments, without adanal or accessory plates but with preanal plate which is continued backward on either side of anus as two prongs ……..*Margaropus* Karsch 1879

8. Basis capituli rectangular dorsally, ventral plates absent in ♀, ♂ both, ornate or in-ornate ……. …… 9

- Basis caputli hexagonal dorsally, ventral plates present or absent, usually inornate …… 10

9. Usually ornate, dentition of hypostome 3/3 …….*Dermacentor* Say, 1821

- Inornate, dentition of hypostome 4/4 ……*Anocentor* Schulzei, 1937

10. Coxae-IV much larger than other coxae, without anterior plates or shield …….. *Rhipicentor* Koch, 1844

- Coxae-IV normal, ♂ with ventral plates and possibly showing a caudal protrusion …… *Rhipicephalus* Koch 1844

11. Palpi long, with II segment about twice as long as broad, ♂ without adanal or sub anal shields ……. …… 12

- Palpi long, with joints sub equal, ♂ with adanal and sub anal shields ……..……..... *Hyalomma* Koch 1844

12. Without eyes, …. …… *Aponomma* Neumann 1899

- With eyes , ……. *Amblyomma* Koch 1844

Male (8 specimens examined): Body**:** ♂: Size medium to large, 4.7–5. 5 long, 3.5–4.5, broad, ovoid, black, narrow in front, broadly rounded behind, maximum breadth at spiracular level.

Capitulum (Fig.A-1): Highly sclerotized structure consisting of basis capituli, median ventral hypostome, a pair of long cylindrical shafts, the chelicerae, and flanked on each side by a pair of palpi with visible three segments, capitulum shorter than that of ♀, dorsal ridge strongly developed, and both it and lateral margins straight and heavily sclerotized, slightly converging posteriorly, hind margin also straight and transverse.

Basis capituli (Fg.A-2): Height 0.70, breadth 0.63, without lateral projections, dorsal lateral-posterior margin convex.

Palpi: Short and robust, borne on the antero-lateral angles of the capitulum, one on either side of the median hypostome, 0.6 long, in argasid ticks three segments of palpi approximately of equal length but in the genera *Ixodes* and *Hyalomma* the I is considerably smaller than the II and III, whereas the IV is reduced lying in a membranous cup-like hollow structure, segment-I visible dorsally, not concave on its inner surface bearing more than five ventro-median setae (Fig.A-3), internal faces of II and III concave, segment-II relatively long with undulate lateral margins, 7-8 setae concentrated along the margins of the mesial concavity or inner margin of the II-segment (Fig.A-4), segment-III also long, maximum width at base where it adjoins the II-segment, apically the former narrows to the apex, which is bluntly rounded, body with 3-4 median and lateral setae (Fig.A-5).

Hypostome: Club-shaped, total length 1.0, width 0.3, outer visible portion 0.6 long, denticulate portion slightly longer than free portion, dentition 3/3 files of 15-18 strong teeth per file (Fig.A-6), the pointed lateral denticles protrude over the lateral edges, the medians rounded, and then 2/2 files of 3-4 crenulations basally, apex of hypostome flattened (Fig. A-7), sides gently curved.

Chelicerae: A pair of long shafts running through the capitulum dorsally, proximally expanded to form a bulbous base, lying partly within the cavity of the capitulum, a rigid prolongation of a shaft with outwardly directed teeth of triangular pattern originates from the base of the segment, the other relatively broader shaft terminates in a bifid-type teeth .

Conscutum (Fig.A-8): Yellowish-brown in color, almost oval in shape, pale marbling, 4.5–5.0 long, 3.8–4.0 broad, widest at mid-length, but slightly narrowing in area of spiracular plates, cervical grooves short but apparent (Fig.A-9), Lateral grooves long but narrow (Fig.A-10), postero-median groove long deep and almost reaches to central festoon (Fig.A-11), paramedian grooves also deep (Fig.A-12), additional lateral depressions also present (Fig.A-13), additional central depression apparent (Fig.A-14), large punctuations very rare (Fig.A-15).

Eyes : Very convex, bulging, on the edge of conscutum (Fig.A-16), at the level of coxae-II.

Genital structure (Fig.A-17): At the level of coxae-II, in the center of the body, just below the capitulum.

Adanal plates: Trapezoidal in shape, body comparatively long and broader (Fig.A-18), almost straight, antero-median part long extending beyond the 1/2 of the body of adanals, median projection obvious (Fig.A-19), postero-median part of adanals convex, hind basal margins with almost rounded ends (Fig.A-20), anterior part of adanal plates almost in line with the anus.

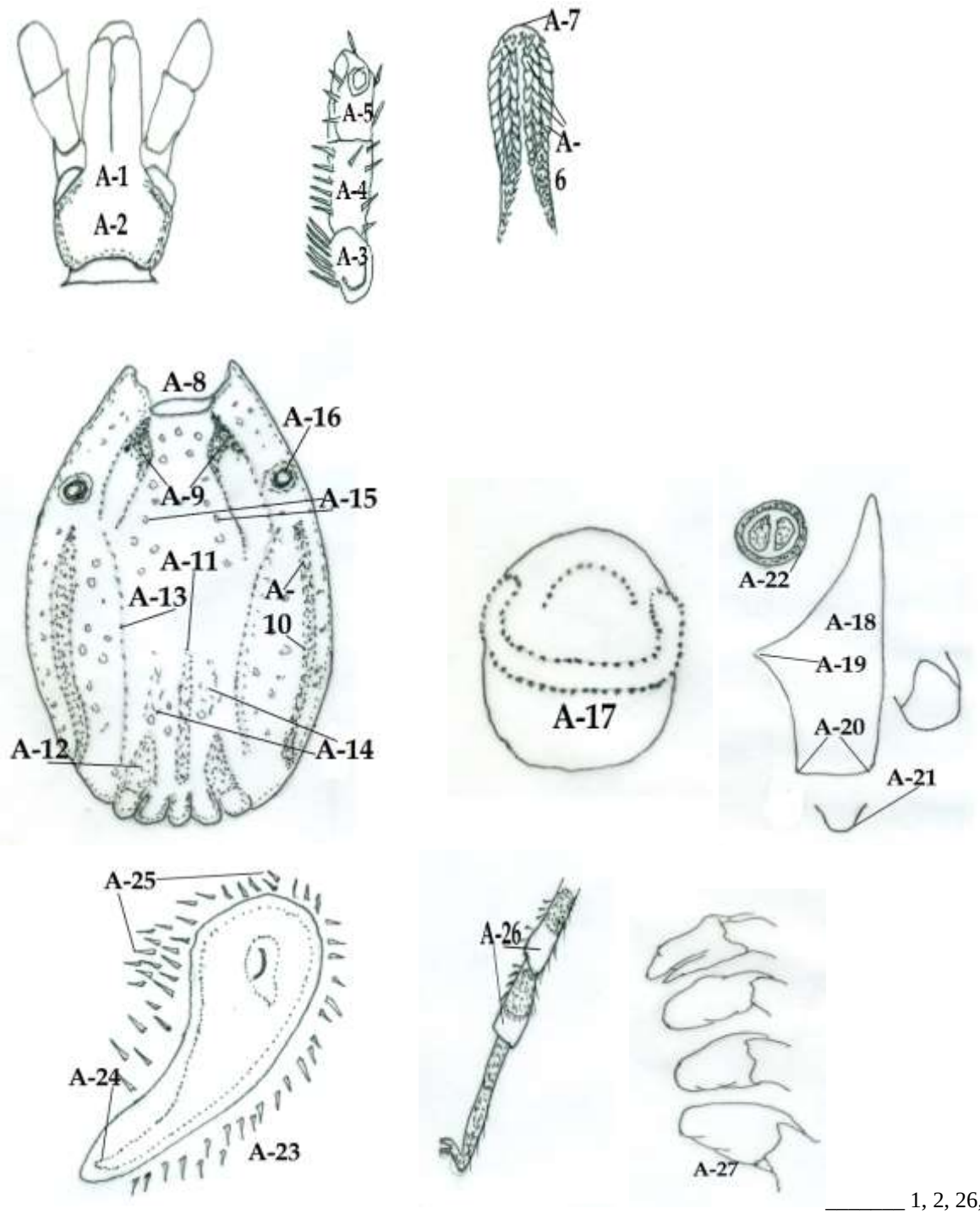
Sub anal plates: Usually vary in shape and size, moderate, alignment with adanal plates and directly posterior of the adanals (Fig.A-21).

Anus: As illustrated (Fig-A-22), almost rounded, borne at 1.0 mm away from the hind end of the body, a thick integumentary wall encircles the anal opening.

Spiracular plates (Fig.A-23): As illustrated, dorsal prolongation of perforated part comparatively long but narrow (Fig.24) so distinct from the remaining body, circum-spiracular setae relatively dense around the main body but specially at the anterior margin (Fig.A-25).

Legs: Of moderate length and thickness, relatively shorter and broader than those of ♀. Legs coloration with broad pale rings and usually cover distal portion of middle segments (Fig.A-26). Coxae (Fig.A-27): Coxa-I with postero-median and postero-lateral spurs long, sub-equal in length (posterolateral spur longer than posteromedian spur), coxae-II-IV each having triangular postero-lateral spur, postero-median spur on coxae II and III not clearly apparent. Tarsus-I with almost parallel sides but tapering sub-apically, apex round, tarsi II-IV sharply humped with apical and sub-apical spurs Claws mildly curved and relatively longer than pulvillae.

Female (6 specimens examined): ♀ are more or less like males in size.



_____ 1, 2, 26, 27

_____ 3-25

Fig. 1. Male *Hyalomma* (*Euhyalomma*) *marginatum issaci* Sharif, 1928: A-1, Capitulum; A-2 Basis Capituli, A-3, Segment-1 of Palpi; A-4, Segment-II of Palpi; A-5, Segment-III; A-6, Hypostome dentition 3/3 per File; A-7, Apex of Hypostome gently flattened; A-8, Conscutum; A-9, Cervical field depressions short but obvious; A-10, Lateral grooves long; A-11, Postero-median groove long, deep and almost reaching the central festoon; A-12, Paramedian grooves also deep; A-13, Additional lateral depressions present; A-14, Additional central depressions present; A-15, Large punctuations rare; A-16, Eyes; A-17, Genital structure; A-18, Adanal plate; A-19, Median projections apparent; A-20, Hind basal ends almost rounded; A-21, Sub-anal plate short and aligned with adanals; A-22, Anus; A-23, spiracular plate; A-24, Narrow dorsal perforated part of spiracular plate; A-25, Circumspiracular setae dense at anterior part of the plate; A-26, Broad pale rings at the middle segments of legs; A-27, Coxae. *Scale bars*: 1, 2, 26, 27=0.1 mm (4X10), 3-25=0.1 mm (10X10).

Capitulum (Fig.B-1) : Relatively longer than that of ♂.

Basis capituli (Fig.B-2): Dorsal lateral projections broad and stretches latero-posteriorly and making a short notch but ventrally lacking, posterior margin concave.

Palpi: Relatively short but broad, shape much as in ♂, segment-I bearing more than five ventro-median setae (Fig.B-3), segment-II relatively long and with 6-8 setae situated at the margin (Fig.B-4), segment-III with few setae but apex slightly rounded (B-5).

Hypostome: Club-shaped, denticulate part slightly longer than that of denticle free part, dentition 3/3 files of 15-18 strong teeth per file (Fig.B-6), apex of hypostome flattened, sides gently curved (Fig.B-7).

Scutum (Fig.B-8): Dark in color, almost as long as broad, but in few specimens slightly longer than wide, heart-shaped, cervical depressions quite obvious (Fig.B-9), deep and almost reaching the hind margin of the scutum, punctuations as in male.

Eyes (Fig.B-10): Prominent, round, convex and peripheral.

Genital structure (Fig.B-11): Genital aperture relatively wider, triangular and almost rounded posteriorly, pre-atrial fold almost bulging, borne between coxae-II and III.

Spiraclular plates (Fig.B-12): Relatively broad, irregularly oval, perforated part of dorsal projections a little curved, long and narrow (Fig.B-13), circumspiracular setae dense especially at anterior margin of plate (Fig.B-14).
Adanal and **sub-anal plates:** Absent.

Legs: Usually long, legs coloration with pale rings and similar to ♂, broad patches of pale color present on the dorsal side of leg segments.

Coxae : Coxa-I with postero-median and postero-lateral spurs long, sub-equal in length, postero-median spur almost narrow but tapering apex, coxae II-IV each having broadly tri-angular postero-lateral spur.

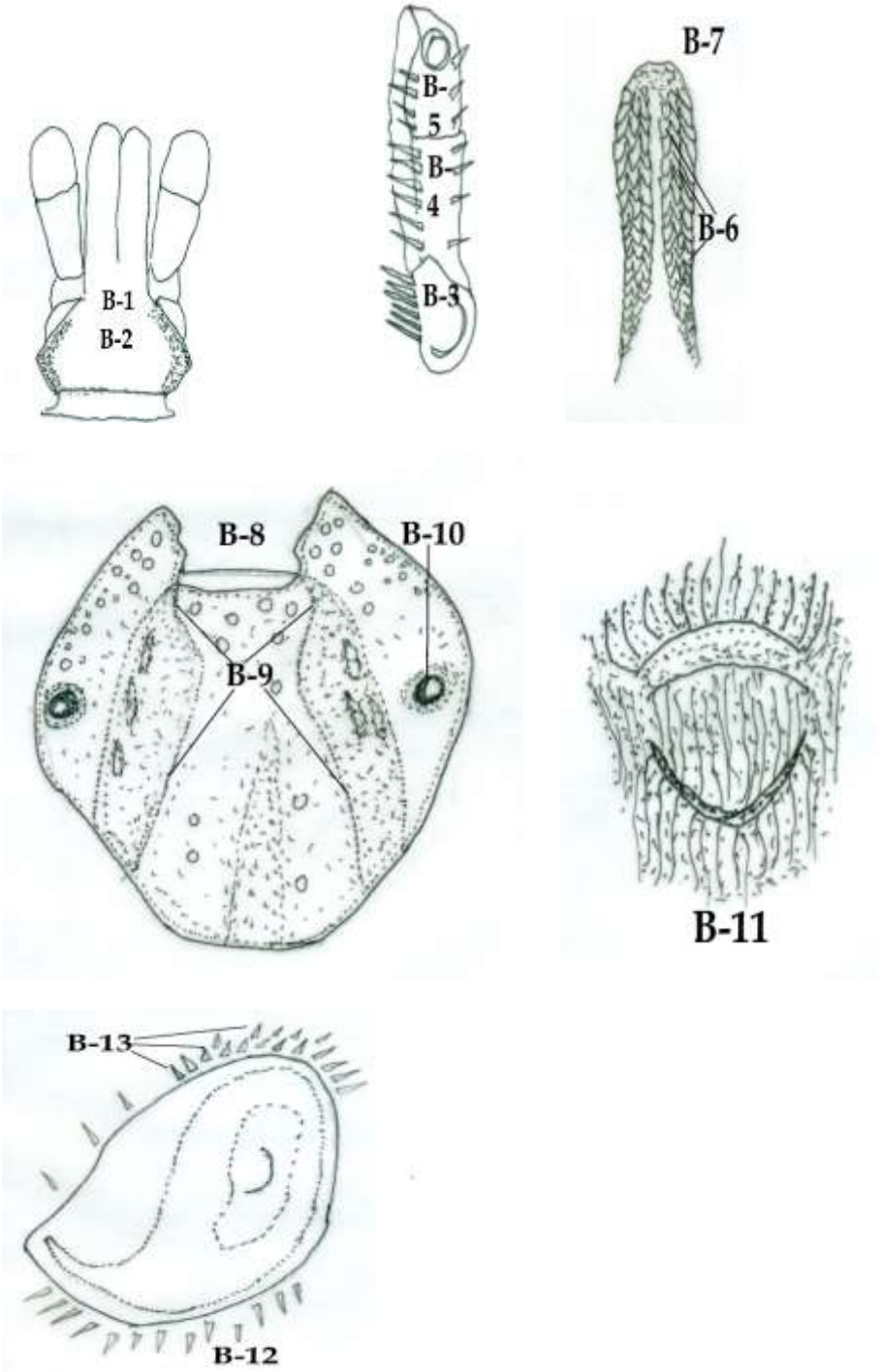
Hosts and Distribution: *H. m. issaci* is a two host species (Hoogstraal, 1979). Large domestic cattle and camels are the most common hosts for adults of this species. New Record: For the first time from Balochistan, Pakistan present specimens were collected from sheep, goats and camels from Chaman, Dalbandin, Kharan, Kuchlagh, Quetta, and Turbat. Afghanistan, Pakistan India (Kaiser and Hoogstraal, 1963; Geevarghese and Dhanda, 1987), China (Teng and Jiang, 1991), Maynmar (Robbins *et al.*, 2002). Turkey (Bursali *et al.*, 2010).

Comparative note: This species shares on the genus level some characters assigned for the genus *Hyalomma.* ♂ of this species can be discriminated from those of other species of the genus *Hyalomma* by their dark scutum color, few large scattered punctuations, cervical grooves stretches anteriorly, long obvious and deep lateral grooves, paramedian grooves and postero-median grooves quite obvious, relatively larger adanal plates with small sub anal plates and perforated part of dorsal prolongation of spiracular plate comparatively narrow, circumspiracular setae dense particularly at antnterior part of the plate. However, ♀ *H. m. issaci* can be very easily identified from those of other species of *Hyalomma* by their scutum relatively longer than wide, deep cervical grooves, genital aperture widely triangular, but it is V-shaped in *H. m. rufipes* Koch, 1844, and circumspiracular setae dense particularly at antnterior part of the plate, setae also dense in *H. m. rufipes* but sparse in *H. m. marginatum* Koch, 1844.

DISCUSSION

The subgenus *Euhyalomma* Filippova, 1984 composed of some 20 species which have been divided into groups of closely related ticks. *Hyalomma marginatum* Koch consists of four subspecies *vitz: H. m. marginatum* Koch, 1844; *H. m. rufipes* Koch, 1844; *H. m. isaaci* Sharif, 1928 and *Hyalomma m. turanicum* Pomerantzev, 1946. Sharif (1928) originally described *H. issaci* as a subspecies whereas Kratz (1940) suggested that this tick should be considered as a full species. Later, Delpy (1949) and Hoogstraal (1956) proposed that *H. aegyptium issaci* is a junior synonym of *H. detritum.* Kaiser and Hoogtraal (1963) after examining a large collection of south Asian

ticks, suggested that *H. aegyotium* issaci is a quite separate taxon but closely related to *H. marginatum*, therefore, it should be considered a subspecies of *H. marginatum.*



1, 2

3-13

Fig. 2. Female *Hyalomma* (*Euhyalomma*) *marginatum issaci* Sharif, 1928: B-1, Capitulum; B-2 Basis Capituli, B-3, Segment-1 of Palpi; B-4, Segment-II of Palpi; B-5, Segment-III; B-6, Hypostome dentition 3/3 per File; B-7, Apex of Hypostome gently flattened; B-8, Conscutum; B-9, Cervical depressions obviously reaching the hind margin of scutum; B-10, Eyes ; B-11, Genital operculum; B-12, Spiracular plate; B-13, Circumspiracular setae dense at anterior part of spiracular plate. *Scale bars*: 1, 2=0.1 mm (4X10), 3-13=0.1 mm (10X10).

Hyalomma m issaci adults usually found attached with domestic animals whereas their immature stages parasitize hares and birds. Since no taxonomic work on *H. m. issaci* has ever been carried out in Pakistan, therefore, present data cannot be compared with it. However, present data are in line with the published data on this species from other countries (Kaiser and Hoogstraal, 1963).

Peoples crossing borders of Afghanistan and Iran usually bring with them herds of sheep, goats and camels from Iran and Afghanistan to Pakistani border areas. The collection sides of present specimen are situated with border areas with Afghanistan and Iran. The present findings of this tick in sheep and goats of Pakistani border areas conforms this fact.

CONCLUSION

The climatic conditions of the country and poor husbandry practices of small holder dairy farmers considerable are making the livestock more prone to tick infestation. Keeping in view of the finding of this study, the farmers and owners of the domesticated animals of the study area should be educated about the prevalence of tick fauna. The precise data on the distribution of tick's fauna in Pakistan especially in Balochistan Province and morphologic taxonomical characters of prevalent ticks facilitating researchers in species identification are still unknown and rightly deserve to be investigated. Till then, it is hoped that taxonomic findings of this study would provide the basis for further taxonomic work on ticks in the Province which is essential for control of the ticks and disease abatement.

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(*Accepted for publication December 2010*)