

RE-DESCRIPTION AND NEW RECORD OF *HYALOMMA (EUHYALOMMA) ANATOLICUM EXCAVATUM* KOCH, 1844 (ACARI: IXODIDAE) FROM NEW LOCALITIES IN BALOCHISTAN, PAKISTAN

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ABSTRACT

Hyalomma (Euhyalomma) anatolicum excavatum Koch, 1844 is re-described and recorded for the first time from new localities in 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 anatolicum excavatum*, Ixodidae, Balochistan, Pakistan.

INTRODUCTION

Ticks are obligate ecto-parasites infesting most vertebrates and are often reservoirs or vectors of pathogenic viruses, rickettsias, bacteria, spirochaetes, protozoa and nematodes (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. Kakar and Kakarsulemankhel (2008) recorded *Hyalomma anatolicum excavatum* from Loralai, Harnai, Ziarat, Qallat, Noshki and Sibi area of Balochistan and was not known in the literature to date from the localities this tick has been collected this time and its important taxonomic characters like capitulum, basis capituli, cheleceral teeth, hypostomal teeth, spiracular plate male and female genitalia from Pakistan were never described nor figured. Since there is incomplete and insufficient literature on taxonomy of Pakistani ticks, therefore, to facilitate zoologists and veterinarians in correct identification of ticks, presently, *Hyalomma anatolicum excavatum* Koch, 1844 is not only re-described here in detail but also illustrated. 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.

MATERIALS 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 first author's collection of ticks.

RESULTS

Hyalomma (Euhyalomma) anatolicum excavatum Koch, 1844

(Male Fig. A-1 to A-30; Female Fig. B-1 to B-12)

Synonyms: *Hyalomma aegyptium typica* Schulze, 1919; *Hyalomma algeriense* Canestrini, 1890; *Hyalomma detritum pavlovskiyi* Schulze & Schlottke, 1930; *Hyalomma excavatum* Koch, 1844; *Hyalomma* (*Hyalomma*) *Anatolicum Excavatum* Kaiser & Hoogstraal, 1964; *Hyalomma lusitanicum algericum* Sonevet, 1928; *Hyalomma* sp. No 2 Near *excavatum* Hoogstraal, 1956; *Hyalomma syriacum typica* Schulze, 1919; *Hyalomma tunesiacum ganorai* Tonelli-Rondelli, 1932; *Hyalomma tunesiacum pavlovskiyi* Kratz, 1940; *Hyalomma tunesiacum* Schulze & Schlottke, 1930 *Hyalomma tunesiacum tunesiacum* Kratz, 1940; *Hyalomma tunesiacum turkmeniense* Kratz, 1940; *Hyalomma tunesiacum zavattarii* Tonelli-Rondelli, 1935; *Hyalomma turkmeniense* Olenev, 1931. (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. aegyptium* (Linnaeus). Its adults parasitize tortoises (Robbins *et al.*, 1998; Siroky *et al.*, 2006; Tavassoli *et al.*, 2007) with characteristics (Apanaskevich, 2003): ♂: any grooves 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 capituli 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 (5 specimens examined): Body: ♂: Size medium to large, 4.5–5.5 long, 3.2–3.7 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 lateral margins straight and heavily sclerotized, slightly converging posteriorly, hind margin also straight and transverse.

Basis capituli (Fig.A-2): Height 0.77, breadth 0.72, lateral projections present (Fig.A-3), dorsal lateral-posterior margin a little concave (Fig.A-4) and short cornua apparent (Fig.A-5).

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-6), 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-7), 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 (Fig.A-8), body with 3-4 median and lateral setae.

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-9), 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-10), 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-11): Black brown in color, almost oval in shape, whitish pigment present, widest at mid-length, but slightly narrowing at spiracular plates, hind margin almost rounded, cervical grooves apparent and moderately (Fig.A-12), lateral grooves short but obvious (Fig.A-13), posterior ridges two in numbers (Fig.A-14), caudal depression apparent, postero-median groove present (Fig.A-15) and separated from parma by a little higher elevations, central festoon present (Fig.A-16) but pale in color, paracentral festoons joined anteriorly (Fig.A-17), paramedian groove small (Fig.A-18), punctuation size large (Fig. A-19) and localized especially in caudal depression area and also in cervical depression area.

Eyes: Very convex, bulging, on the edge of conscutum (Fig.A-20), at the level of coxae-II.

Genital structures (Fig.A-21): 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-22), almost straight, median projections quite obvious (Fig.A-23), hind-median margin almost straight but lateral margins a little convex, antero-median margin almost concave, hind basal margins with square ends (Fig.A-24), anterior part of adanal plates almost in line with the anus.

Sub anal plates: Usually vary in shape and size, moderate, distinct, alignment with adanal plates and directly posterior of the adanals (Fig.A-25).

Anus: As illustrated (Fig.A-26), 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-27): As illustrated, dorsal prolongation comparatively long and narrow, macula anteriorly placed, perforated portion of prolongation slightly curved throughout its length (Fig.A-28), circum-spiracular setae sparse (not shown in Fig).

Legs: Of moderate length and thickness, relatively shorter and broader than those of ♀. Legs usually dark but with pale-patches apparent at the middle segments of legs (Fig.A-29). Coxae (Fig.A-30): 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, in few specimen ivory-colored enamel band apparent on distal part of

each segment of legs. 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.

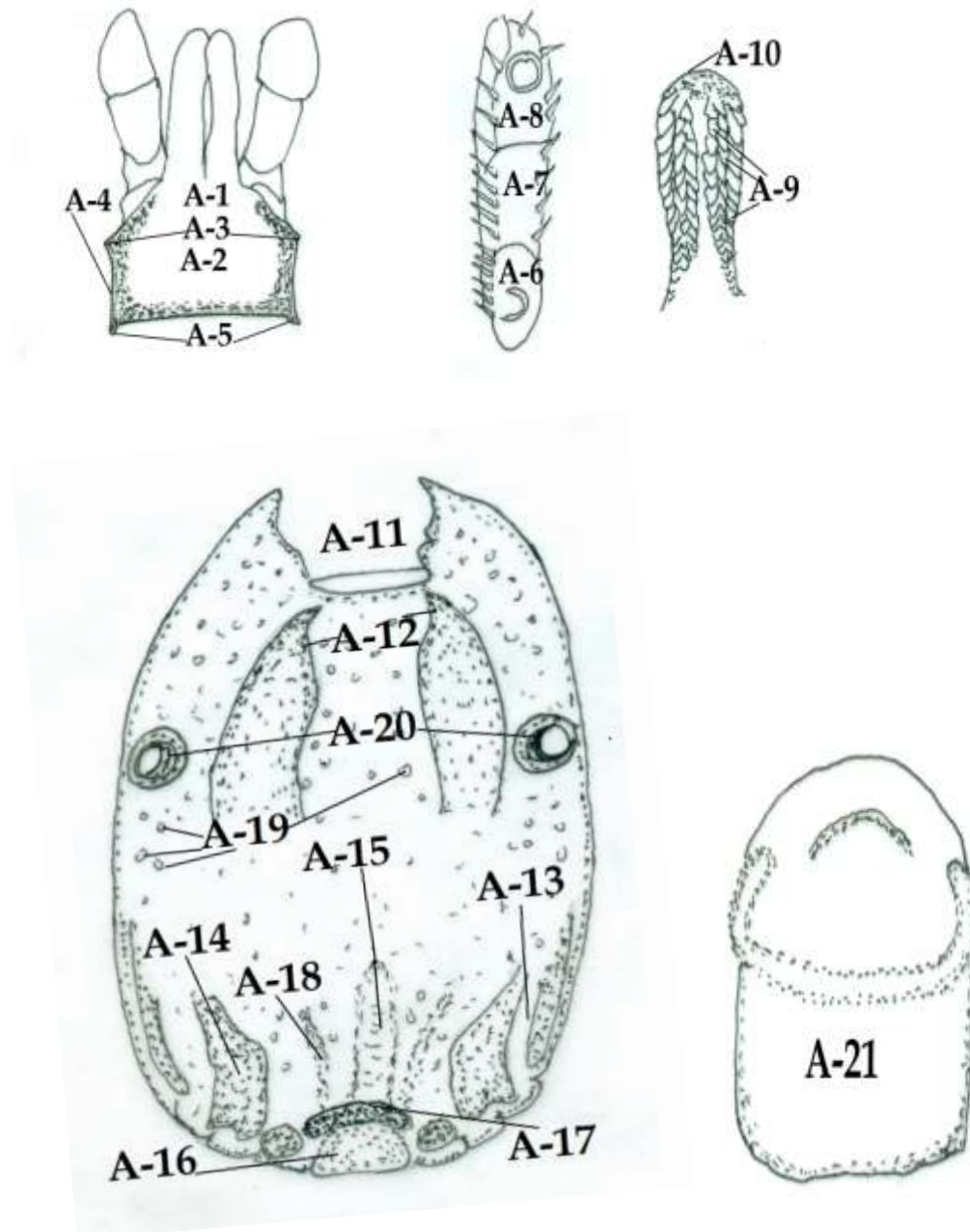
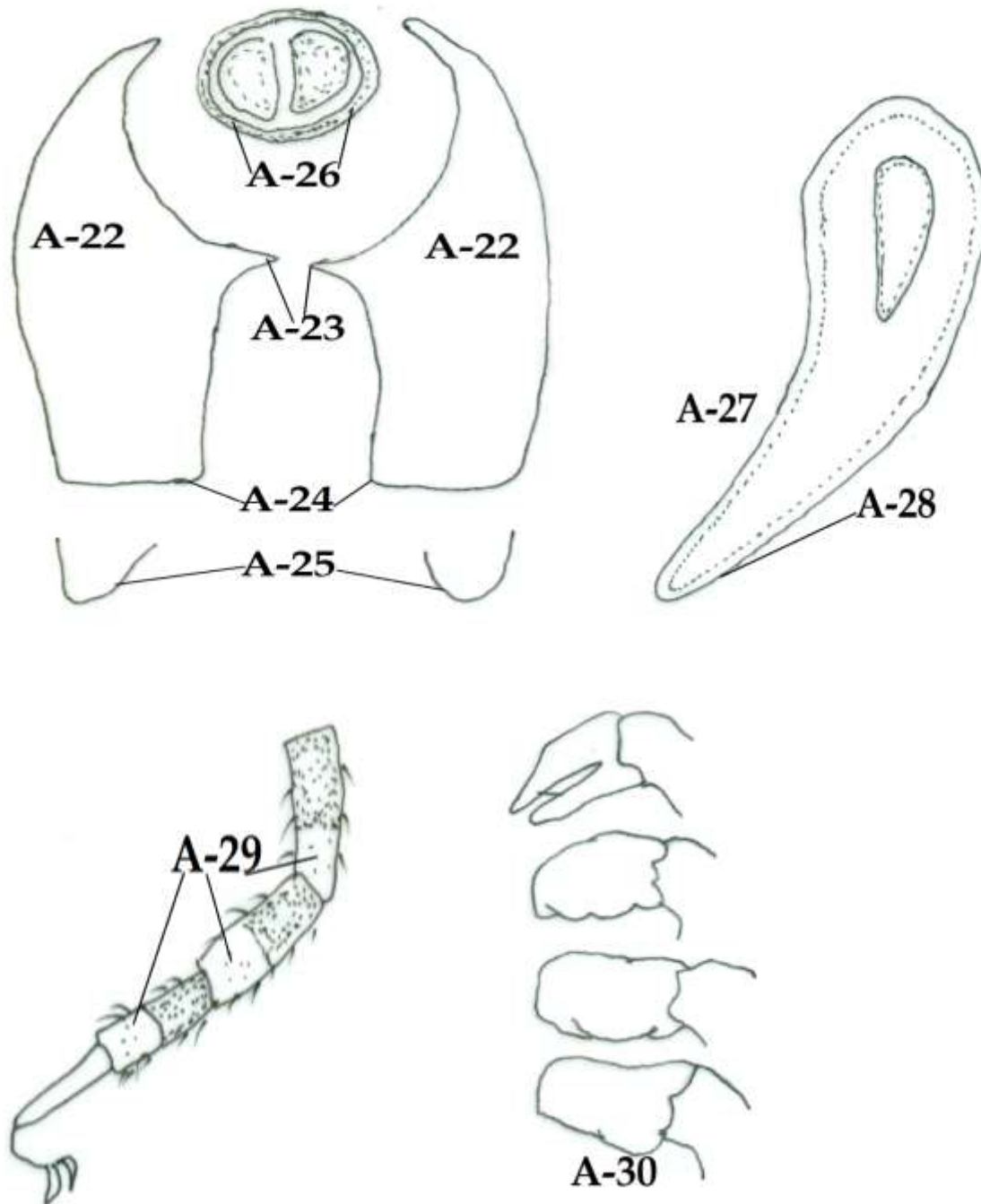
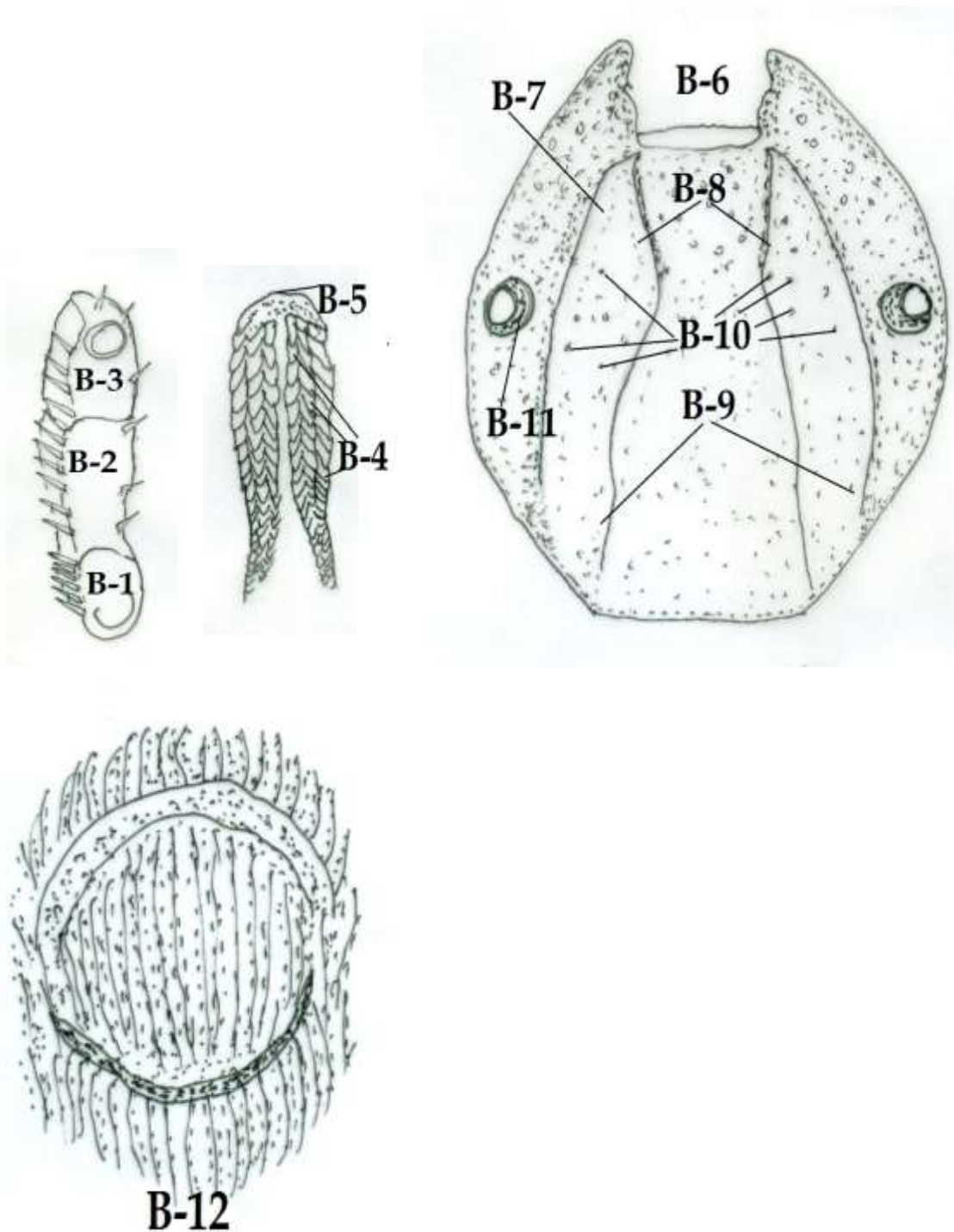


Fig. 1. Male *Hyalomma (Euhyalomma) anatolicum excavatum* Koch, 1844: A-1, Capitulum; A-2 Basis Capituli, A-3, Lateral dorsal projections present; A-4, Lateral posterior margins a little concave; A-5, Short cornua present; A-6, Segment-I of Palpi; A-7, Segment-II of Palpi; A-8, Segment-III of Palpi; A-9, Hypostome dentition 3/3 per File; A-10, Apex of Hypostome gently flattened; A-11, Consutum; A-12, Cervical grooves moderately long; A-13, Lateral grooves short; A-14, Posterior ridges two in numbers; A-15, Postero-median groove present; A-16; Central festoon present; A-17, Paracentral festoons joined anteriorly in the form of an arch; A-18, Paramedian grooves small; A-19, Punctations size relatively large but localized; A-20, Eyes; A-21, Genital structure;



_____ 1, 2, 29, 30
 _____ 3-28

Fig. 1. Male *Hyalomma (Euhyalomma) anatolicum excavatum* Koch, 1844: A-22, Adanal plates; A-23, Median projections apparent; A-24, Hind basal margins almost with square ends; A-25, Sub-anal plates short and aligned with adanals; A-26, Anus; A-27, Spiracular plate; A-28, Perforated part of dorsal prolongation slightly curved throughout its length; A-29, Broad yellow patches at the middle segments of legs; A-30, Coxae. Scale bars: 1, 2, 29, 30=0.1 mm (4X10), 3-28 =0.1 mm (10X10).



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Fig. 2. Female *Hyalomma (Euhyalomma) anatolicum excavatum* Koch, 1844 : B-1, Segment-I of Palpi; B-2, Segment-II of Palpi; B-3, Segment-III of Palpi; B-4, Hypostome dentition 3/3 per File; B-5, Apex of Hypostome gently flattened; B-6, Scutum; B-7, Scapular depressions almost reaching to the hind margin of the scutum; B-8, Cervical grooves; B-9, Lateral grooves; B-10, Punctations size small; B-11, Eyes; B-12, Genital operculum. Scale bars: 1-12=0.1 mm (10X10).

Female (4 specimens examined): ♀ are more or less like males in size.

Capitulum: Relatively longer than that of ♂.

Basis capituli: Dorsal lateral-posterior part broad but angular, posterior margin straight but moderately concave, dorsal cornua not clearly visible.

Palpi: Relatively long but broad, shape much as in ♂, segment-I bearing more than five ventro-median setae (Fig.B-1), segment-II relatively long and with 6-8 setae (Fig.B-2) situated at the margin, segment-III with few setae but apex slightly rounded (Fig.B-3).

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-4), apex of hypostome flattened (Fig. B-5), sides gently curved.

Scutum (Fig.B-6): Almost as long as broad, heart-shaped, black brown in color, whitish pigment usually present, covers only a portion of the dorsal surface at the anterior end, maximum width in front of eyes, almost as broad as long but in few specimens longer than wide, scapular depressions obvious (Fig.B-7) and almost reaching to the hind margin of scutum, cervical grooves (Fig.B-8) and lateral grooves (Fig.B-9) more deeper anteriorly and shallow posteriorly, punctuations size small (Fig.B-10) but localized mostly in anterior region of scutum.

Eyes (Fig.B-11): Prominent, round, convex and peripheral.

Genital structures (Fig.B-12): Genital aperture posterior lips broad U-shaped, anterior groove of genital operculum deep, pre-atrial fold a little convex and obviously bulging, borne between coxae-II and III.

Spiracular plates: Relatively broad, irregularly oval, perforated part of dorsal projections a little curved but narrow, tail relatively narrow, circumspiracular setae present but sparse.

Adanal and sub-anal plates: Absent.

Legs: Usually long, legs coloration with pale rings and similar to ♂.

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. anatolicum excavatum* is a two host or 3-hosts species (Schuler,1979). Large domestic cattle (sheep, goats, donkeys and horses) 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. This tick is mostly prevalent in Mediterranean and North African regions (Tunisia: Bouattour *et al.*, 1996). Central Asia (Uzbekistan: Rasulov, 2007). Saudi Arabia (Al-Khalifa *et al.*, 1983, 1984). Yemen (Pegram *et al.*, 1982). Turkey (Aktas *et al.*, 2004; Gunes, 2006). Afghanistan (Kaiser and Hoogstraal, 1963). Iran (Nabian and Rahbari, 2008; Nabian *et al.*, 2009; Dehaghi *et al.*, 2010). Pakistan (Kakar and Kakarsulemankhel, 2008).

Comparative note: This species shares on the genus level some characters assigned for the genus *Hyalomma*. *Hyalomma anatolicum anatolicum* Koch is closely similar to *H. anatolicum excavatum*. ♂ of this species discriminated from those of other species of the genus *Hyalomma* by their comparatively larger size (*Hyalomma anatolicum anatolicum* comparatively small in size), conscutum wide oval in shape, whereas ♂ conscutum of *Hyalomma anatolicum* is narrow. whitish pigments often present whereas white pigments absent on scutum and conscutum of ♂ and ♀ of *Hyalomma anatolicum*, posteromedian groove separated from parma by a little higher elevations, paracentral festoons joined anteriorly to form an arch, whereas paracentral festoons are separated anteriorly in *Hyalomma anatolicum*, punctuations size large (small in *Hyalomma anatolicum*), hind basal margins of adanal plates with square ends (rounded in *Hyalomma anatolicum*). However, ♀ *H. anatolicum anatolicum* can be very easily identified from those of other species of *Hyalomma* by their relatively larger size than of *Hyalomma anatolicum*, scutum with black brown in color, scutum sides steep and margins curved than female scutum of *Hyalomma anatolicum*, scapular grooves deep whereas shallow in *Hyalomma anatolicum*, whitish pigment present,

posterior-lateral ridges of scutum quite obvious, cervical and lateral grooves relatively more deeper than in *Hyalomma anatolicum*, genital operculum anterior groove is deep (shallow in *Hyalomma anatolicum*).

DISCUSSION

Hyalomma anatolicum excavatum also known as the larger Anatolian Hyalomma and its adults usually found attached with domestic animals whereas their immature stages parasitize rodents, hares and hedgehogs. Since no taxonomic work on *H. anatolicum excavatum* 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 sites 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.

Disease relationships of *Hyalomma anatolicum excavatum* is poorly known however, Gunes (2006) has been found this tick in Turkey to transmit virus responsible for causing Crimean-Congo Hemorrhagic fever (CCHF). However, this fact is yet to be explored in the Province Balochistan by initiating research in this direction.

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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