Scanning electron microscopy and morphometrics of nymph and larva of the tick *Hyalomma rufipes* Koch, 1844 (Acari: Ixodidae)

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Abstract The genus *Hyalomma* comprises the most ixodid tick species that parasitize camels in Egypt. Although the immature stages of tick species play an important role in distribution of ticks and tick-borne diseases, the identification depends mainly on the adult stage. Therefore, this study tries to identify the specific characteristics of both nymph and larva of *Hyalomma rufipes* Koch, 1844 using scanning electron microscopy and morphometric analysis in order to differentiate them easily from those of other *Hyalomma* spp. described before in Egypt. Results showed that the nymph and larva of *H. rufipes* can be easily identified from those of *H. excavatum* Koch, 1844, *H. dromedarii* Koch, 1844 and *H. impressum* Koch, 1844 but they are strongly close to *H. marginatum* Koch, 1844. The nymph of *H. rufipes* can be distinguished from *H. marginatum* by the number and distribution of dorsal and ventral idiosomal setae and the distribution of sternal setae. All morphological characteristics of *H. rufipes* larva resemble those of *H. marginatum* larva. The measurements of nymph and larva structures of *H. rufipes* are significantly lower than those of *H. marginatum*.

Keywords Morphology · Systematic ·
Hyalomma excavatum · *Hyalomma dromedarii* ·
Hyalomma marginatum · *Hyalomma impressum* ·
Hyalomma rufipes

Introduction

Immature stages of ixodid ticks play an important role in the distribution of ticks (Ghosh et al. 2007; Ica et al. 2007; Rahbari et al. 2007; Hartelt et al. 2008) and transmission of tick-borne diseases (Hubalek and Halouzka 1998; Murrell et al. 2003; Adham et al. 2009; Aktas et al. 2009; Ramos et al. 2010) because they infest small mammals (Kiffner et al. 2011) and birds (Literak et al. 2007; Ogrzewalska et al. 2010) which either move from place to place as rodents or migrate across different countries as migratory birds. In Egypt, the genus *Hyalomma* comprises the most ixodid tick species that parasitize camels. The most dominant species were *H. dromedarii*, *H. impeltatum* Schulze et Schlottke, 1930, *H. excavatum*, *H. anatolicum* Koch, 1844, *H. truncatum* Koch, 1844, *H. marginatum*, *Hyalomma rufipes*, *H. turanicum* Hoogstraal et Kaiser, 1960, *H. schulzei* Olenev, 1931 and *H. impressum* (Diab et al. 2001; El Kammah et al. 2001; Abdel-Shafy et al. 2011).

Scanning electron microscope (SEM) provides an excellent tool for studying the undistorted form and fine structure of ticks. Use of this instrument allows recognizing similarities and differences between ticks never seen previously by light microscope (LM) (Homsher and Sonenshine 1975). SEM and morphometric analysis were used for description of the immature stages of some *Hyalomma* spp. parasitizing camels in Egypt such as *H. excavatum*, *H. dromedarii*, *H. marginatum* (Abdel-Shafy 2008a, b) and *H. impressum* (Abdel-Shafy et al. 2011). The morphological characteristics which aid to identify nymph tick species from others are chaetotaxy of dorsal and ventral idiosoma, chaetotaxy of scutum, the position of sternal setae especially front of coxae II and III, the shape and size of spurs on coxae and the shape of spiracular plate and intensity of its pores (Abdel-Shafy 2008a). It was found that the nymph

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of *H. marginatum* and *H. impressum* could be easily identified. However, *H. excavatum* is close to *H. dromedarii* but the most measurements of the later are significantly higher. In larva, in spite of the similarities between *Hyalomma* species are higher than in nymph stage, the two species *H. marginatum* and *H. impressum* could also be identified. The most obvious character showing the differences between *Hyalomma* species is the scutum that has hexagonal shape in *H. marginatum*, pentagonal shape in *H. impressum* and tetragonal shape in each of *H. excavatum* and *H. dromedarii*. The larva of the latter is larger than that of *H. excavatum* (Abdel-Shafy 2008b; Abdel-Shafy et al. 2011).

Hyalomma rufipes is the widest spread *Hyalomma* in Africa. It also occurs in Southern Europe and extends eastwards to Asia. The main hosts of adults are camels, cattle, sheep, goats, horses and wild ungulates (El Kammah et al. 2001; Estrada-Peña et al. 2004). The immature stages feed on hares and ground frequenting bird. It has two-host life cycle. It is a vector of the virus causing Crimean Congo Haemorrhagic Fever in humans. It also transmits the bacterium *Anaplasma marginale* to cattle causing bovine anaplasmosis, the bacterium *Rickettsia conorii* causing tick typhus in humans and the protozoa *Babesia occultans* to cattle. Immature stages of *H. rufipes* were previously described by using SEM (Arthur 1975a, b) and LM (Apanaskevich and Horak 2008). The first author omitted completely the chaetotaxy of dorsal and ventral idiosoma, while the second authors gave a morphological description for *H. marginatum* complex and only measurements of some structures in either nymph or larva of *H. rufipes*. Therefore, the present study aimed to clarify in details by using SEM and morphometrics the morphological description of nymph and larva of *H. rufipes* and to give a morphological comparison between the immature stages of this tick species and other *Hyalomma* spp. that most recorded in Egypt. Some important additional taxonomic characters may be added that help in distinguishing nymph and larva of *H. rufipes* from other *Hyalomma* species.

Materials and methods

Specimens of larvae and nymphs

Specimens of *H. rufipes* Koch, 1844 were collected from camel at camel market, Giza, Egypt. Ticks were identified according to Hoogstraal (1956) and Estrada-Peña et al. (2004). A single engorged female was incubated at 27 °C and 75 % RH and checked daily to obtain the eggs. Eggs were placed in a new cup and incubated at the same condition until they hatched to larvae. One week post hatching, larvae were divided into two groups. The first

group was fed on rabbits, checked daily to follow the engorgement of larvae and observed their moulting to nymphs that occurred on rabbit. The second group of larvae and nymphs moulted from the engorged larvae of the first group were placed in water at 70 ± 10 °C, washed with 0.9 % KCl several times and preserved in 70 % ethanol (Famadas et al. 1997).

Preparation of larvae and nymphs for scanning electron microscopy

Larvae and nymphs were thoroughly cleaned by overnight immersion in water–glycerol–KCl solution at 40 °C (Homsher and Sonenshine 1977). This solution composed of 96.6 % (by weight) glycerol combined with 0.05 % (by weight) of potassium chloride (KCl) and 3.35 % (by weight) of distilled water (Brody and Wharton 1971). Specimens were washed in tap water again using the ultrasonic cleaner. Then they were taken through graded series of alcohol/water (25, 50, 75 and 100 % ethyl alcohol) remaining 1 h in each dilution except 10 min for 100 % ethanol (Keirans et al. 1976). Following this, specimens were glued by their dorsal and ventral surfaces to the SEM stub, and were dried by the dryer (Blazer Union, F1-9496 Blazer/Fürstentun Liechtenstein), using liquid carbon dioxide. Specimens mounted on SEM stubs were coated with gold by using a S150A Sputter Coater. Coated larvae and nymphs were examined by SEM.

Preparation of larvae and nymphs for morphometric measurements

Larvae and nymphs preserved in 70 % alcohol were put in lactic acid for 24 h without heating for clearing. Internal organs of specimens were removed with fine sharp needle under a dissecting microscope after which they were washed with distilled water. These specimens were taken through gradual series of alcohol/water as above, transferred to 1:1 absolute alcohol:xylene for 5 min and mounted on clean slides using Canada Balsam. Slides were put on hot plate (30 °C) for 48 h. Measurements of 10 specimens from each larva and nymph were given in millimeters by using optical microscope.

Many structures of larvae and nymphs were measured as follows; body length from apex of palpi to posterior end of idiosoma, body width or idiosoma width between two lateral sides' behind coxae III for larvae and coxae IV for nymphs, idiosoma length from scapula to posterior end of idiosoma, scutum length across longitudinal axis from scapula to posterior end of scutum, scutum width across transverse axis including eyes, basis capituli length from base of hypostome to posterior end of basis capituli dorsally and ventrally, basis capitulum width across the widest

transverse axis, hypostomal length from the apex of hypostome to the last denticle of the outer file posteriorly, hypostomal width and palpal length from the base of segment I to the apex of segment III.

Results

Nymph

Dorsal idiosoma

Body Length (including gnathosoma or capitulum) is 1.181 mm, shorter than those of all *Hyalomma* spp., insignificantly comparing with *H. excavatum* and significantly comparing with other *Hyalomma* spp. Idiosoma length is 1.010 mm, approximately equal that of *H. impressum*, significantly longer than that of *H. excavatum* and shorter than those of *H. dromedarii* and *H. marginatum*. Idiosoma width is 0.743 mm, significantly wider than that of *H. excavatum* and narrower than those of other *Hyalomma* species. Idiosoma length/idiosoma width ratio is 1.359 ± 0.031 , which is significantly higher than those of *H. marginatum* and *H. impressum* and lower than those of *H. excavatum* and *H. dromedarii*. The body showed maximum width behind scutum and narrowing noticeably anteriorly; alloscutum with furrowed surface, with large number of uniformly punctuations and 48 pairs of strong setae (excluding scutum) dense peripherally (Table 1; Fig. 1a–d).

Scutum Slightly wider than length; the length is 0.515 ± 0.003 mm, approximately equal to that of *H. excavatum*, significantly longer than that of *H. impressum* and shorter than those of *H. dromedarii* and *H. marginatum*. The width is 0.601 ± 0.003 mm, approximately equal to those of *H. impressum* and *H. dromedarii*, significantly wider than that of *H. excavatum* and narrower than that of *H. marginatum*. The ratio of scutum length/scutum width is 0.858 ± 0.004 , significantly higher than that of *H. impressum* and lower than those of other *Hyalomma* species. The scutum has a pair of circular eyes at its greatest width; posterior margin is broadly rounded, postero-lateral margins are slightly concave behind the level of eyes, anterolateral margins are mildly convex and convergent to broadly round scapulae. Cervical grooves are convergent and deep for short distance anteriorly then divergent as shallow grooves until the edges of postero-lateral margins; surface reticulately patterned, convex between the grooves. Moreover, thirteen pairs of small setae are demonstrated, one central, one in front of postero-lateral margins, two adjacent to the eyes, two anterior and seven lateral at the edge of antero-lateral margins (Table 1; Fig. 1b, c).

Ventral idiosoma

The surface is furrowed with many small punctuations and few large punctuations. The ventral number of setae are 30 pairs (excluding coxal setae): sternal (six pairs), preanal (three pairs), anal (three pairs), premarginal (nine pairs) and marginal (nine pairs). The position of sternal setae according to coxae is: one setae front of coxa I and IV, two horizontal setae front of coxa II and two vertical setae front of coxa III. Each coxa with three setae; coxa I with two long narrow subtriangular spurs nearly equal in length, cleft between internal and external spurs forms large triangular, spurs narrowly rounded apex; coxae (II–IV) with moderate external spur for each; the spurs are consequently decreasing in size from coxa II to IV. Spiracle is egg shaped, broad blunt at apex, macula antero-mesial with large pores around it, remaining pores are smaller and more numerous (Fig. 2a–d).

Gnathosoma

Palpus The external margins are straight, segment 2 broadest distally and narrowing to insertion with segment I. The internal margin is convex, broad rounded apically. The suture lines between palpal segments are discernible. The palpus do not project beyond the hypostome. Surface of segment II with prominent oblique furrow; with ten setae dorsally and six setae ventrally. Statistical analysis for palpal length revealed that *H. rufipes* (0.206 ± 0.001 mm) is significantly smaller than all *Hyalomma* species (Table 1; Fig. 3a–b).

Hypostome It is cylindrical in shape, dental formula is 2/2, teeth number per file (excluding small basal and apical teeth) is nine in the outer file and eight in the inner file, a pair of posthypostomal setae, flattened apically, pair of closely set depressions between posthypostomal setae. The hypostomal length is 0.179 mm which is approximately equal to that of *H. excavatum*, significantly longer than that of *H. impressum* and significantly shorter than those of *H. marginatum* and *H. dromedarii*. The hypostomal width is 0.079 mm which is approximately equal to that of *H. excavatum*, significantly narrower than those of *H. marginatum* and significantly wider than those of *H. impressum* and *H. dromedarii* (Table 1; Fig. 3b).

Basis capitulum Dorsally, it is subtriangular in shape without setae, the posterior margin is straight, the postero-lateral margins are straight and forming sharp angles with antero-lateral margins. The length is 0.137 ± 0.002 mm, significantly longer than those of *H. impressum* and *H. excavatum*, significantly shorter than those of *H. marginatum* and *H. dromedarii*. The width is 0.329 ± 0.001 mm, approximately equal to those of *H. excavatum* and *H. dromedarii*, significantly wider than that of *H. impressum*,

Table 1 Morphometric of different structures of *Hyalomma rufipes* nymphs in comparison with those in other *Hyalomma* species

Character	Measurements (mm) $\pm$ SE					F value	P value	Sig.
	<i>H. rufipes</i>	<i>Hyalomma</i> spp. (Abdel-Shafy 2008a; Abdel-Shafy et al. 2011)						
	<i>H. impressum</i>	<i>H. excavatum</i>	<i>H. dromedarii</i>	<i>H. marginatum</i>				
Body-length	1.181 $\pm$ 0.000a	1.264 $\pm$ 0.006b	1.219 $\pm$ 0.010a	1.472 $\pm$ 0.014c	1.589 $\pm$ 0.025d	160.340	0.000	**
Idiosoma-length	1.010 $\pm$ 0.000b	1.000 $\pm$ 0.006b	0.936 $\pm$ 0.005a	1.239 $\pm$ 0.018c	1.335 $\pm$ 0.024d	158.915	0.000	**
Idiosoma-width	0.743 $\pm$ 0.000b	1.005 $\pm$ 0.005d	0.633 $\pm$ 0.007a	0.795 $\pm$ 0.019c	1.148 $\pm$ 0.019e	273.669	0.000	**
Idiosoma-length/idosoma-width	1.359 $\pm$ 0.031c	0.995 $\pm$ 0.001a	1.480 $\pm$ 0.018d	1.564 $\pm$ 0.028e	1.164 $\pm$ 0.020b	176.839	0.000	**
Scutum-length	0.515 $\pm$ 0.003b	0.473 $\pm$ 0.003a	0.531 $\pm$ 0.010b	0.573 $\pm$ 0.008c	0.717 $\pm$ 0.026d	50.609	0.000	**
Scutum-width	0.601 $\pm$ 0.003b	0.608 $\pm$ 0.003b	0.552 $\pm$ 0.010a	0.603 $\pm$ 0.018b	0.749 $\pm$ 0.022c	30.148	0.000	**
Scutum-length/Scutum-width	0.858 $\pm$ 0.004b	0.778 $\pm$ 0.007a	0.965 $\pm$ 0.025c	0.955 $\pm$ 0.018c	0.967 $\pm$ 0.052c	9.386	0.000	**
Palal-length	0.206 $\pm$ 0.001a	0.263 $\pm$ 0.003b	0.270 $\pm$ 0.000c	0.300 $\pm$ 0.000d	0.300 $\pm$ 0.003d	514.194	0.000	**
Length of dorsal basis capitulum	0.137 $\pm$ 0.002c	0.109 $\pm$ 0.001a	0.126 $\pm$ 0.004b	0.144 $\pm$ 0.002c,d	0.145 $\pm$ 0.002d	36.586	0.000	**
Length of ventral basis capitulum	0.148 $\pm$ 0.002a	0.154 $\pm$ 0.001a	0.186 $\pm$ 0.002b	0.219 $\pm$ 0.002c	0.215 $\pm$ 0.004c	152.931	0.000	**
Width of basis capitulum	0.329 $\pm$ 0.001b	0.270 $\pm$ 0.000a	0.333 $\pm$ 0.002b	0.324 $\pm$ 0.002b	0.369 $\pm$ 0.007c	111.081	0.000	**
Hypostom-length	0.179 $\pm$ 0.000b	0.158 $\pm$ 0.003a	0.186 $\pm$ 0.002b	0.204 $\pm$ 0.002c	0.244 $\pm$ 0.005d	127.230	0.000	**
Hypostom-width	0.079 $\pm$ 0.000c	0.068 $\pm$ 0.000a	0.081 $\pm$ 0.002c	0.073 $\pm$ 0.003b	0.090 $\pm$ 0.001d	49.009	0.000	**

Different letters within the same row are significant according to Duncan test

** High significant at $P < 0.01$

significantly narrower than that of *H. marginatum*. Ventrally, it is tetragonal in shape with two pairs of setae laterally; posterior and postero-lateral margins forms bow-shaped; the length is 0.148 ± 0.002 mm which approximately equal to that of *H. impressum* and shorter than those of other *Hyalomma* species (Table 1; Fig. 3a, b).

Larva

Dorsal idiosoma

Body Length (including gnathosoma or capitulum) is 0.468 ± 0.003 mm, idiosoma length is 0.371 ± 0.003 mm, idiosoma width is 0.308 mm and the ratio of idiosoma length/idiosoma width is 1.204 ± 0.009 . These measurements are significantly lower than those in the other *Hyalomma* species except the ratio of idiosoma length/idiosoma width is insignificantly higher than that of *H. dromedarii* and significantly higher than those of other *Hyalomma* species. The body is elongated oval shaped, widest at midlength, narrowest anteriorly across the scapulae, broadly rounded posteriorly. The dorsal arrangement of setae (chaetotaxy) resembles that on the other tick species. Dorsal larva has 13 pairs of setae; eight marginal, two central and three scutal (one in lateral field, one in anterior and one in central). The

posterior margin is divided into nine festoons which resembles that in the other tick species (Table 2; Fig. 4a, b).

Scutum The length is 0.237 ± 0.003 mm, approximately equal to those of *H. impressum* and *H. excavatum*, significantly longer than that of *H. dromedarii* and shorter than that of *H. marginatum*. The width is 0.269 mm, significantly narrower than those of all *Hyalomma* species. The ratio of scutum length/scutum width (0.882) is significantly higher than those of all *Hyalomma* species. Anterolateral margins are mildly convex, broader than longer; cervical grooves are narrow, deep extending for less than half distance from margin to eyes, convergent posteriorly; the eyes have distinct oval, convex, peripheral and at greatest width of scutum. It is approximately hexagonal in shape with reticulate surface. Posterior margin is straight or slightly convex, posterolateral margins are straight (Table 2; Fig. 4a, b).

Ventral idiosoma

The ventral number of setae is 15 pairs (excluding coxae): three sternal, two preanal, one anal, four premarginal and five marginal. Coxa I has large spur with rounded apex; coxae II–III has smaller rounded apex spur for each. The spurs are consequently decreasing in size from coxa I to III. Coxa I has three setae. Coxa II and III has two setae for each (Fig. 5a–c).

Fig. 1 Dorsal view of *Hyalomma rufipes* nymph: **a** the entire idiosoma, **b** scutum, **c** the half left of scutum, **d** the posterior half left of idiosoma

Fig. 2 Ventral view of *Hyalomma rufipes* nymph: **a** the entire idiosoma, **b** coxae, **c** the posterior half left of idiosoma, **d** spiracle

Fig. 3 Capitulum of *Hyalomma rufipes* nymph: **a** dorsal view, **b** ventral view

Gnathosoma

Palpus External margins are straight; internal margins are convex, broad and rounded apically; suture lines between palpal segments are not discernible; palpi are not projected beyond the hypostome, surface with few transverse furrows, with eight setae dorsally, three setae ventrally and one seta apically. Statistical analysis for palpal length revealed that *H. rufipes* (0.109 ± 0.002 mm) is approximately equal to that of *H. excavatum*, significantly longer than that of *H. dromedarii* and *H. marginatum* (Table 2; Fig. 6a, b).

Hypostome It is cylindrical in shape; the dental formula is 2/2, teeth number per file (excluding small basal and apical teeth) is 9 in the outer file and 8 in the inner file. The hypostomal length is 0.065 mm which is significantly shorter than that in other *Hyalomma* species. The hypostomal width is 0.034 mm which is significantly wider than that of *H. impressum* and narrower than that of the others (Table 2; Fig. 6b).

Basis capitulum Dorsally, it is subtriangular in shape without setae; posterior margin is straight, posterolateral margins are straight and forming pointed edges with anterolateral margins those are sinuous and convergent to

bases of external cheliceral sheaths. The length is 0.030 ± 0.001 mm, significantly shorter than that in other *Hyalomma* species. The width is 0.133 ± 0.001 mm, significantly wider than that of *H. impressum* and narrower than that in other *Hyalomma* species. Ventrally, it is tetragonal in shape, with one pair of post hypostomal setae; posterior margin slightly convex, lateral margins undulate. The length is 0.074 ± 0.001 mm which approximately equal to *H. dromedarii*, significantly shorter than that in other *H. marginatum*, significantly longer than that of each *H. impressum* and *H. excavatum* (Table 2; Fig. 6a, b).

Discussion

The genus *Hyalomma* comprises the most ixodid tick species that parasitize domestic animals in Egypt (El Kammah et al. 2001). Although the immature stages of tick species play an important role in the distribution of ticks and tick-borne diseases, the identification depends mainly on the adult stage. Therefore, the present study tries to identify the specific characteristics of both nymph and larva of *H. rufipes* in order to differentiate them easily from other *Hyalomma* spp. that described before in Egypt (Abdel-Shafy 2008a, b; Abdel-Shafy et al. 2011). These characters may have taxonomic importance and aid in distinguish nymph and larva of *H. rufipes* from those of other *Hyalomma* species. There are two publications introduced brief descriptions for the immature stages of *H. rufipes*, one of them used SEM (Arthur 1975a, b) and another used LM (Apanaskevich and Horak 2008).

Regarding to nymph description, the most features of dorsal idiosoma agreed with those recorded before by Arthur (1975a) excepting the length of idiosoma, he found that it ranged between 1.7 and 1.8 mm longer than that recorded in the present study (1.01 mm). This difference may be attributed to the differences between hosts which were used in rearing ticks to obtain the immature stages. The structures added and not recorded before in dorsal idiosoma of nymph were the number of alloscutum setae (48 pairs), idiosomal length (1.01 mm), idiosomal width (0.74 mm) and the ratio of length to width (1.36). The number of setae on dorsal idiosoma (excluding scutum) or alloscutum was lower (48 pairs) than those in *H. marginatum* (67 pairs) and *H. dromedarii* (55 pairs) and higher than those in *H. excavatum* (43 pairs) and *H. impressum* (27 pairs) (Abdel-Shafy 2008a; Abdel-Shafy et al. 2011). Moreover, the most structures of scutum agreed with those recorded by Arthur (1975a) except the number and distribution of scutal setae. He observed only four pairs of small setae in the median field of scutum and omitted the others. In this study, the scutal setae are 13 pair distributed as, one central, one in front of postero-lateral margins, 2 adjacent

Table 2 Morphometric of different structures of *Hyalomma rufipes* larvae in comparison with those in other *Hyalomma* species

Character	Measurements (mm) $\pm$ SE					F value	P value	Sig.
	<i>H. rufipes</i>	<i>Hyalomma</i> spp. (Abdel-Shafy 2008b; Abdel-Shafy et al. 2011)						
		<i>H. impressum</i>	<i>H. excavatum</i>	<i>H. dromedarii</i>	<i>H. marginatum</i>			
Body-length	0.468 $\pm$ 0.003a	0.513 $\pm$ 0.006b	0.620 $\pm$ 0.011c	0.672 $\pm$ 0.008d	0.741 $\pm$ 0.004e	277.166	0.000	**
Idiosoma-length	0.371 $\pm$ 0.003a	0.426 $\pm$ 0.002b	0.527 $\pm$ 0.016c	0.575 $\pm$ 0.005d	0.582 $\pm$ 0.009d	124.987	0.000	**
Idiosoma-width	0.308 $\pm$ 0.000a	0.426 $\pm$ 0.002b	0.486 $\pm$ 0.014c	0.487 $\pm$ 0.011c	0.522 $\pm$ 0.015d	64.125	0.000	**
Idiosoma-length/idosoma-width	1.204 $\pm$ 0.009c	1.000 $\pm$ 0.007a	1.085 $\pm$ 0.019b	1.185 $\pm$ 0.025c	1.123 $\pm$ 0.034b	14.550	0.000	**
Scutum-length	0.237 $\pm$ 0.003b	0.240 $\pm$ 0.003b	0.234 $\pm$ 0.005b	0.221 $\pm$ 0.004a	0.327 $\pm$ 0.006c	100.799	0.000	**
Scutum-width	0.269 $\pm$ 0.000a	0.300 $\pm$ 0.003b	0.344 $\pm$ 0.005c	0.359 $\pm$ 0.004d	0.401 $\pm$ 0.004e	188.052	0.000	**
Scutum-length/Scutum-width	0.882 $\pm$ 0.012d	0.801 $\pm$ 0.014c	0.681 $\pm$ 0.010b	0.615 $\pm$ 0.009a	0.816 $\pm$ 0.008c	104.840	0.000	**
Palal-length	0.109 $\pm$ 0.002b	0.096 $\pm$ 0.002a	0.105 $\pm$ 0.000b	0.105 $\pm$ 0.000b	0.150 $\pm$ 0.000c	223.636	0.000	**
Length of dorsal basis capitulum	0.030 $\pm$ 0.001a	0.039 $\pm$ 0.001b	0.045 $\pm$ 0.000c	0.054 $\pm$ 0.002d	0.066 $\pm$ 0.002e	69.866	0.000	**
Length of ventral basis capitulum	0.074 $\pm$ 0.001b	0.065 $\pm$ 0.002a	0.068 $\pm$ 0.000a	0.072 $\pm$ 0.001b	0.092 $\pm$ 0.001c	76.974	0.000	**
Width of basis capitulum	0.133 $\pm$ 0.001b	0.120 $\pm$ 0.000a	0.147 $\pm$ 0.002c	0.146 $\pm$ 0.002c	0.156 $\pm$ 0.003d	62.399	0.000	**
Hypostom-length	0.065 $\pm$ 0.000a	0.069 $\pm$ 0.002b	0.090 $\pm$ 0.000c	0.090 $\pm$ 0.000c	0.135 $\pm$ 0.000d	643.917	0.000	**
Hypostom-width	0.034 $\pm$ 0.000b	0.030 $\pm$ 0.000a	0.036 $\pm$ 0.001c	0.044 $\pm$ 0.001d	0.045 $\pm$ 0.001d	101.75	0.000	**

Different letters within the same row are significant according to Duncan test

** High significant at $P < 0.01$

to the eyes, 2 anterior and 7 lateral at the edge of antero-lateral margins. Arthur (1975a) also omitted the measurements of scutum, while Apanaskevich and Horak (2008) measured length and width of scutum which were higher than those recorded in this study. Scutum length is close to that of *H. excavatum* and *H. impressum* and it is obviously shorter than those in *H. marginatum* and *H. dromedarii*. Scutum width is lower than that in *H. marginatum* and close to those in other *Hyalomma* species. The number of scutal setae of *H. rufipes* is higher than that in *H. impressum* (8 pairs) and lower than those in other *Hyalomma* spp.; *H. excavatum* (14 pairs), *H. dromedarii* (15–17 pairs) and *H. marginatum* (14 pairs). Therefore, it can easily identify the scutum of *H. rufipes* from *H. excavatum*, *H. dromedarii* and *H. impressum*, while its shape is close to that of *H. marginatum*. The scutum of *H. rufipes* has two specific characteristics that help in identification of this tick species from *H. marginatum*. The first is postero-lateral margin which is slightly concave behind the level of eye in *H. rufipes*, while it is straight in *H. marginatum*. The second is the scutal setae near to the postero-lateral margins; one pair in *H. rufipes* and 2 pairs in *H. marginatum*. *Ventral idiosoma* Although the numbers of setae on ventral surface and its distribution have taxonomic important between *Hyalomma* nymphs, Arthur (1975a) and Apanaskevich and Horak (2008) fully omitted them. Ventral idiosom (excluding coxae) carries 30 pairs of setae, higher than

those in *H. dromedarii* (28 pairs) and *H. impressum* (28 pairs), and lower than those in *H. excavatum* (38 pairs) and *H. marginatum* (33 pairs). The distribution of setae resembles that in *H. dromedarii* and *H. impressum* with two additional pre-marginal pairs of setae. Chaetotaxy can help us in identification of *H. rufipes* from *H. excavatum* by one additional sternal pair of setae in *H. rufipes* and 6 additional marginal pairs of setae in *H. excavatum*. *H. marginatum* carries one additional pair of setae on each pre-anal, pre-marginal field comparing with those in *H. rufipes*. Furthermore, the most important character which helps in identification of *H. rufipes* from other *Hyalomma* species is the setal pattern on sternum especially setae those in front of coxae II and III. There is a pair of setae in front of each coxa I and IV like those in other *Hyalomma* species. *Hyalomma rufipes* has two horizontal pairs of setae in front of coxa II and two vertical pairs of setae in front of coxa III. There are two vertical pairs of setae in front of each coxa II and III in *H. dromedarii* and *H. impressum*. There are two horizontal pairs of setae in front of each coxa II and III in *H. marginatum*. There are one pair of setae in front of each coxa II and two vertical pairs of setae in front of coxa III in *H. marginatum*. Spurs and setae on coxae agreed with those observed before in *H. rufipes* (Arthur 1975a; Apanaskevich and Horak 2008). It was also found that the morphological characteristics of coxae resemble with those in other *Hyalomma* species described by Abdel-

Fig. 4 Dorsal view of *Hyalomma rufipes* larva: **a** the entire idiosoma, **b** scutum

Shafy (2008a) and Abdel-Shafy et al. (2011). Spiracle has egg-shape in agreement with the description of Arthur (1975a) and Apanaskevich and Horak (2008). On the other hand, this shape was observed in spiracle of *H. dromedarii*, *H. excavatum* and *H. impressum*. However, *H. marginatum* has semicircular spiracle. Therefore, the shape of spiracle helps in identification of *H. rufipes* from *H. marginatum*. **Palpus** Morphological characteristics of palpus agreed with those observed before by Arthur (1975a). It has 10 setae dorsally and 6 setae ventrally. It almost resembles that in other *Hyalomma* species. The length of palpus is smaller than that in other *Hyalomma* species. **Hypostome** Its description agreed with that recorded before by Arthur (1975a) and Apanaskevich and Horak (2008). It also resembles that of other *Hyalomma* species excepting the large hypostomal teeth those are 9 on the outer file and 8 on the inner file like those in *H. marginatum* with an additional tooth per file more than those in each *H. dromedarii*, *H. excavatum* and *H. impressum*. **Basis capitulum** Dorsally, it is subtriangular in shape like that in other *Hyalomma* species, while its postero-lateral margins are straight and forming sharp angles with antero-lateral margins like that in *H. marginatum*. Postero-lateral margins are also straight in *H. impressum* but they form blunt angle with antero-

Fig. 5 Ventral view of *Hyalomma rufipes* larva: **a** the entire idiosoma, **b** coxae, **c** the posterior half left of idiosoma

lateral margins. It can easily identify from *H. excavatum* and *H. dromedarii* by postero-lateral margins those are concave in these two species. Ventrally, it resembles all other *Hyalomma* specis. Then it can separate *H. rufipes* from other *Hyalomma* species through the dorsal capitulum excepting *H. marginatum* that has dorsal capitulum strongly resembles *H. rufipes*.

Regarding to larva description, the dorsal idiosoma resembles fully the dorsal idiosoma of either *H. rufipes* described by Arthur (1975b) and other *Hyalomma* spp. described by Abdel-Shafy (2008b) and Abdel-Shafy

Fig. 6 Capitulum of *Hyalomma rufipes* larva: **a** dorsal view, **b** ventral view

et al. (2011). The number of setae and its distribution on the dorsal surface of *H. rufipes* larvae are similar as in other *Hyalomma* species. So, there is no any specific character for *H. rufipes* on the dorsal surface of larvae. Only the measurements of body length, idiosomal length and idiosomal width significantly are the lowest: Morphological characteristics of scutum agreed with the description of Arthur (1975b). Apanaskevich and Horak (2008) measured only the length and width of scutum and they found the measurements were higher than those recorded in this study. It can identify easily scutum of *H. rufipes* from those in *H. excavatum* and *H. dromedarii* which have tetragonal shape scutum and *H. impressum* that have pentagonal shape scutum, while, it is difficult to distinguish the scutum of *H. rufipes* from that of *H. marginatum* because both of them have hexagonal shape scutum. The morphological characteristics and chaetotaxy of ventral idiosoma agreed with those described before in *H. rufipes* (Arthur 1975b) and in other *Hyalomma* species (Abdel-Shafy 2008b; Abdel-Shafy et al. 2011). Morphological characteristics, chaetotaxy and measurements of Palpus agreed with the descriptions of Arthur (1975b) and Apanaskevich and Horak (2008). The palpal setal pattern also agreed with that observed in other *Hyalomma* species (Abdel-Shafy 2008b;

Abdel-Shafy et al. 2011). The description of Hypostome agreed with that of Arthur (1975b). It is cylindrical shape like that in *H. impressum* and *H. marginatum*. It can be easily differentiated from those in *H. dromedarii* and *H. excavatum* because they have club-shape hypostome. Furthermore, the hypostome of *H. rufipes* has 9 and 8 teeth per outer and inner file like those in *H. marginatum*. However, other *Hyalomma* species have 8 and 7 teeth per outer and inner file for each. Basis capitulum agreed morphologically with that described by Arthur (1975b), its width also agrees with that of Apanaskevich and Horak (2008). Also it resembles that of other *Hyalomma* species that described before by Abdel-Shafy (2008b) and Abdel-Shafy et al. (2011).

Conclusion

The nymph and larva of *H. rufipes* can be easily identified from those of *H. excavatum*, *H. dromedarii* and *H. impressum* but they are strongly close to *H. marginatum*. The nymph of *H. rufipes* can be distinguished from *H. marginatum* by the number and distribution of dorsal and ventral setae and the distribution of sternal setae. All morphological characteristics of *H. rufipes* larva resemble those of *H. marginatum* larva. The measurements of nymph and larva of *H. rufipes* are significantly lower than those of *H. marginatum*.

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