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Creative Commons-BY-NC-ND which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.
SCANNING ELECTRON MICROSCOPY AND COMPARATIVE MORPHOLOGY OF HYALOMMA ANATOLICUM EXCAVATUM, H. DROMEDARII AND H. MARGINATUM MARGINATUM (ACARI: IXODIDAE) BASED ON LARVAE

BY S. S. ABDEL-SHAFY 1
(Accepted November 2007)

SUMMARY: The morphological differentiations between larvae of Hyalomma anatolicum excavatum, H. dromedarii and H. marginatum marginatum were studied by Scanning Electron Microscopy (SEM) and morphometric analysis. Results showed that H. m. marginatum is easy differentiated from other two tick species while H. a. excavatum is close to H. dromedarii. Scutum is tetragonal shape in H. a. excavatum and H. dromedarii, hexagonal shape in H. m. marginatum, scutal posterior margin sinuous in H. a. excavatum and widely rounded in H. dromedarii. Hypostome is club shape in H. a. excavatum and H. dromedarii, cylindrical shape in H. m. marginatum, the dental formula is 2/2 for each tick species, the teeth numbers per file are 7/6 for each H. a. excavatum and H. dromedarii and 9/8 for H. m. marginatum. Presence of well-developed spur on each coxa II and III for H. m. marginatum while this spur is very small or reduced in others. The measurements of body, scutal, palpi, basis capituli and hypostome of H. m. marginatum larvae were significantly higher than others. H. dromedarii was significantly higher than H. a. excavatum in case of body length, scutal width, length of basis capitulum and hypostomal width.

INTRODUCTION

The important ixodid tick genera infesting farm animal in Egypt are Hyaloma, Boophilus and Rhipicephalus. The genus Hyalomma includes a large number of tick species infesting a wide variety of hosts. The most abundant species belonging to this genus are Hyalomma anatolicum excavatum, H. dromedarii and H. marginatum marginatum (ABDEL-SHAFY S., 1994 & 2000).

H. a. excavatum is a parasite of cattle, horse, donkey, camel sheep, goat and swine. It also attacks man and dogs (HOOGSTRAAL, 1956). It feeds as a two- or three-host tick depending on availability of hosts. Larvae feed on horses, hedgehogs, rodents, lizards, birds and man (HOOGSTRAAL, 1956 and APANASKEVICH, 2004). It is found in North Africa from Mauritania to Egypt, Sudan, Ethiopia, Eritrea, Iran, Turkey, Europ in Italy and Greece (ESTRADA-Peña et al., 2004). It is the vector of the causative disease agents

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H. dromedarii is chiefly hosted by camel, but cattle, sheep, goat and horse are also infested (EL KAMMAH et al., 2001, AHMED et al., 2005 and GEBRE & KAAYA, 2006). The larvae feed on small borrowing animals, hares, reptiles, wild birds, more rarely on lizards. Man is uncommonly attacked by adults but more frequently by larvae. It is a two-host or three-host tick (HOOGSTRAAL, 1956, and APANASKEVICH, 2004). It is distributed in Africa and the near, middle and far east as far as India, Mongolia and Tibet (ESTRADA-PEÑA et al., 2004). It transmit Theileria annulata to cattle experimentally (ESTRADA-PEÑA et al., 2004), Anaplasma marginale, Coxiella burnetti, Rickettsia aeschlimannii, Ehrlichia spp. (LOFTIS et al., 2006) and bacteria (MONTASSER, 2005).

H. m. marginatum is a parasite only on domestic animals especially cattle, horse, sheep and camel (HOOGSTRAAL, 1956, CASTELLA et al., 2001, and TORINA et al., 2006). Birds are chief hosts of larvae (HOOGSTRAAL, 1956, OSACAR-JIMENEZ et al., 1998 and PAPADOPOULOS et al., 2001). Larvae feed also on small mammals such as hares and rabbits (HOOGSTRAAL, 1956). It is a two-host tick (APANASKEVICH, 2004). This species occurs in the Humid Mediterranean Climate of Northern Africa and Southern Europe and Steppe Climate further East wards (EL-KAMMAH et al., 2001, ESTRADA-PEÑA et al., 2004 and TORINA et al., 2006). It is the causative disease agents such as babesiosis, theileriosis, rickettsiosis and Crimean Congo hemorrhagic fever (MEISSNER et al., 2006).

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 microscopy (HOMSHER & SONENSHINE, 1975). The differentiations between immature stages of H. a. excavatum, H. dromedarii and H. m. marginatum are still difficult and literature data are inadequate. Therefore, this study clarify by use of SEM, morphological characters of the larval stage. The nymphaal stage will be investigated morphologically in the subsequent study.

Material and Methods

1- Specimens of Hyalomma Larvae: The specimens of Hyalomma spp. (commonly on farm animals in Egypt) were collected as engorged females from various localities and reared in laboratory to obtain their larvae. These species were H. anatolicum excavatum Koch, 1844; H. dromedarii Koch, 1844, from local camel, Marsa Matrouh, Egypt; and H. marginatum marginatum Koch, 1844, from Nikhil, Sinai, Egypt. Ticks were identified according to HOOGSTRAAL et al., (1956) HOOGSTRAAL et al., (1981) and ESTRADA-PEÑA et al., 2004. A single engorged female for each species 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 placed in water at 70 ± 10 °C, washed with normal saline 0.9% KCI several times and preserved in 70% ethanol (FAMADAS et al., 1997).

2- Preparation of Larvae for Scanning Electron Microscopy: Larvae were cleaned by overnight immersion in water-glycerol-KCI solution at 40 °C (HOMSHER & SONENSHINE, 1977), (96.6% (by weight) glycerol combined with 0.05% (by weight) of potassium chloride (KCI) and 3.35% (by weight) of distilled water) (BRODY & WHARTON, 1971). Specimens were washed in tap water again using the ultrasonic cleaner, then taken through a graded series of alcohol/water (25%, 50%, 75% and 100% ethyl alcohol) remaining one hour in each dilution (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/Fürsentunt Li weighted), using liquid carbon dioxide. Specimens mounted on SEM stubs were coated with gold by using a S150A Sputter Coater. Coated larvae were examined by SEM.

3- Preparation of Larvae for Morphometric Measurements: Specimens, preserved in 70% alcohol were put in lactic acid for 24 h without heating for clearing. Internal organs of larvae were removed with
SCUTUM: capitulum) is elongate oval shape, widest midlength, anteriorly, pal pal length from the base of segment I to the hypostome to the last denticle of the outer file posteriorly, from scapula to posterior end of idiosoma, scutum and ventrally, basis capitulum width across the widest axis including eyes, basis capituli length from base of length across longitudinal axis from scapula to posterior end of basis capituli dorsally for each species were given in millimeters by using These larvae were taken through a gradual series of plates using Canada Balsam. Slides were put on hot optical microscopy. Ralal-length 0.105 Width of basis capitulum Scutum-length/Scutum-width Scutum-width Scutum-length Signific a nt a t P 0.01**, NS for 48 h. Measurements for specimens revealed that larvae were washed with distilled water. These larvae were taken through a gradual series of alcohol/water as above, transferred to 1:1 absolute alcohol: xylene for 5 minutes and mounted on clean slides using Canada Balsam. Slides were put on hot plate (30°C) for 48 h. Measurements for 10 specimens for each species were given in millimeters by using optical microscopy.

Many structures of Hyalomma species were measured as follows: body length from apex of palpi to posterior end of idiosoma, body width between two lateral sides behind coxae III, 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, palpal length from the base of segment I to the apex of segment III.

RESULTS

1- BODY: The body of Hyalomma larvae (including capitulum) is elongate oval shape, widest midlength, narrow est anteriory across the scapulae. Dorsal larva includes 13 pairs of setae: 8 marginal (Md1-8), 2 central (Cd1-2) and 3 scutal (Sc1-3), 2 in lateral field and one in central. The posterior margin is divided into 9 festoons for each tick species (Fig.1). Ventral larva includes 15 pairs of setae: 3 sternal (St1-3), 2 preanal (Pa1-2), 1 anal (A), 4 pre-marginal (Pm1-4) and 5 marginal (Mv1-5), (Fig. 2). Statistical analysis for larval length revealed that H. m. marginatum was the longest followed by H. dromedarii and then H. a. excavatum while the difference between width of larvae were insignificant. (TABLE 1).

2- SCUTUM: The following scutal characters are common in the three species: anterolateral margins mildly convex, broader than longer, cervical grooves narrow and shallow extending about one third of the scutal length, eyes distinct oval, convex, peripheral and at greatest width of scutum. The scutum of H. a. excavatum and H. dromedarii is approximately tetragonal in shape with smooth surfaces. Posterior margin is sinuous laterally in H. a. excavatum and widely rounded in H. dromedarii (Fig. 3 A&B). The scutum of H. m. marginatum is hexagonal with distinct shallow cells on its surface. Posterior margins form wide U shape, with straight postero-lateral margins. (Fig. 3 C). Statistical analysis for scutal measurements revealed that H. m. marginatum was longer and

<table>
<thead>
<tr>
<th>Character</th>
<th>Measurements (mm) ± SE</th>
<th>H. a. excavatum</th>
<th>H. dromedarii</th>
<th>H. m. marginatum</th>
<th>F. value</th>
<th>P. value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body-length</td>
<td>0.620 ± 0.011</td>
<td>0.672 ± 0.008</td>
<td>0.741 ± 0.004</td>
<td>60.134</td>
<td>0.000</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Idiosoma-length</td>
<td>0.527 ± 0.016</td>
<td>0.575 ± 0.005</td>
<td>0.582 ± 0.009</td>
<td>8.084</td>
<td>0.002</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Idiosoma-width</td>
<td>0.486 ± 0.014</td>
<td>0.487 ± 0.011</td>
<td>0.522 ± 0.015</td>
<td>2.301</td>
<td>0.119</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Idiosoma-length/Idiosoma-width</td>
<td>1.085 ± 0.019</td>
<td>1.185 ± 0.025</td>
<td>1.123 ± 0.034</td>
<td>3.540</td>
<td>0.043</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Scutum-length</td>
<td>0.234 ± 0.005</td>
<td>0.221 ± 0.004</td>
<td>0.327 ± 0.006</td>
<td>143.584</td>
<td>0.000</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Scutum-width</td>
<td>0.344 ± 0.005</td>
<td>0.339 ± 0.004</td>
<td>0.401 ± 0.004</td>
<td>43.833</td>
<td>0.000</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Scutum-length/Scutum-width</td>
<td>0.681 ± 0.010</td>
<td>0.615 ± 0.009</td>
<td>0.816 ± 0.008</td>
<td>134.318</td>
<td>0.000</td>
<td>**</td>
<td></td>
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<tr>
<td>Palal-length</td>
<td>0.105 ± 0.000</td>
<td>0.105 ± 0.000</td>
<td>0.150 ± 0.000</td>
<td>25.320</td>
<td>0.000</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Length of dorsal basis capitulum</td>
<td>0.045 ± 0.000</td>
<td>0.054 ± 0.002</td>
<td>0.066 ± 0.002</td>
<td>27.750</td>
<td>0.000</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Length of ventral basis capitulum</td>
<td>0.068 ± 0.000</td>
<td>0.072 ± 0.001</td>
<td>0.092 ± 0.001</td>
<td>170.541</td>
<td>0.000</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Width of basis capitulum</td>
<td>0.147 ± 0.002</td>
<td>0.146 ± 0.002</td>
<td>0.156 ± 0.003</td>
<td>6.888</td>
<td>0.004</td>
<td>*</td>
<td></td>
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<tr>
<td>Hypostom-length</td>
<td>0.090 ± 0.000</td>
<td>0.090 ± 0.000</td>
<td>0.135 ± 0.000</td>
<td>36.320</td>
<td>0.000</td>
<td>**</td>
<td></td>
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<tr>
<td>Hypostom-width</td>
<td>0.036 ± 0.001</td>
<td>0.044 ± 0.001</td>
<td>0.045 ± 0.001</td>
<td>3.875</td>
<td>0.000</td>
<td>**</td>
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</table>

a, b, c means within the same column with different letters are significant according to Duncan test.
* Significant at P < 0.05. ** high significant at P < 0.01. NS, insignificant.

TABLE 1: Morphometric of different structures of Hyalomma larvae.
FIG. 1: *H. dromedarii*: Body dorsal view includes Sc = Scutal setae (1-3), Md = Marginal dorsal setae (1-8), Cd = Central dorsal setae (1-2). X 220.
**FIG. 2:** *H. m. marginatum*: Body ventral view includes; St = Sternal setae (1-3), Pa = Preanal setae (1-2), A = Anal seta, Pm = Premarginal setae (1-4), Mv = Marginal ventral setae (1-5). X200.
Fig. 4: Dorsal capitulum view. A. — *H. a. excavatum* (X650). B. — *H. dromedarii* (X650). C. — *H. m. marginatum* (X450).
Fig. 5: Ventral capitulum view: A. — *H. a. excavatum* (X700). B. — *H. dromedarii* (X700). C. — *H. m. marginatum* (X330). D. — Palpal article IV (X3500).
wider than other two species. There were not significant difference between the length of the scutum of \( H. a. \) excavatum and \( H. \) dromedarii while the later was wider than \( H. a. \) excavatum. The ratio of scutal length to scutal width was arranged as \( H. m. \) marginatum, \( H. a. \) excavatum and \( H. \) dromedarii. (TABLE 1).

3- PALPUS: The morphological characters and chaetotaxy are similar. External margins straight, internal margins convex, broad rounded apically, suture lines between palpal segments not discernible, palpi not project beyond the hypostome, with 8 setae dorsally, 3 setae ventrally and one seta apically (FIG. 4 A-C and FIG. 5 A-C). Palpal article IV arise from palpal article III ventrally and with 12 stout hairs: 8 apically and 4 basally (FIG. 5 D). Statistical analysis for palpal length revealed that \( H. m. \) marginatum was the longest while \( H. a. \) excavatum was equal to \( H. \) dromedarii (TABLE 1).

4- BASIS CAPITULUM: Dorsally: it is triangular shape, without setae, posterior margin straight, postero-lateral margins divergent, short, antero-lateral margins strongly convergent with junction bend down-wards to form lateral projection in all tick species (FIG. 4 A-C). Ventrally: it is rectangular shape, with post hypostomal setae anteriorly for each tick species (FIG. 5 A-C). Statistical analysis for the length and width of the dorsal and ventral of basis capitulum revealed that \( H. m. \) marginatum was longer and wider than other two species while \( H. d. \) dromedarii was longer than \( H. a. \) excavatum. There were not significant difference between the width of \( H. a. \) excavatum and \( H. \) dromedarii (TABLE 1).

5- HYPOSTOME: It is club shape in \( H. a. \) excavatum and \( H. \) dromedarii while it is cylindrical shape in \( H. m. \) marginatum. The dental formula is 2/2 for each tick species. The dental number per file are 7 in the outer file and 6 in the inner file for each
Fig. 7: Coxae: A. – *H. dromedarii* (X400). B. – *H. m. marginatum* (X350).
H. a. excavatum and H. dromedarii while there are 9 in the outer file and 8 in the inner file for H. m. marginatum. Two additional rows of reduced or small teeth are found for each tick species posteriorly. The outer edge of corona on the apex of hypostome is rounded for all tick species. The corona has a varying number of small hooks (Fig. 6 A-C). Statistical analysis for the hypostomal length revealed that H. m. marginatum was the longest while H. dromedarii was equal to H. a. excavatum. H. m. marginatum and H. dromedarii were approximately equal in width and both significantly wider than H. a. excavatum (Table 1).

Section 6: Coxae: Coxa I with posterio-external large triangular spur that it more large in H. m. marginatum and more tapering apically in H. a. excavatum and H. dromedarii. Each of coxae II and III carries posterio-median spur, well developed in H. m. marginatum and very small or reduced in both H. a. excavatum and H. dromedarii. Chaetotaxy is similar for all tick species, Coxa I with 3 setae, Coxa II and III with 2 setae for each (Fig 7A & B).

DISCUSSION

The most common Hyalomma species which were found at different localities in Egypt among a wide range of hosts (farm animals) especially cattle, camels, horses and sheep were H. a. excavatum, H. dromedarii and H. m. marginatum (Abdel-Shafy, 1994 and El Kammah et al., 2001). It was found that these tick species carried a wide variety of protozoal and bacterial disease agents (El Kammah et al., 2007). Therefore this study tries to give a morphological differentiation between the larvae of these tick species depending on SEM to facilitate the identification especially when more than one species are collected in a single field collection. Additionally larvae play an important role on the distribution of these tick species and transmission of pathogens because they infest small mammals and birds which either move from place to place as rodent or migrate across different countries such as migratory birds. As well as a few number of literatures described the larvae of these tick species without any comparison between of them: H. a. excavatum (El Kammah, 1969, Snow, 1971 and Apanaskevich & Horak, 2005), H. dromedarii (Abdel-Shafy, 1994, Apanaskevich, 2002 and Montasser, 2006) and H. m. marginatum (Apanaskevich, 2003). All the previous mentioned authors used light microscope to describe the larvae except Montasser (2006) used SEM but he did not concentrate on the important taxonomic characters which facilitate the recognition of H. dromedarii from other Hyalomma species, as well as the printed photos did not clear.

No previous study gave a morphological differentiation between the larvae of H. a. excavatum, H. dromedarii and H. m. marginatum. So, this study is considered firstly do that and it is the first study describe the larvae of H. a. excavatum and H. m. marginatum in detail by using SEM. However, the morphological differentiations between the larvae of H. anatolicum subspecies (anatolicum & excavatum) and H. marginatum subspecies (marginatum, rufipes, turanicum & isaaci) were only described by light microscope (El Kammah, 1969, Snow, 1971, Apanaskevich, 2003 and Apanaskevich & Horak, 2005). Generally, most morphological characters which either described or measured in this study agree with the results of above mentioned authors who used light microscope except some discrepancies. Snow, 1971 found that the hypostomal denticles per file of H. a. excavatum exceed 2-5 denticles than this study, may be by counting the small basal denticles or small apical hooklets. Also, he referred to the number of festoon of H. a. excavatum decreased two festoon than this study. Apanaskevich & Horak (2005) mentioned that the hypostomal denticles per inner file for H. a. excavatum were 5 comparing with 6 in this study and they did not remember the denticle number of outer file. Abdel-Shafy (1994) recorded 6 denticles in outer file of hypostome of H. dromedarii instead of 7 in this study. Despite of larval palp chaetotaxy was similar in the three species and agrees with other authors, only Montasser (2006) referred that palp included 8-10 setae instead of 12 setae in this study and other authors. He distributed the stout hairs of palpal article IV as, 7 apically and 5 basally, whereas this study confirms that the distribution of these setae is: 8 apically and 4 basally in all larvae of the studied tick species. Apanaskevich (2002) omitted important taxonomic characters for larvae of H. dromedarii.
such as hypostome and chaetotaxy of body and palpi. Apanaskevich (2003) described solely the larva of *H. m. marginatum* and compared it with other *H. marginatum* subspecies. Unfortunately, this study was written in Russian language. These characters agreed with the correspondent in this study.

CONCLUSION

The larva of *H. m. marginatum* is easy differentiated from other two tick species by its large size, shape of scutum and hypostom, the number of hypostomal teeth per file and spurs on coxae. In spite of *H. dromedarii* is very close to *H. a. excavatum*, it can be differentiated by the measurement of body length, scutum width and posterior margin of scutum.

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