Global Veterinaria 10 (1): 01-08, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.10.1.71150

Scanning Electron Microscopy of Nymphal and Larval Stages of The Cattle Tick *Rhipicephalus* (*Boophilus*) *annulatus* (Say) 1821(Acari: Ixodidae)

Sobhy Abdel-Shafy, Salwa M. Habeeb, Amira H. El Namaky and Hala A.A. Abou-Zeina

Department of Parasitology and Animal Diseases,
Veterinary Research Division, National Research Center, Giza, Egypt

Abstract: The cattle tick *Rhipicephalus* (*Boophilus*) *annulatus* is the only one-host tick species infesting domestic animals in Egypt. It is common on cattle and occasionally found on horses, sheep and buffaloes. The aim of this study was to describe the nymphal and larval stages of this species using scanning electron microscope (SEM) and morphometric analysis. *R. annulatus* nymph could be identified by the following characteristics; idiosoma with 19 pairs of setae dorsally and 30 pairs ventrally; scutum without cervical grooves and sinuous posterolateral margins forming a wide V-shape; palpi noteched externally with 9 setae dorsally and 8 setae ventrally; basis capitulum hexagonal shape dorsally without setae, ventrally: margins rounded posteriorly, curved anteriorly; distinct auriculae present; hypostome with dental formula 3,3 and the number of teeth per file 7,7,6. *R. annulatus* larva can be identified by the following characteristics; the dorsal surface carries 13 pairs of setae; scutum with two cervical grooves; palpi concaved externally with 9 setae dorsally and 3 setae ventrally; basis capitulum rectangular in shape without setae dorsally and hypostome with dental formula 2/2 and the number teeth per file 6,5. It is concluded that further studies are needed to describe other boophilid nymphs by SEM to identify the main characteristics for *R. annulatus* nymph. The characteristics of *R. annulatus* larva resemble those recorded in other boophilids species and their measurements were slightly smaller than those recorded with other boophilids. Then, it is difficult to distinguish *R. annulatus* larva from other boophilid species. Therefore, further molecular studies are needed to identify *R. annulatus* larva accurately.

Key words: Systematic · Morphology · Boophilids · Egypt

INTRODUCTION

Cattle are the main hosts of *Rhipicephalus* (*Boophilus*) *annulatus* (Say) 1821. It also parasitizes horses, sheep, goats and wild ungulates with successful completion of its life cycle. The preferred feeding sites are legs, belly, neck and dewlap but in heavy infestations the tick may be found over the back and shoulders [1-4]. It is a one-host tick. The feeding period on cattle is approximately three weeks which may extend to two months. Six generations per year are reported under conditions optimum conditions of temperature and humidity. In the Mediterranean region the tick's activity starts in late summer with a peak in autumn [2]. *R. annulatus* survives mainly in Mesomediterranean climates. It has been recorded from Portugal, Spain, Italy, Greece and Turkey. It is common in northern Africa [5-11]. It was also recorded in Saudia Arabia, Mexico, USA, Benin, Sudan and Iran [1, 12-16]. *R. annulatus* transmits the protozoans *Babesia bigemina* and *Ba bovis* to cattle, the cause of bovine babesiosis [17-18]. It also transmits the bacterium *Anaplasma marginale* to cattle causing bovine anaplasmosis [19]. It transmits *Borrelia theileri* [20] and Crimean Congo haemorrhagic fever virus (CCHFV) [21]. Heavy infestations cause damage to hides and probably lead to a reduction in the rate of growth of cattle [2].

There are few studies had described the immature stages of boophild species either by light microscope (LM) or SEM. Clifford *et al*. [22] have described only larval stage of both *R. annulatus* and *R. microplus* by LM. Also, Abdel-Shafy [6] used LM in his description of

Corresponding Author: Sobhy Abdel-Shafy, Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Center, Post Box, 12622, El-Behouse Street, Dokki, Giza, Egypt.

nymph and larva of *R. annulatus*. However, Estrada-Peňa *et al.* [23] described adults and larva of *R. australis* by SEM and gave measurements for larvae of four boophilds strains; *R. australis* from Australia, *R. australis* from Caledonia, *R. microplus* from Argentina and *R. microplus* from Australia. Therefore, the aim of this study was to describe the nymphal and larval stages of the cattle tick *R. annulatus* by scanning electron microscope and morphometric analysis. This description probably helps to distinguish clearly nymph and larva of *R. annulatus* from other boophilid species.

MATERIALS AND METHODS

Specimens of Larvae and Nymphs: The specimens of *R. annulatus* (Say) 1821 were collected as engorged females from a cow at Giza, Egypt. Ticks were identified according to Hoogstraal *et al.,* [1] and Estrada-Peňa *et al.* [2]. 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 container and incubated at the same conditions until they hatched. One week post hatching, larvae were divided into two groups. The first group was processed for scanning electron microscopy and morphometric analysis, while the second one was fed on rabbits, checked daily to monitor the engorgement of larvae and their subsequent moulting to nymphs that occurred on rabbit. The larvae and nymphs were placed in water at 70±10°C, washed with normal saline 0.9% KCl several times and preserved in 70% ethanol [24].

Preparation of Larvae and Nymphs for Scanning Electron Microscopy: Larvae and nymphs were prepared for scanning electron microscope (SEM) according to the method described by Abdel-Shafy *et al.* [25]. Specimens were cleaned by water-glycerol-KCl solution and washed in distilled water. Then they were taken through a graded series of alcohol/water. 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 S15OA Sputter Coater. Coated larvae and nymphs were examined by SEM.

Preparation of Larvae and Nymphs for Morphometrics: 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 microscopy after which they were washed with distilled water. These specimens were taken through a gradual series of alcohol/water, 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 for ten specimens for each larvae and nymphs were given in millimetres by using optical microscopy.

Many structures of larvae and nymphs were measured as follows: idiosoma, body width between two lateral sides' behind the last coxae, 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, 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

Nymph

Dorsum (Fig. 1, A-C): Idiosoma: It has rectangular shape; widest at midlength, broadly rounded posteriotly; without festoons; narrowest anteriorly across scapulae; 0.992±0.030 mm long x 0.728 ± 0.025 mm wide, length/width equal 1.4 and 19 pairs of setae (excluding scutum), 2 on median field and 17 on marginal field. Scutum: it has heart-shape; smooth surface; few small punctuations; no cervical grooves; indistinct oval eyes, length approximately equal width (0.462±0.027 mm long x 0.476±0.039 mm wide); anterolateral margins diverge to midlength then convert to form wide V-shape; with five pairs of small setae; one central, two marginal and two anterior. Palpus: it is notched externally, sinuous internally and humped apically; deep furrow-like between palpal article II and III; 0.157±0.005 mm long the palpus; with 9 setae dorsally. Basis capitulum: it is hexagonal shape; without setae; the posterior margin slightly convex, posterolateral margins concave and forming blunt edges with anterolateral margins; 0.167±0.005 mm long x 0.376±0.013 mm wide.

Venter: (Fig. 2. A-D and Fig. 3, A-D): Idiosoma: It has 30 pairs of setae (excluding coxal setae); sternal (8 pairs), preanal (2 pairs), anal (3 pairs), premarginal (11 pairs) and marginal (6 pairs). Sternal setae: one seta front of each coxa I and II while three horizontal setae front of each coxa III and IV. Each coxa has 3 setae and a spur which

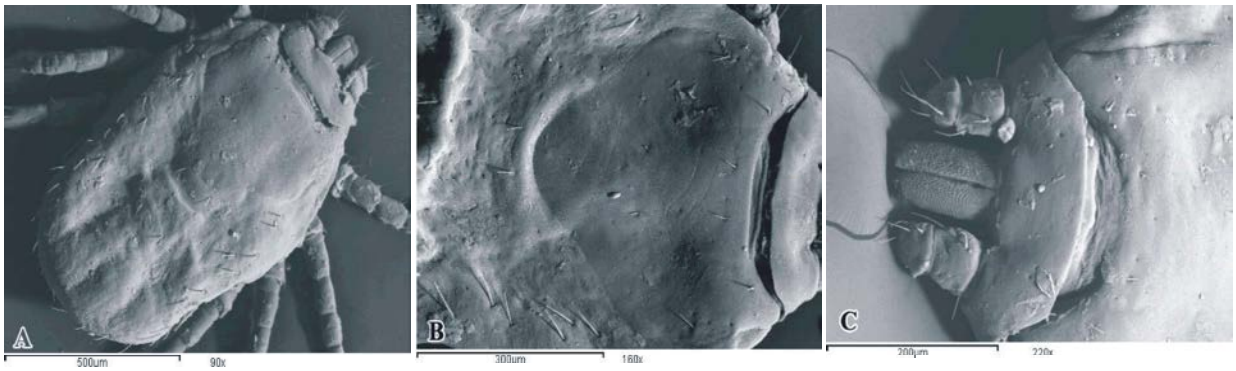

Fig. 1: Dorsal view of *Rhipicephalus* (*Boophilus*) *annulatus* nymph: A) whole body, B) Scutum, C) Capitulum (gnathosoma)

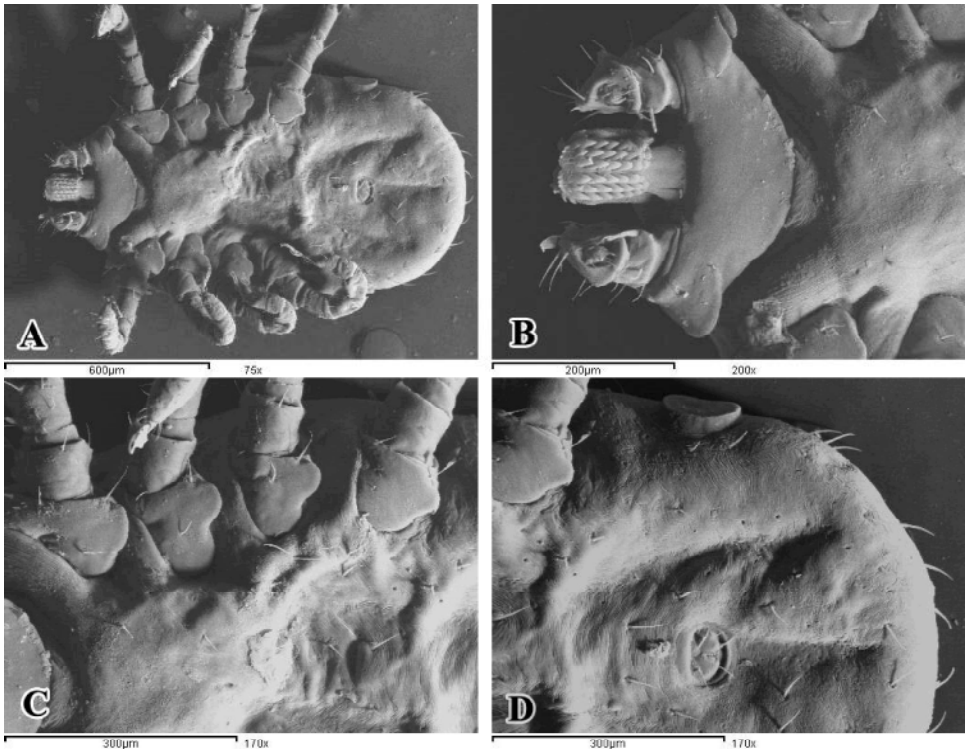

Fig. 2: Ventral view of *Rhipicephalus* (*Boophilus*) *annulatus* nymph: A) whole body, B) Capitulum (gnathosoma), C) Coxae, D) Half body area behind coax IV

larger in Coxa I and decrease gradually in Coxae II, III and IV. Palpus: it has 8 setae; one on article I, 3 on article II and 4 on article III. Basis capitulum: has auriculae; 3 pairs of setae, 2 laterally and 1 posthypostome. Palpal article IV: arise from palpal article III ventrally and it carries 11 stout hairs, 8 apically and 3 basally. Hypostome: it is cylindrical in shape; 0.136±0.003 mm long x 0.087±0.003 mm wide; slightly longer than palpi; 3/3 dental formula; teeth number per file (excluding small basal and apical teeth) 7, 7, 6 in the outer, middle and inner file, respectively. Spiracle: it is oval shaped, with numerous pores.

Larva

Dorsum: (Fig. 4, A-D): The body: It is elongated oval shaped, widest at midlength, narrowest anteriorly across the scapulae. Idiosoma: it has 13 pairs of setae; 8 marginal, 2 central and 3 scutal that distributed as one seta for each lateral, anterior and central area; 0.467±0.020 mm long x 0.425±0.016 mm wide; length/width equal 1.1.; posterior margin free of festoons. Scutum: it has narrow and shallow cervical grooves that deep anteriorly and extending to midlength; 0.278±0.010 mm long x 0.403±0.013 mm wide; length/width equal 0.6; distinct oval eyes and

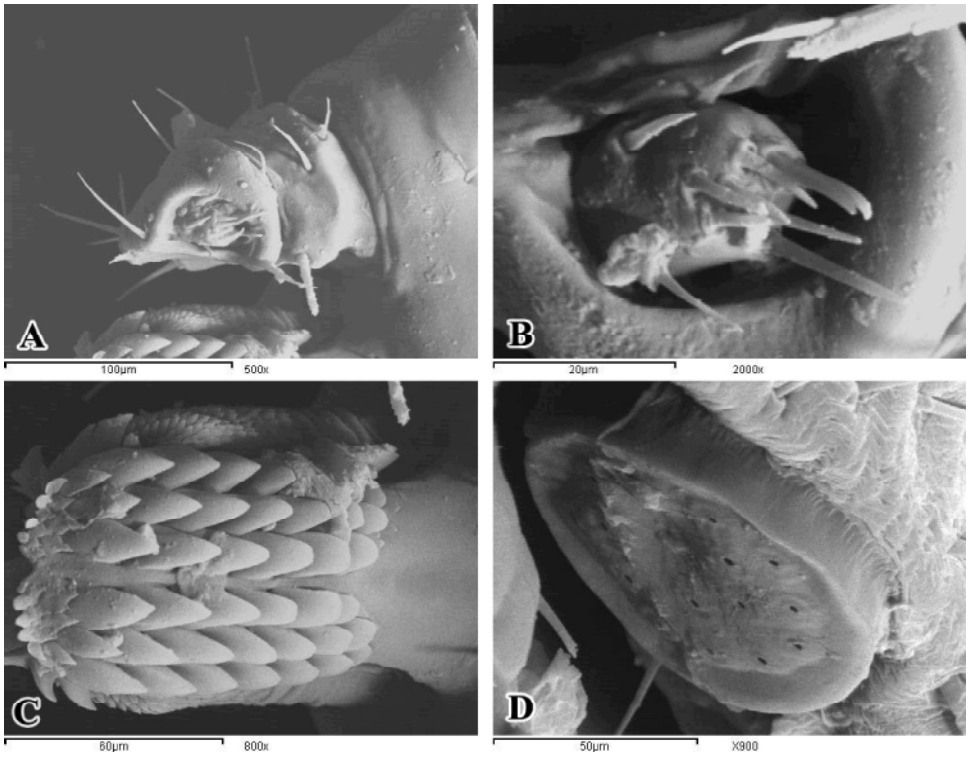

Fig. 3: High magnification of *Rhipicephalus* (*Boophilus*) *annulatus* nymph: A) Palpus, B) Palpal Article IV, C) Hypostome, D) Spiracular plate

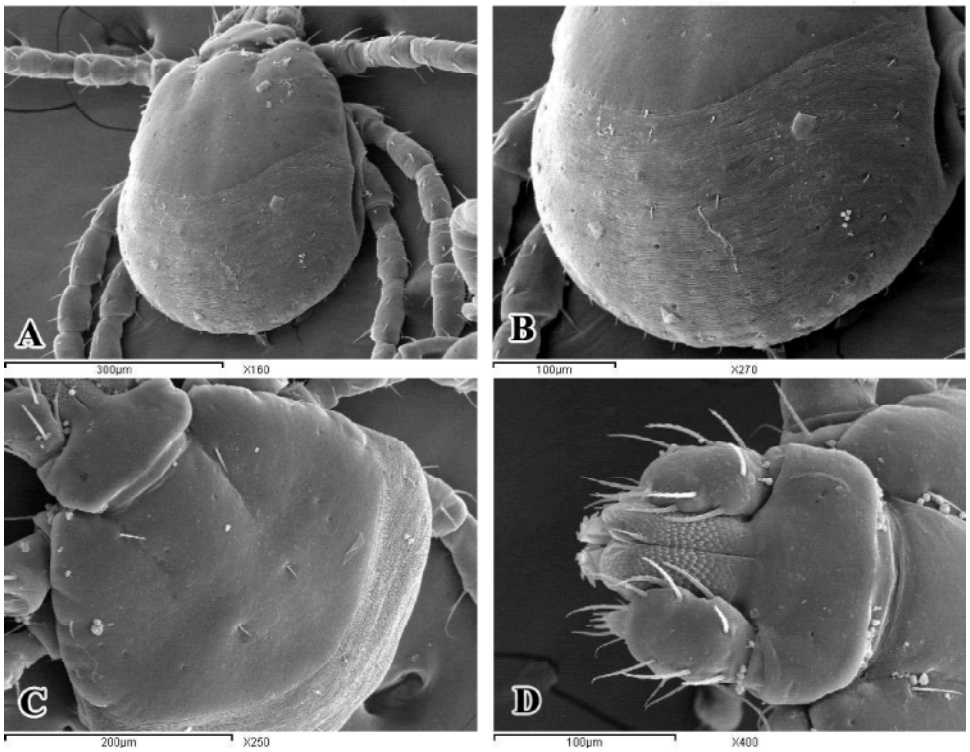

Fig. 4: Dorsal view of *Rhipicephalus* (*Boophilus*) *annulatus* larva: A) whole body, B) Alloscutum, C) Scutum, D) Capitulum (gnathosoma)

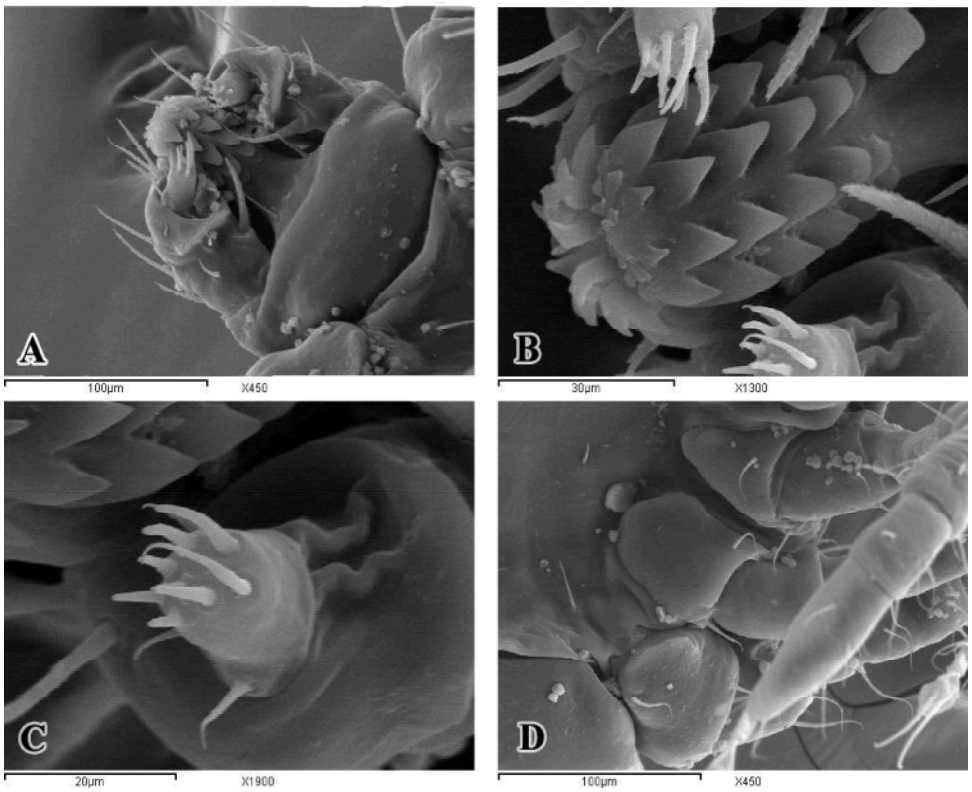

Fig. 5: Ventral view of *Rhipicephalus* (*Boophilus*) *annulatus* larva: A) Capitulum (gnathosoma), B) Hypostome, C) Palpal article IV, D) Coxae

Table 1: Morphometrics of nymphs and larvae of *Rhipicephalus (Boophilus) annulatus* and larvae of boophilid spp.

	Measurements (mm)							
			Boophilid spp.					
	Nymph	Larvae	Clifford *et al.* [22] [Mean (Range)]		Estrada-Peña *et al.* [23] [Mean± SE]			
Characteristic	*R. annulatus* [Mean± SE]	*R. annulatus* [Mean± SE]	*R. annulatus*	*R. microplus*	*R. australis* (Australia)	*R. australis* (Caledonia)	*R. microplus* (Argentina)	*R. microplus* (Uruguay)
Idiosoma-length	0.992±0.030	0.467±0.020	0.463 (0.429-0.502)	0.431 (0.403-0.508)	0.451±0.020	0.449±0.013	0.494±0.023	0.525±0.023
Idiosoma-width	0.728±0.025	0.425±0.016	0.445 (0.429-0.462)	0.424 (0.396-0.442)	0.420±0.011	0.427±0.009	0.425±0.011	0.466±0.016
Scutum-length	0.462±0.027	0.278±0.010	0.303 (0.290-0.330)	0.279 (0.264-0.297)	0.285±0.013	0.295±0.009	0.282±0.002	0.300±0.028
Scutum-width	0.476±0.039	0.403±0.013	0.445 (0.429-0.462)	0.421 (0.396-0.455)	0.399±0.008	0.400±0.009	0.413±0.008	0.427±0.016
Palal-length	0.157±0.005	0.090±0.004	0.096 (0.092-0.102)	0.096 (0.092-0.106)	0.101±0.001	0.098±0.003	0.104±0.004	0.104±0.004
Length of dorsal basis capitulum	0.167±0.005	0.078±0.005	0.072 (0.066-0.086)	0.069 (0.158-0.172)	0.096±0.007	0.095±0.003	0.097±0.001	0.094±0.005
Width of basis capitulum	0.376±0.013	0.153±0.006	0.165 (0.158-0.175)	0.165 (0.158-0.175)	0.168±0.005	0.174±0.004	0.172±0.002	0.173±0.005
Hypostom-length	0.136±0.003	0.070±0.004	0.068 (0.059-0.073)	0.087 (0.075-0.092)	0.078±0.005	0.0.079±0.003	0.078±0.001	0.088±0.001
Hypostom-width	0.087±0.003	0.045±0.003	-	-	0.053±0.002	0.054±0.002	0.054±0.002	0.054±0.002

very few small punctuations. Palpus: it is concaved externally; convex internally; rounded apically; 0.090±0.004 mm long; suture lines between palpal segments not discernible; with 9 setae, 1 central, 3 lateral and 5 internal. Basis capitulum: it is quadrangular shape; 0.078 ± 0.005 mm long x 0.153 ± 0.006 mm wide; margins widely rounded; without setae; straight posterior margin and sinuous lateral margins.

Venter: (Fig. 5, A-D): Palpus: It carries 3 setae ventrally and one seta apically. Palpal article IV arise from palpal article III ventrally and it carries 11 stout hairs, 8 apically and 3 basally. Basis capitulum: it has wide rectangular without auriculae and one pair of post hypostomal setae. Hypostome: it has dental formula 2/2; teeth number per file (excluding small basal and apical teeth) 6 in the outer file and 5 in the inner file; 0.070±0.004 mm long x

0.045±0.003 mm wide. Coxae: Coxa I with broadly rounded spur and three setae; Coxa II with a rounded spur near the inner edge of posterior border and 2 setae; coxa III similar to coxa II.

DISCUSSION

The cattle tick *R. annulatus* is the only one-host tick species common on cattle and occasionally on horses, sheep and buffaloes [6, 7]. It can spread from location to another and country to another through animal trading where quarantine measures are not enforced. The immature stages of *R. annulatus* are very small and not easily seen especially when they are between skin animal folds. Therefore, this study was designed to describe in details morphological characteristics of the nymph and larva of *R. annulatus* using SEM and morphometric analysis. This study would help to obtain an accurate identification of this species through immature stages and predict the pathogens that will import to a location or country. This is the first study that described the immature stages of *R. annulatus* by SEM. There is no study described nymph and larva of any boophlid species together using SEM. A unique study conducted by Estrada-Peña *et al.* [23] used SEM in description of adults and larva of *R. australis*. They did not describe the nymphal stage because they thought that it was difficult to obtain this stage. There are two studies used LM in description of the immature stages of boophild species. The first study conducted by Clifford *et al.* [22] who described only the larvae of *R. annulatus* and *R. microplus*. They did not define any specific characteristics for each larva help us in differentiation between of them. The second study described nymph and larvae of *R. annulaus* together [6]. This is the only study that described *R. annulatus* nymph in details by SEM. Therefore, SEM was used as more accurate advanced tool in order to describe nymph and larva of *R. annulatus*. It probably provide definitive characteristics for this species differ than those recorded before in other boophilids.

In nymphal description, it was observed that most characteristics agree with those observed by Abdel-Shafy [6]. There are some important morphological structures which were too difficult to observe by LM such as number of setae, number of teeth on hypostome and the ventral shape of basis capitulum. SEM showed that nymphal idiosoma carries 19 pairs of setae dorsally and 30 pairs ventrally comparing with 21 pairs dorsally and 38 pairs ventrally observed by LM [6]. This discrepancy may attribute to the overlapping between dorsal and ventral surface during the examination by LM. The distribution of setae on dorsal and ventral idiosoma was clarified in this study that may help better in identification of *R. annulatus* nymph from other nymph of boophilids species those need further investigation by SEM. All characteristics of scutum, coxae and palpal article IV of *R. annulatus* nymph observed by SEM in this study agree with those recorded before by LM. The palpal chaetaxy of nymph agree with that observed by LM dorsally, while it has 3 additional setae ventrally. With the same manner, basis capitulum of nymph agrees with that illustrated before by LM dorsally but it differs completely when it photos ventrally by SEM (it has auriculae). This structure was too difficult to illustrate by LM. The number of hypostomal teeth per file that observed by SEM was, 7,7,7 but it was 7,7,6 in case of investigation by LM.

In larval description, it was found that the number of setae on the dorsal idosomal surface and its distribution resemble with those recorded before on *R. annulatus* and *R. microplus* [22] and *R. australis* [23]. However, the number of setae on the ventral idosomal surface was not investigated in this study. Clifford *et al.,* [22] and Estrada-Peña *et al.* [23] gave the same findings in this point. They observed 15 pairs of setae on the ventral idosomal surface those distributed as 3 sternal, 2 preanal, 4 premarginal, 5 marginal and one anal. The SEM photo of scutum exhibited well distinctive cervical grooves in agreement with those observed before on *R. annulatus* and *R. microplus* [22] and *R. australis* [23]. This feature was not observed by Abdel-Shafy [6] may due to clear larvae greatly before examination by LM. The description of hypostome agrees with that recorded before on boophilid species [22, 23] except the denital formula of *R. australis* was 6/6 [23] compared with 6/5 in this study and Clifford *et al.* [22]. The characteristics of palpi, basis capitulum, papla article IV and coxae agree with those observed before by Clifford *et al.* [22], Abdel-Shafy, [6] and Estrada-Peña *et al.* [23]. The most structures of *R. annulatus* larvae those measured in this study were slightly smaller than those of other boophilid species measured by Clifford *et al.* [22] and Estrada-Peña *et al.* [23].

CONCLUSION

R. annulatus nymph can be identified by the following characteristics; idiosoma with 19 pairs of setae dorsally and 30 pairs ventrally; scutum without cervical grooves and sinuous posterolateral margins those formed

together wide V shape; palpi noteched externally with 9 setae dorsally and 8 setae ventrally; basis capitulum hexagonal shape dorsally without setae and it has auriculae ventrally; hypostome with dental formula 3,3 and the number teeth per file 7,7,6. *R. annulatus*. Larva can be identified by the following characteristics; the dorsal surface carries 13 pairs of setae; scutum with two cervical grooves; palpi concaved externally with 9 setae dorsally and 3 setae ventrally; basis capitulum rectangular in shape without setae dorsally and hypostome with dental formula 2/2 and the number teeth per file 6,5. Further studies are needed to describe other boophilid nymphs by SEM to identify the main characteristics of *R. annulatus*. The characteristics of *R. annulatus* larva resemble those recorded in other boophilids species. The measurements of these characteristics were slightly smaller than those recorded with other boophilids. Then, it is difficult to distinguish *R. annulatus* larva from other boophilid species. Therefore, further molecular studies are needed to identify *R. annulatus* larva accurately.

REFERENCES

1. Hoogstraal, H., H.Y. Wasseff and W. Buttiker, 1981. Ticks (Acarina) of Saudia Arabia Fam. Argasidae, Ixodidae. Fauna of Saudia Arabia, 32: 25-110.
2. Estrada-Peňa, A., A. Bouattour, J.L. Camicas and A.R. Walker, 2004. Ticks of domestic animals in the Mediterranean region. A guide to identification of species, 1st edn. Bioscience Reports, Edinburgh, pp: 131.
3. Bursali, A., S. Tekin, A. Keskin, M. Ekici and E. Dundar, 2011. Species diversity of Ixodid Ticks feeding on humans in Amasya, Turkey: Seasonal abundance and presence of crimean-congo hemorrhagic fever virus. J. Med. Entomol., 48: 85-93.
4. Gargili, A., S. Kar, N. Yilmazer, O. Ergönül and Z. Vatansever, 2011. Different abundances of human-biting ticks in two neighboring provinces in Turkey. Kafkas Universitesi Veteriner Fakultesi Dergisi, 17: S93-S97.
5. Khoury, C., G. Manilla and M. Maroli, 1994. Parasitic horse ticks in Italy. Observations on their distribution and pathogenic role. Parassitologia, 36: 273-279.
6. Abdel-Shafy, S., 1994. Morphological description of ixodid immature stages and research of blood parasites in farm animals in Egypt. M.S.Thesis, Fac. Agric. Cairo Univ.
7. Abdel-Shafy, S., 2000. Microbiological and control studies on ticks infesting farm animals and poultry. Phd Thesis, Fac. Agr. Cairo Univ.
8. Loftis, A.D., W.K. Reeves, D.E. Szumlas, M.M. Abbassy, I.M. Helmy, J.R. Moriarity and G.A. Dasch, 2006. Rickettsial agents in Egyptian ticks collected from domestic animals. Exp. Appl. Acarol., 40: 67-81.
9. Benchikh-Elfegoun, M.C., A. Benakhla, B. Bentounsi, A. Bouattour and R. Piarroux, 2007. Identification and seasonal kinetics of parasitic bovine ticks in the Taher area (Jijel) Algeria. Annales de Medecine Veterinaire, 151: 209-214.
10. Pavlidou, V., S. Gerou, M. Kahrimanidou and A. Papa, 2008. Ticks infesting domestic animals in northern Greece. Exp. Appl. Acarol., 45: 195-198.
11. Santos-Silva, M.M., L. Beati, A.S. Santos, R. De Sousa, M.S. Núncio, P. Melo, M. Santos-Reis, C. Fonseca, P. Formosinho, C. Vilela and F. Bacellar, 2011. The hard-tick fauna of mainland Portugal (Acari: Ixodidae): An update on geographical distribution and known associations with hosts and pathogens. Exp. Appl. Acarol., 55: 85-121.
12. Estrada-Peňa, A. and J.M. Venzal, 2006. High-resolution predictive mapping for *Boophilus annulatus* and *B. microplus* (Acari: ixodidae) in Mexico and Southern Texas. Vet. Parasitol., 142: 350-358.
13. Farougou, S., M. Kpodekon, H. Adakal, R. Sagbo and C. Boko, 2007. Seasonal abundance of ticks (Acari: Ixodidae) infesting sheep in the southern area of Benin. Revue De Medecine Veterinaire, 158: 627-632.
14. Salih, D.A., I.I. Julla, S.M. Hassan, A.M. El Hussein and F. Jongejan, 2008. Preliminary survey of ticks (Acari: Ixodidae) on cattle in Central Equatoria State, Southern Sudan. Onderstepoort J. Vet. Res., 75: 47-53.
15. Lohmeyer, K.H., J.M. Pound, M.A. May, D.M. Kammlah and R.B. Davey, 2011. Distribution of *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *annulatus* (Acari: Ixodidae) Infestations Detected in the United States Along the Texas/Mexico Border. J. Med. Entomol., 48: 770-774.
16. Asgarian, F., A.A. Enayati, A. Amouei and J.Y. Charati, 2011. Fauna, geographical distribution and seasonal activity of hard ticks from Sari township in 2007-2008. J. Mazandaran Univ. Med. Sci., 21: 24-33.

17. Adham, F.K., E.M. Abd-El-Samie, R.M. Gabre and H.E. Hussein, 2009. Detection of tick blood parasites in Egypt using PCR assay I- *Babesia bovis* and *Babesia bigemina*. Parasitol. Res., 105: 721-730.
18. Silva, M.G., P.X. Marques and A. Oliva, 2010. Detection of *Babesia* and *Theileria* species infection in cattle from Portugal using a reverse line blotting method. Vet. Parasitol., 174: 199-205.
19. Scoles, G.A., M.W. Ueti, S.M. Noh, D.P. Knowles and G.H. Palmer, 2007. Conservation of transmission phenotype of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) strains among *Dermacentor* and *Rhipicephalus* ticks (Acari: Ixodidae). J. Med. Entomol., 44: 484-491.
20. Trees, A.J., 1978. The transmission of *Borrelia theileri* by *Boophilus annulatus* (SAY, 1821). Trop. Anim. Health Prod., 10: 93-94.
21. Gunes, T., O. Poyraz and Z. Vatansever, 2011. Crimean-Congo hemorrhagic fever virus in ticks collected from humans, livestock and picnic sites in the hyperendemic region of Turkey. Vector-Borne Zoonotic Dis., 11: 1411-1416.
22. Clifford, C.M., G. Anastos and A. Elbl, 1961. The larval ixodid ticks of the eastern United States (Acarina-Ixodidae). Misc. Publ. Entomol. Soc. Am., 5: 214-237.
23. Estrada-Peňa, A., J.M. Venzal, S. Nava, A. Mangold, A.A. Guglielmone, M.B. Labruna and J. de la Fuente, 2012. Reinstatement of *Rhipicephalus* (*Boophilus*) *australis* (Acari: Ixodidae) with Redescription of the Adult and Larval Stages. J. Med. Entomol., 49: 794-802.
24. Famadas, K.M., N.M. Serra-Freire and R.M. Landfredi, 1997. Redescription of the larvae of *Amblyomma cajennense* (Fabricius) (Acari: Ixodidae) using optical and scanning electron microscopy. Acarologia, 38: 100-109.
25. Abdel-Shafy, S., A.H. El Namaky and F.A. Khalil, 2011. Scanning electron microscopy and morphometrics of nymph and larva of the tick *Hyalomma impressum* (Acari: Ixodidae). Parasitol. Res., 109: 1509-1518.