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DESCRIPTION OF IMMATURE STAGES OF THE BROWN DOG TICK *RHIPICEPHALUS SANGUINEUS* (ACARI: IXODIDAE) USING SCANNING ELECTRON MICROSCOPY

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ABSTRACT

The brown dog tick Rhipicephalus sanguineus is a three-host tick that feeds primarily on dogs and occasionally on other hosts, including humans. Therefore, this study aimed to describe in details the immature stages (larva and nymph) of R. sanguineus using scanning electron microscope (SEM) and morphometeric analyses. The measurements of nymph and larva structures are relatively close to those of Hyalomma excavatum. The number and distribution of setae on either the dorsal or ventral surfaces of nymph differ from those of other ixodid ticks. Eyes have an elongated shape in nymph and larva. The position of sternal setae in nymph resembles the position of these setae in H. dromedarii. The shape and position of spurs on coxae in nymph and larva are considered the unique characters that can differentiate this species from other three species of R. sanguineus group (R. camicasi, R. turanicus and R. sulcatus). Palpi in nymph and larva are tending to each other forming triangular shape. The apices of palpi in nymph and larva are tapering. Basis capitulum has hexagonal shape dorsally in nymph and larva. Basis capitulum of nymph has a pair of spur-like ventrally. Further studies are needed in details on the immature stages of other tick species of R. sanguineus group such as R. camicasi, R. turanicus and R. sulcatus using advanced techniques such as SEM or molecular biology. These techniques may provide additional characteristics which may facilitate the identification of the immature stages of R. sanguineus group.

Keywords: Larva, Morphology, Nymph, *Rhipicephalus*, SEM, Taxonomy.

Contribution/ Originality

This study is one of very few studies which have investigated the immature stages of *Rhipicephalus sanguineus* morphologically by scanning electron microscope.

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1. INTRODUCTION

Rhipicephalus sanguineus, commonly known as the brown dog tick, is a three-host tick that feeds primarily on dogs and occasionally on other hosts, including humans. The brown dog tick *R. sanguineus* is widely distributed around the world and it is known vector of many pathogens such as *Babesia canis*, *Ehrlichia canis*, and *Rickettsia conorii*. Itbelongs to the family Ixodidae (hard ticks). It is small and has elongated body shape, usually inornate and has short palps. Eyes and festoons are present. Coxa I has deeply cleft and spiracular plates are comma-shaped in males. An identifying character for the brown dog tick is the hexagonal basis capituli. Newly hatched larvae or "seed ticks" are small (length, 0.54 mm; width, 0.39 mm) and have only three legs. Nymphs have four pairs of legs and resemble adults except they are smaller (length, from 1.14 to 1.3 mm; width, from 0.57 to 0.66 mm) and sexually immature, i.e., they have no genital aperture. Adults have four pairs of legs like nymphs, but they are lager and sexually mature [1, 2].

Electron microscopy (EM) has detected differences, usually minor, that are not easily detected with traditional phenotypic methods. Further studies on the *R. sanguineus* group would be established to determine the status of the members of this group. These studies should be based on both EM and traditional methods. A better understanding of identification would be useful for the improvement of the control strategies against *R. sanguineus* ticks and their pathogens [2].

There is a unique study on the using of scanning electron microscope (SEM) in morphological description of the immature stages (larva and nymph) of *R. sanguineus*) published by Pegram, et al. [3]. They gave a brief morphological description and showed that *R. sanguineus* larva has the following features: lateral angles of basis capituli are very short and blunt, width of palps at their juncture with basis capituli is equal to width of basis; external margins of palpi are slightly convex but broad at apex. Scutum is only mildly convex posteriorly, coxa I has broad salient ridge posteriorly; coxa II has small spur and coxa III has mere indication of spur. They also revealed that *R. sanguineus* nymph has the following features: capitulum is somewhat broader than long, anterolateral margins of basis capituli and external margins of palpi are slightly curved, apices of palpi are slightly hunched in outline with external margins of palpal article 3 slightly convex, posterointernal margin of coxa I has external spur slightly longer and narrower than internal spur; coxae II and III each has a very small spur; coxa IV has a mere indication only of a spur.

Therefore, this study aimed to describe in details the immature stages (larva and nymph) of the brown dog tick *R. sanguineus* using scanning electron microscope (SEM) and morphometeric analyses. This may provide additional characteristics that help in the identification of this tick species and its potential pathogens that may be transmitted without waiting the development of the immature stages into adult stages (male and female). This in turn helps in improving control strategies methods for ticks and pathogens to take a control decision at an appropriate time.

2. MATERIALS AND METHODS

2.1. Specimens of Larvae and Nymphs

Specimens *Rhipicephalus sanguineus* (Latreille, 1806) were collected from dog in Giza, Egypt. Ticks were identified according to Pegram, et al. [3] and Estrada-Peña, et al. [4]. A single engorged female was incubated at 27 °C and 75% relative humidity (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 and second groups of larvae were from larvae that hatched from eggs which laid from one female of the brown dog tick *R. sanguineus*. The first group was fed on rabbits according the methodology Abdel-Shafy, et al. [5], checked daily to follow the engorgement of larvae and observed their moulting to nymphs that occurred out of rabbit. This stage (nymph) was used for scanning electron microscope preparation and light microscope for measurements. The second group of larvae was used directly for scanning electron microscope and light microscope for measurements without feeding on rabbits. 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 [6].

2.2. Preparation of Larvae and Nymphs for Scanning Electron Microscopy

Larvae and nymphs preserved in 70% ethanol were thoroughly cleaned by overnight immersion in water-glycerol-KCl solution at 40 °C [7]. 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 [8]. 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 one hour in each dilution except 10 min for 100% ethanol [9]. 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.

2.3. 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 speciemens 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 minutes 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; idiosoma length from scapula to posterior end of idiosoma, idiosoma width between two lateral sides' behind coxae III, scutum length across longitudinal axis from scapula to posterior end of scutum, scutum width across transverse axis including eyes, palpal length from the base of segment I to the apex of segment III, palpal width, 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 posthypostomal setae and hypostomal width.

3. RESULTS

3.1. Nymph

3.1.1. Dorsal Idiosoma

Idiosoma length is 1.102 mm, idiosoma width is 0.742 mm, Idiosoma length/idiosoma width ratio is 1.457. The body showed maximum width behind scutum and narrowing noticeably anteriorly; alloscutum with furrowed surface, with moderate number of uniformly punctuations and 31 pairs of strong setae (excluding scutum) dense peripherally. Idiosoma has nine festoons posteriorly, each has one seta dorsally except parma without setae (Table 1 and Figs. 1, A-C). Scutum: almost width is equal length; the length is 0.535 mm, the width is 0.550 mm, the ratio of scutum length/scutum width is 0.987. The scutum has a pair of elongated eyes at its greatest width; posterior margin is well convex, postero-lateral margins are straight, anterolateral margins are straight and slightly convergent to broadly round scapulae. Scutum has pentagonal shape and few small punctuation. Cervical grooves are convergent, deep for midlength then divergent as shallow grooves until the edges of postero-lateral margins; surface reticulately patterned more pronounced in the area of cervical grooves, convex between the grooves. Moreover, thirteen pairs of small setae are demonstrated, two central, one in front of postero-lateral margins, two adjacent to the eyes, two anterior and six lateral at the edge of antero-lateral margins (Table 1 and Figs. 2, A&B).

3.1.2. Ventral Idiosoma

The surface is furrowed with many small punctuations and few large punctuations. The ventral number of setae is 26 pairs (excluding anal and coxal setae): sternal (six pairs), preanal (two pairs), premarginal (thirteen pairs) and marginal (five pairs). The position of sternal setae according to coxae is: one setae front of either coxa I or IV, and two vertical setae front of either coxa II or III. Each coxa with three setae; coxa I with external spur slightly longer and narrower than internal spur, 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 (Figs. 3, A-B & 4, A-B).

3.1.3. Gnathosoma

Palpus: palpal length is 0.290 mm, palpal width is 0.055 mm, palpal length/width ratio is 5.300, the palpi are tending to each other forming triangular shape, the external margins are straight, the internal margins are convex, tapering apically, and the suture lines between palpal segments are discernible. The palpus do not project beyond the hypostome. Surface of segment II has eight setae (4 dorsally and 4 ventrally). Surface of segment III has seven setae (4 dorsally and 3 ventrally). (Table 1 and Figs. 5, A&B).

Basis capitulum: Dorsally, it is hexagonal in shape without setae, the posterior margin is straight, the postero-lateral margins are concave and forming sharp angles with antero-lateral margins. The dorsal length is 0.208 mm, the width is 0.345 mm, width/length ratio is 1.635 Ventrally, it is tetragonal in shape with one pairs of setae laterally; posterior and postero-lateral margins forms bow-shaped; it has a pair of spur-like on its postero-lateral margins (Table 1 and Fig. 5, A&B).

Hypostome: The hypostomal length is 0.240 mm, the hypostomal width is 0.077 mm and hypotomal length/width is 3.122. It is cylindrical in shape, rounded apically, dental formula is 2/2, teeth number per file (excluding apical teeth) is eight in either outer file or in inner file, a pair of posthypostomal setae (Table 1 and Fig. 5C).

3.2. Larva

3.2.1. Dorsal Idiosoma

Idiosoma length is 0.527 mm, idiosoma width is 0.420 mm and the ratio of idiosoma length/idiosoma width is 1.260. The body is semicircular shape, widest at midlength, narrowest anteriorly across the scapulae, broadly rounded positeriorly. 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 (Table 1 and Figs 6 A&B).

Scutum: It has tetragonal shape and its length is 0.230 mm, the width is 0.343 mm, the ratio of scutum length/scutum width (0.663). 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 elongate shape, convex, at greatest width of scutum. It is approximately tetragonal in shape. Posterior margin is slightly convex (Table 1 and Figs 6 B).

3.2.2. 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 broad salient ridge posteriorly; coxae II has small spur and coxa III has mere indication. Coxa I has three setae; coxa II and III have two setae for each (Fig. 7).

3.2.3. Gnathosoma

Palpus: palpal length is 0.120 mm, palpal width is 0.039 mm, palpal length/width ratio is 3.000, palpi are tending to each other forming triangular shape, external margins are straight; internal margins are convex, tapering apically; suture lines between palpal segments are not discernible; palpi are not projected beyond the hypostome, with eight setae dorsally, three setae ventrally and one seta apically. (Table 1 and Fig. 8, A&B).

Basis capitulum: The length is 0.070 mm, the width is 0.150 mm and the width/length ratio is 2.100. Dorsally, it is hexagonal in shape without setae; posterior margin is straight, posterolateral margins are straight, long, divergent laterally and forming pointed edges with anterolateral margins those are short and curved. Ventrally, it is tetragonal in shape, with one pair of post hypostomal setae; posterior margin slightly convex. Lateral angles are very short and blunt. (Table 1 and Fig. 8, A&B).

Hypostome: The hypostomal length is 0.087 mm, hehypostomal width is 0.030 mm and the length/width is 2.850. It is cylindrical in shape; the dental formula is 2/2, teeth number per file (excluding small apical teeth) is five in either outer file or in the inner file (Table 1 and Fig. 8B).

4. DISCUSSION

The genera *Hyalomma* and *Rhipicephalus* (including formerly *Boophilus*) comprise the most ixodid tick species that parasitize domestic animals in Egypt [10]. 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 aimed to identify the specific characteristics of both nymph and larva of the brown dog tick *Rhipicephalus sanguineus* in order to differentiate them from other immture stages of ixodid tick species that described before in Egypt such as *Hyalomma* spp., *Rhipicephalus* (*Boophilus*) *annulatus* [11-15] as well as *R. sanguineus* group in abroad [3, 16]. There are two publications introduced brief descriptions for the immature stages of *R. sanguineus* group (including the brown dog tick *R. sanguineus*) by using SEM [3, 16]. They provided full descriptions for males and females of a numerous tick species belong to *R. sanguineus* group and brief descriptions for immature stages of *R. sanguineus*, *R. camicasi*, *R. turanicus* and *R. sulcatus*.

In this study, both length and width measurements of idiosoma of *R. sanguineus* are close to those of *H. rufipes* and *R.* (*B.*) *annulatus* in nymph [14, 15] but they are close to *H. excavatum* and *R. (B.) annulatus* in larvae [12, 14]. They are lower than *H. dromedarii* and *H. marginatum* in nymph and larva [11, 12]. The idiosoma of *R. sanguineus* nymph are close to *H. excavatum* and *H. impressum* in length but it is wider than *H. excavatum* and narrower than *H. impressum* [11, 13]. In larva of *R. sanguineus*, idiosoma is longer and wider than *H. impressum* and *H. rufipes* [13, 15]. In description of the immature stages of *R. sanguineus* group that established by Pegram, et al. [16], Pegram, et al. [3] the measurements of nymphs and larvae were completely omitted. The number of dorsal setae in *R. sanguineus* nymph (31 pairs excluding scutum) is lower than that of *H.*

excavatum (55 pairs) *H. dromedarii* (43 pairs), *H. marginatum* (67 pairs) and *H. rufipes* (48 pairs) [11, 15]. It is higher than that of *H. impressum* (26/27 pairs) and *R.* (*B.*) *annulatus* (19 pairs) [13, 14]. The number of ventral setae in *R. sanguineus* nymph (26 pairs) is lower than that of other tick species (28-35 pairs) which were recorded by Abdel-Shafy [11]; Abdel-Shafy, et al. [13]; Abdel-Shafy, et al. [14]; Abdel-Shafy, et al. [15]. The number of dorsal (13 pairs including scutum) and ventral (15 pairs) setae on the idiosoma of *R. sanguineus* larva and their distribution are exactly like that recorded before on *Hyalomma* spp. and *R. (B.) annulatus*. Posterior margin has nine festoons like *Hyalomma* spp, while *R. (B.) annulatus* has not festoons on posterior margin of its larva [12-15]. Therefore, it is too difficult to distinguish *R. sanguineus* larvae from other *Hyalomma* spp. by the dorsal or ventral chaetotaxy of idiosoma. The chaetotaxy was also omitted in the description of immature stages of *R. sanguineus* group which published by Pegram, et al. [16], Pegram, et al. [3].

The length and width of scutum in *R. sanguineus* close to those of *H. excavatum* in nymph and larva. The length of scutum in *R. sanguineus* larva closes to *H. impressum* and *H. rufipes*. Scutum in *R. sanguineus* is longer than that of *H. rufipes*, *H. impressum* and *R. (B.) annulatus* in nymph but it is wider than *R. (B.) annulatus* in larva, shorter than *H. dromedarii* in nymph and *H. marginatum* in nymph and larva, wider than *R. (B.) annulatus* in nymph and *H. impressum* and *H. rufipes* in larva and narrower than *Hyalomma* species in nymph and *H. dromedarii* and *H. marginatum* in larva. Scutum of *R. sanguineus* larva has tetragonal shape like that of *H. excavatum* and *H. dromedarii* but it differs from those have bentagonal shape like *H. impressum* and *R. (B.) annulatus* or hexagonal shape such *H. rufipes* and *H. marginatum*. Posterior margin of scutum in *R. sanguineus* larva resembles with that of *H. excavatum* or *H. dromedarii* [11-15]. Eyes have elongated shape in nymph and larva of *R. sanguineus* comparing with oval or circular shape in those of *Hyalomma* spp or *R. (B.) annulatus*. The number of small scutal setae in *R. sanguineus* nymph and its distribution on scutum surface are different from those of *Hyalomma* spp. and *R. (B.) annulatus*. The number of scutal setae in nymph is equal to *H. rufipes* (13 pairs), higher than *H. impressum* (8 pairs) and *R. (B.) annulatus* (5 pairs) and lower than *H. excavatum*, *H. dromedarii* and *H. marginatum* (14-17 pairs). The scutum of *R. sanguineus* nymph has 2 central setae while scutum of *H. rufipes* nymph has one central seta. Cervical grooves on scutum of *R. sanguineus* nymph almost resemble those of *Hyalomma* species while they are absent in *R. (B.) annulatus*nymph. Posterior margin of scutum in *R. sanguineus* nymph always tend to be close to *Hyalomma* spp. but it differs in scutum of *R. (B.)annulatus* (V shape) [13-15]. Pegram, et al. [16], Pegram, et al. [3] did not provide details about scutum of nymph and larva of *R. sanguineus* in their descriptions of *R. sanguineus* group.

The number and position of sternal setae are the most important characteristics in distinguishing of *R. sanguineus* nymph from *Hyalomma* spp. and *R. (B.) annulatus*. The number of sternal setae in *R. sanguineus* nymph is 6 pairs, equal to that of *H. impressum*, *H. rufipes*, *H. dromedarii* and *H. marginatum*, but higher than that of *H. excavatum* (5 pairs) and lower than that of *R. (B.) annulatus* (8 pairs). The position of sternal setae in *R. sanguineus* nymph resembles that of

H. dromedarii, but it can discriminate *R. sanguineus* from *H. dromedarii* by other structures that almost are different. The position of sternal setae in *R. sanguineus* nymph according to coxae is: one setae front of coxa I and IV, two vertical setae front of coxa II and III. The shape and position of spurs on coxae of *R. sanguineus* nymph are considered the unique characters that can differentiate this species from other three species of *R. sanguineus* group (*R. camicasi*, *R. turanicus* and *R. sulcatus*) which described briefly by Pegram, et al. [16], Pegram, et al. [3]. Pegram, et al. [3] described spurs on coxae of *R. sanguineus* nymph as follow; external spur slightly longer and narrower than internal spur on coxaI; coxae II and III each with a very small spur; coxa IV with a mere indication only of a spur. Spurs on coxae of *R. sanguineus* larvae are considered the main characteristics that help in identification larvae from other ixodid tick species even between *R. sanguineus* group. In agreement with Pegram, et al. [3], *R. sanguineus* larva has; coxa I with broad salient ridge posteriorly, Coxa II with small spur and coxa III with mere indication of spur. Moreover, spurs on coxae of *Hyalomma* spp. larvae are well developed but *R. (B.) annulatus* has coxa I with broadly rounded spur, coxa II with rounded spur and coxa III without spur.

In this study, it was found that palpi in nymph and larva of *R. sanguineus* tending to each other forming triangular shape. This character does not observed in the immature stages of *Hyalomma* spp. and *R.* (*B.*) *annula*tus which described before [11-15] as well as it is a common character in *R. sanguineus* group that photographed using SEM by Pegram, et al. [16], Pegram, et al. [3]. The other specific character for immature stages of *R. sanguineus* nymph is the tapering apices of palpus. This character is also observed in *R. (B.) annulatus* nymph only [14] but the external margins of palpi are notched in the nymph of this species and they are straight in *R. sanguineus* nymph. However, the apices of palpi in nymph and larvae of *Hyalomma* species and larvae of *R. (B.) annulatus* are rounded [11-15]. Moreover, Pegram, et al. [3] reported that the papal apices in nymph and larva of *R. sanguineus* are slightly hunched in outer line with external margin of palpal article 3. This difference may be attributed to the geographical habitats between the Egyptian tick *R. sanguineus* described in this study and Zambian tick *R. sanguineus* described by Pegram, et al. [3]. The shape of palpus in immature stages *R. sanguineus* nymph relatively resembles that of tick species belong to *R. sanguineus* group described by Pegram, et al. [16], Pegram, et al. [3]. The palpus of *R. sanguineus* nymph (length 0.290 mm) closes to *Hyalomma* spp. (range of length 0.206 – 0.300 mm), while it is obviously longer than that of *R.* (*B.*) *annulatus*(0.157 mm). Whereas, the palpi in larva of *R. sanguineus* are shorter (0.070 mm) than those of *Hyalomma* spp. and *R. (B.) annulatus* (0.090 – 0.150 mm) [11, 13-15].

Basis capitulum which has hexagonal shape dorsally in nymph and larva of *R. sanguineus* is in agreement with that in immature stages of *R. sanguineus* which described by Pegram, et al. [16], Pegram, et al. [3] and *R. (B.) annulatus* nymph which described by Abdel-Shafy, et al. [14] but it has tetragonal shape in larva of *R. (B.) annulatus*. The basis capitulum of *R. (B.) annulaus* nymph is obviously wider and shorter dorsally than that of *R. sanguineus*. However, basis capitulum in immature stages of *Hyalomma* species has triangular shape dorsally [11, 13, 15]. Pegram, et al.

[3] stated that anterolateral margins of basis capituli slightly curved in nymphs of *R. sanguineus*. Basis capitulum of *R. sanguineus* nymph has a pair of spurs ventrally on its antero-lateral margins. This character is not found in nymphs of *Hyalomma* spp. and *R.* (*B.*) *annulatus* and Pegram, et al. [16], Pegram, et al. [3] did not refer to this character for any species of *R. sanguineus* group. In *R. sanguineus* group, the basis capituli in larvae of *R. sanguineus* and *R. camicasi* are similar, they have very short and blunt lateral angles [3].

The shape of hypostome in *R. sanguineus* nymph is cylindrical resembling that in *Hyalomma* spp and *R. (B.) annulatus* nymphs, while hypostome has club-shape in larva of *R. sanguineus* resembling that of *H. dromedarii*, *H. excavaum*, *H. impressum* and *R. (B.) annulatus*. The dental formula of hypostome in nymph and larva of *R. sanguineus* is 2/2 like that in the nymphs and larvae of *Hyalomma* spp. and larvae of *R. (B.) a*nnulatus, but it differs from that in *R. (B.) annulatus* nymph (3/3). The number of hypostomal teeth on outer/inner files is 8/8 in *R. sanguineus* nymph comparing with 8/7 or 8/8 in *Hyalomma* spp. while it is 7/7/6 teeth per file in *R. (B.) annulatus* nymph. Whereas, the number of hypostomal teeth on outer/inner files larvae is 5/5 in *R. sanguineus* comparing with 9/8 in either *H. marginatum or H. rufipes*, 6/7 in *H. impressum*, *H. excavatum* and *H. dromedarii* and 6/5 in *R. (B.) annulatus*. No descriptions were recorded before by Pegram, et al. [16], Pegram, et al. [3] on the hypostome in immature stages of *R. sanguineus*.

5. CONCLUSION

It is easy to distinguish nymph at the species level while in larvae there are some difficulties especially between tick species that belong to the same group such as *R.sanguineus* group. Therefore identification of larvae may need to additional diagnostic tools such as molecular biology. Further studies in details are needed on the nymphs and larvae of other tick species of *R. sanguineus* group such as *R. camicasi*, *R. turanicus*, *R. sulcatus*…etc using advanced tools like SEM or molecular biology. These tools may provide additional characteristics facilitate the identification processing of immature stages of this group.

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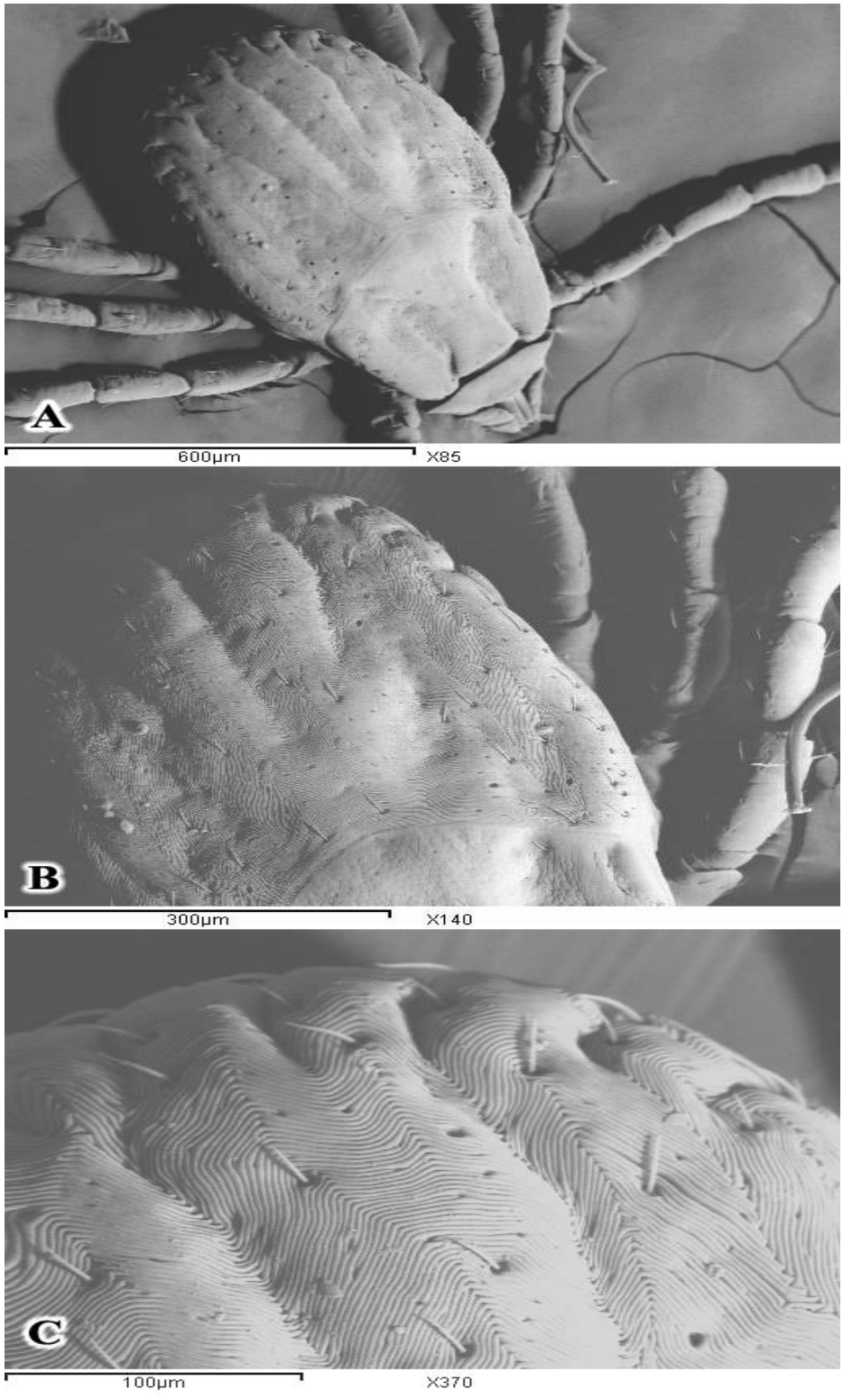

Fig-1. Dorsal view of *Rhipicephalus sanguineus* nymph: A. The entire idiosoma, B. The half left of idiosoma, D. The posterior half left of idiosoma.

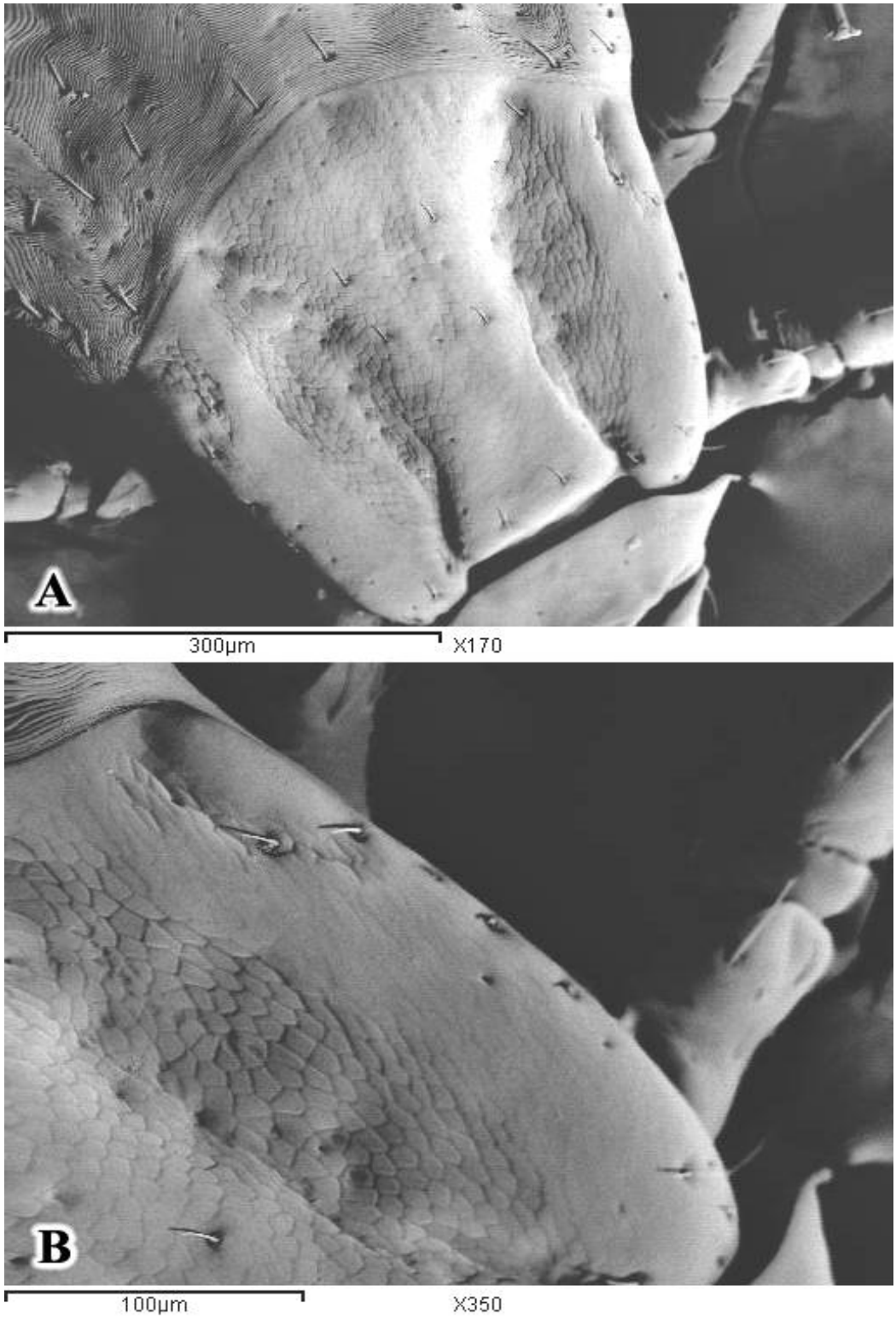

Fig-2. Dorsal view of *Rhipicephalus sanguineus* nymph: A. Scutum, B. The half left of scutum.

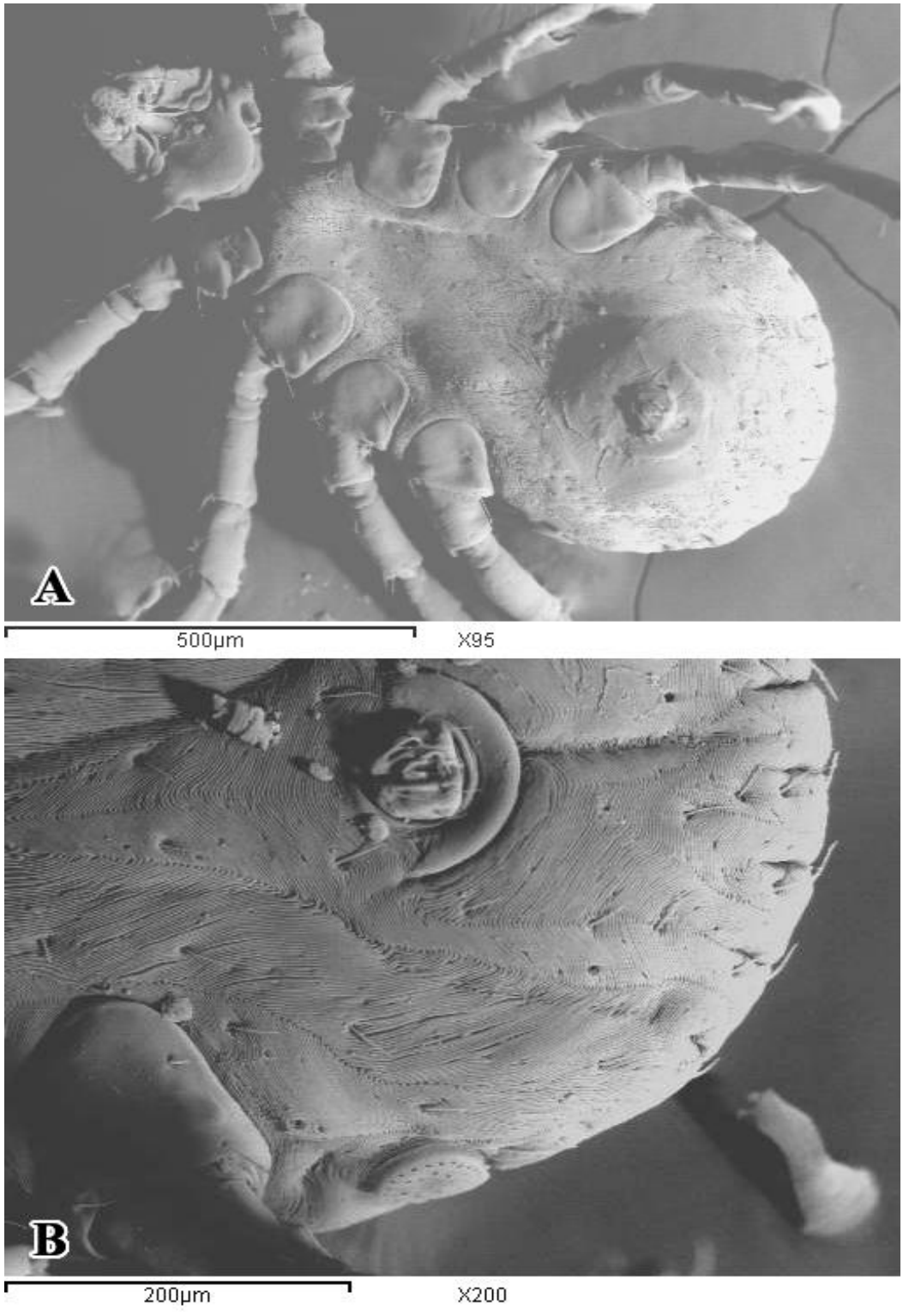

Fig-3. Ventral view of *Rhipicephalus sanguineus* nymph: A. The entire idiosoma, B. The posterior half left of idiosoma.

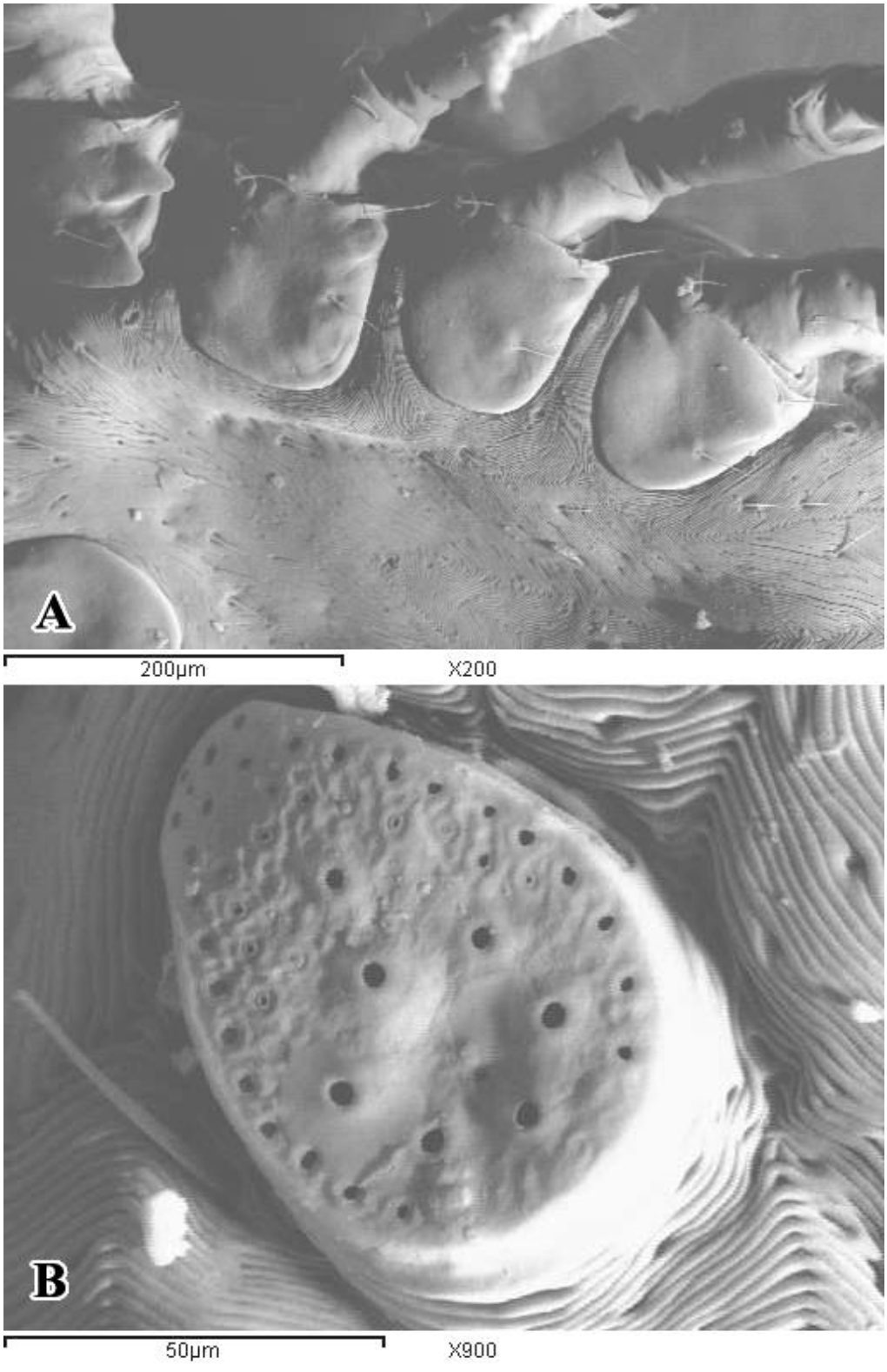

Fig-4. Ventral view of *Rhipicephalus sanguineus* nymph: A. Coxae, B. Spiracle.

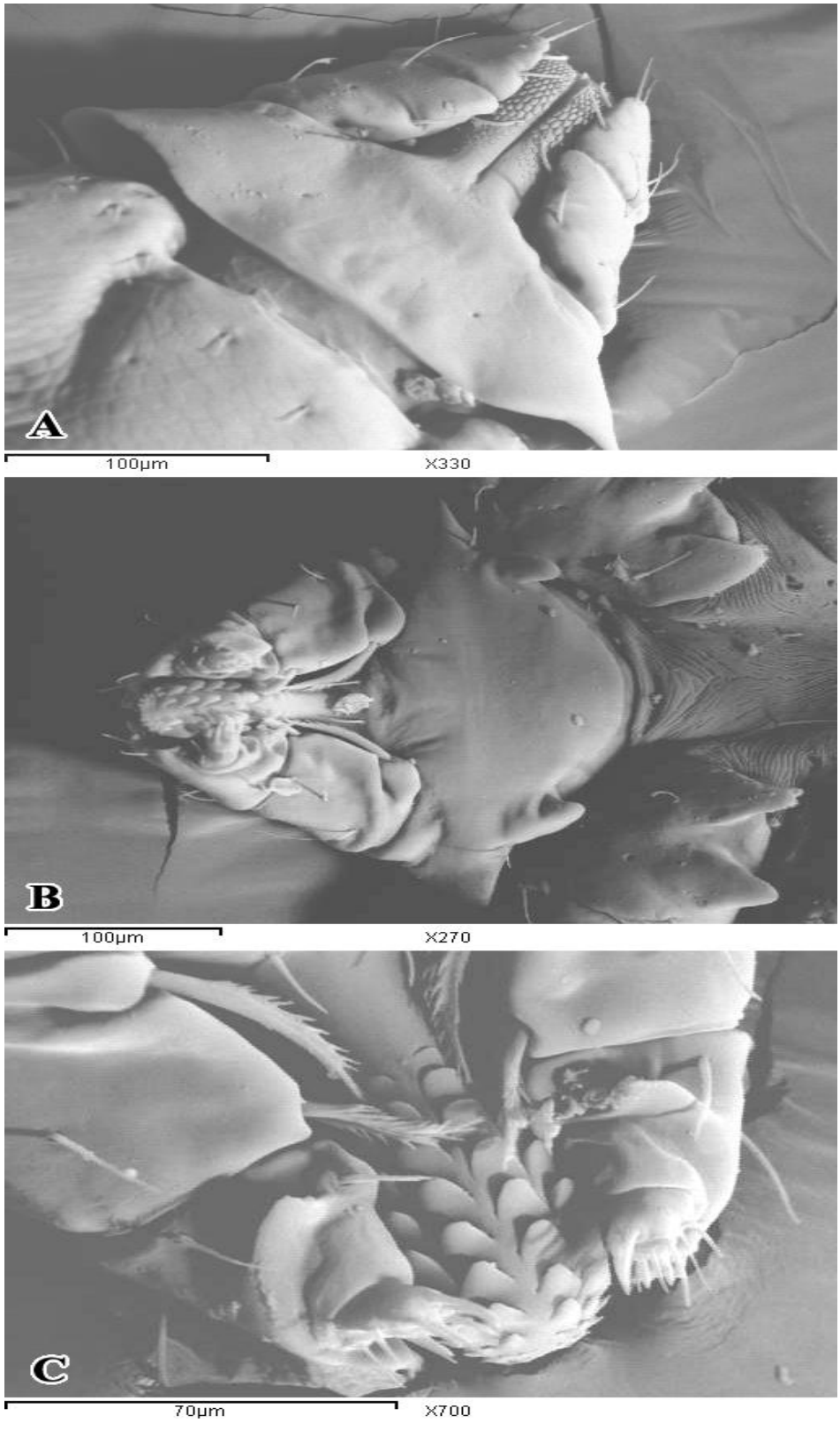

Fig-5. Capitulm of *Rhipicephalus sanguineus* nymph: A. Dorsal view, B. Ventral view, C.Hypostome

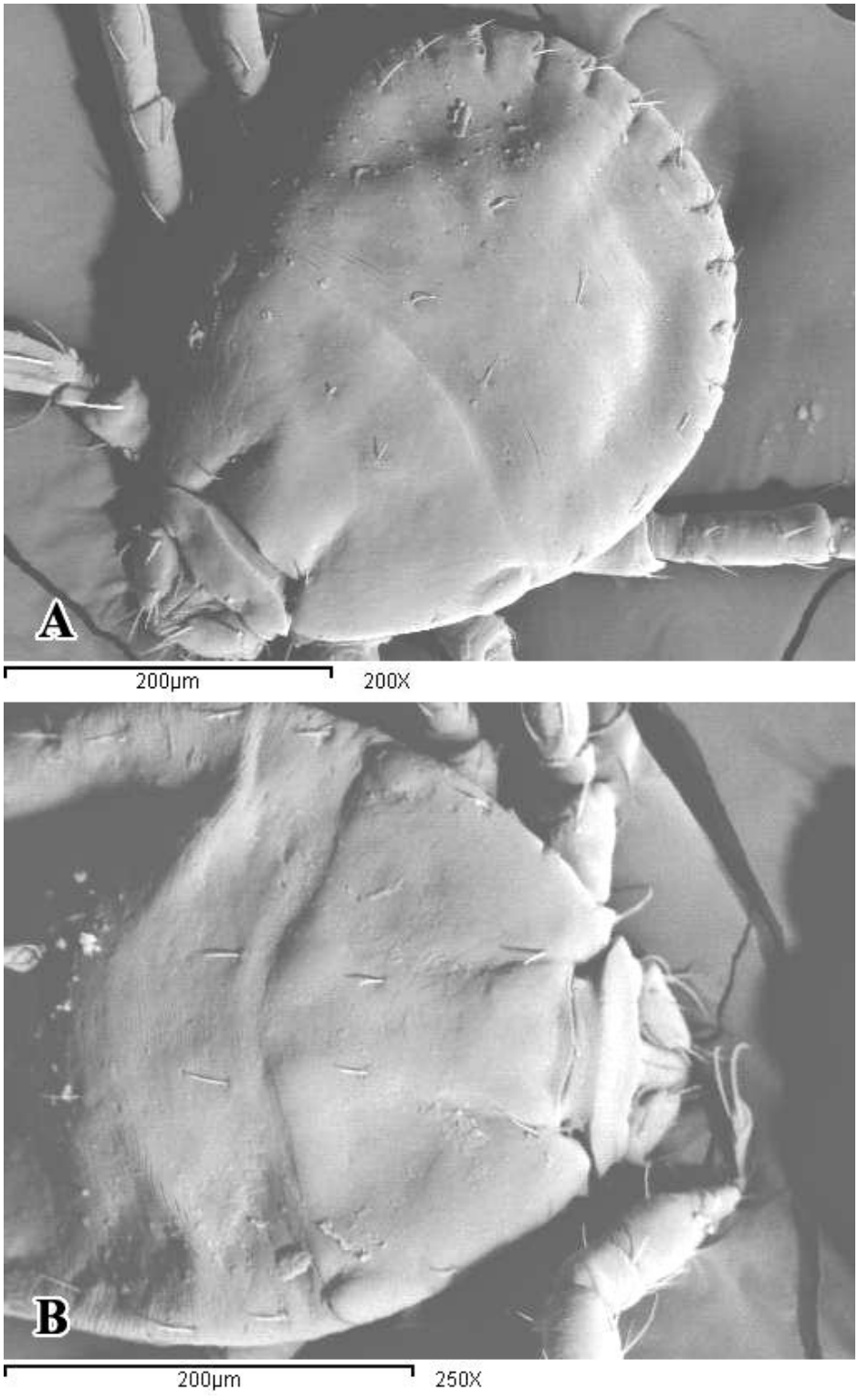

Fig-6. Dorsal view of *Rhipicephalus sanguineus* larva: A. The entire idiosoma, B. Scutum.

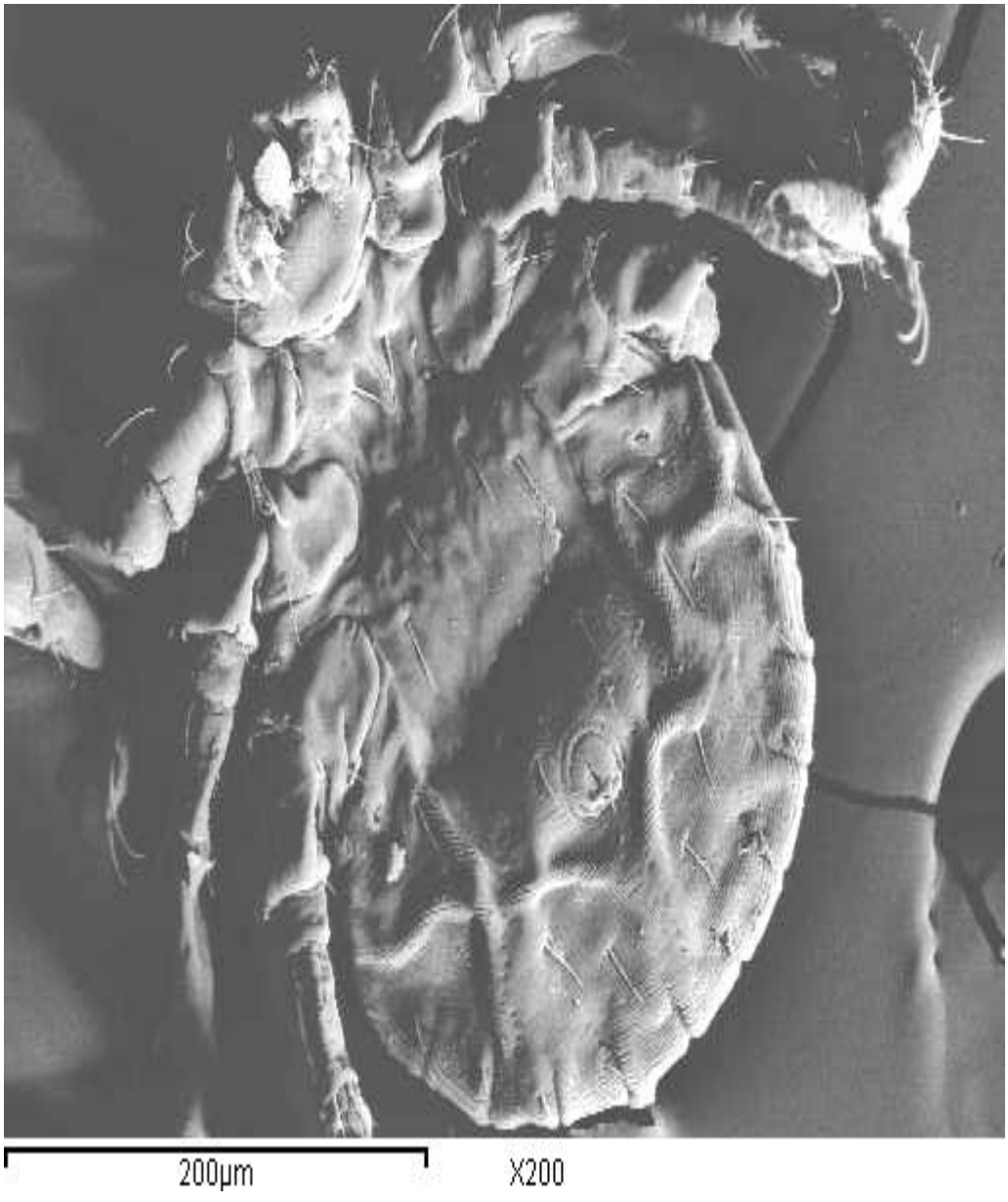

Fig-7. Ventral view of *Rhipicephalus sanguineus* larva.

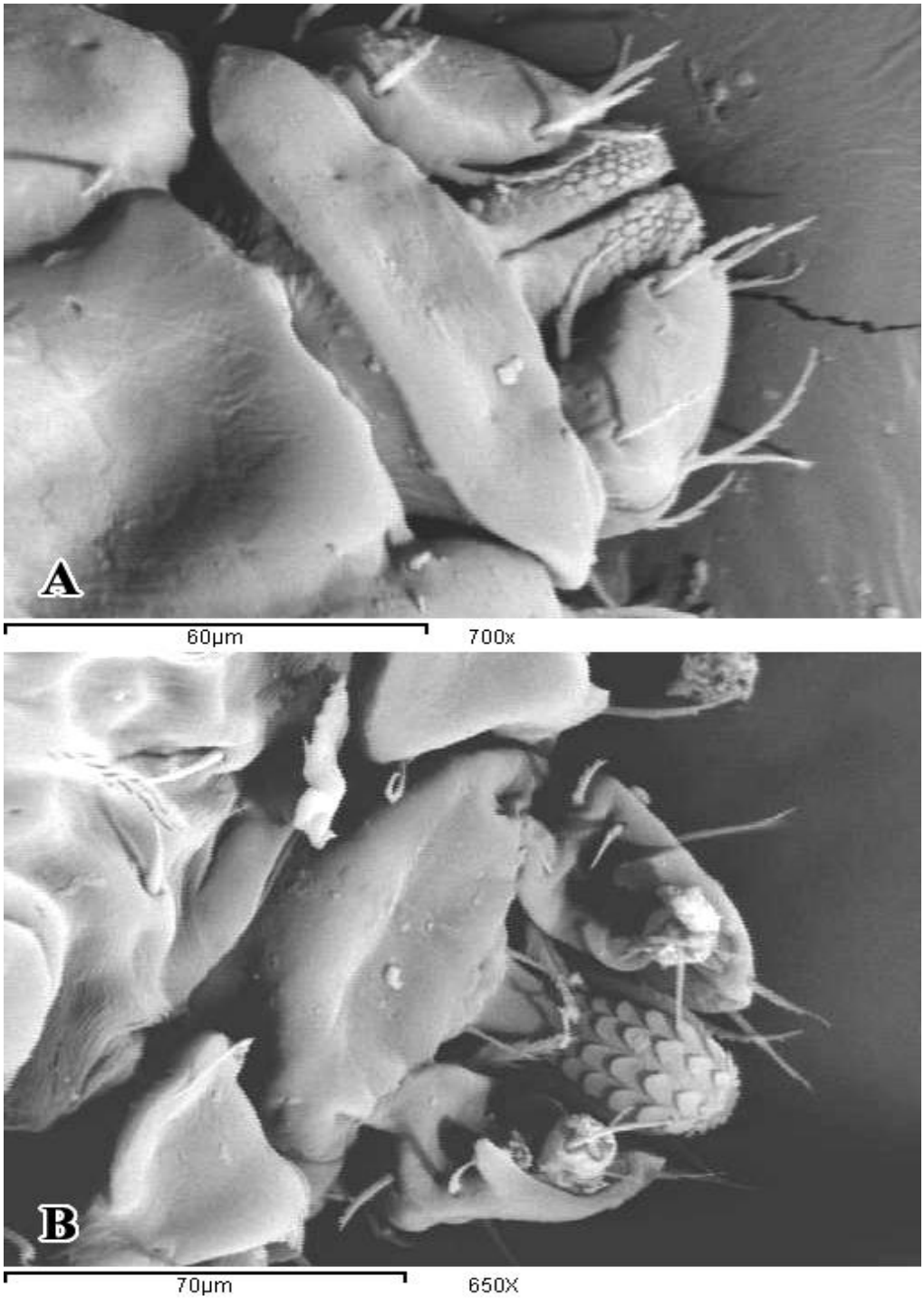

Fig-8. Capitulm of *Rhipicephalus sanguineus* larva: A. Dorsal view, B. Ventral view.

Table-1. Morphometric of different structures for nymphs and larvae of the Egyptian brown dog tick *Rhipicephalus sanguineus.*

Character	Measurements (mm) ± SE	
	Nymph	Larva
Idiosoma-length	1.102±0.025	0.527±0.013
Idiosoma-width	0.742±0.016	0.420±0.015
Idiosoma-length/idosoma-width	1.457±0.027	1.260±0.048
Scutum-length	0.535±0.019	0.230±0.004
Scutum-width	0.550±0.014	0.343±0.008
Scutum-length/Scutum-width	0.987±0.043	0.663±0.013
Palal-length	0.290±0.004	0.120±0.004
Palal-width	0.055±0.002	0.039±0.001
Palal-length/ Palal-width	5.3000±0.2333	3.000±0.029
Basis capitulum-length	0.208±0.006	0.070±0.004
Basis capitulum-width	0.345±0.008	0.150±0.004
Basis capitulum-width / Basis capitulum-length	1.635±0.018	2.100±0.066
Hypostom-length	0.240±0.006	0.087±0.002
Hypostom-width	0.077±0.002	0.030±0.000
Hypostom-length/ Hypostom-width	3.122±0.113	2.850±0.103