SCANNING ELECTRON MICROSCOPY AND COMPARATIVE MORPHOLOGY OF *HYALOMMA ANATOLICUM EXCAVATUM*, *H. DROMEDARII* AND *H. MARGINATUM MARGINATUM* (ACARI: IXODIDAE) BASED ON NYMPHS

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HYALOMMA
NYMPHS
MORPHOLOGY AND SCANNING ELECTRON MICROSCOPY

SUMMARY: The morphological differentiations between nymphs of *Hyalomma anatolicum excavatum*, *H. dromedarii* and *H. marginatum marginatum* were studied by Scanning Electron Microscopy (SEM) and morphometric analysis. Results showed the morphological characters which aide to identify tick-species from each 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 spur on coxae II-IV and the shape of spiracular plates and intensity of its pores. *H. m. marginatum* easy differentiated from other two tick species because it has large size and distinct morphological characters obviously differ from other tick species. Although *H. a. excavatum* close to *H. dromedarii* in some characters, it differs easily from *H. dromedarii*.

INTRODUCTION

The most common *Hyalomma* species infesting farm animals in Egypt were *H. anatolicum excavatum*, *H. dromedarii* and *H. marginatum marginatum* (ABDEL-SHAFY, 1994 & 2000).

H. a. excavatum infests cattle, horse, donkey, camel sheep, goat and swine. It also attacks man and dogs, nymphs feed on horses, hedgehogs, rodents, lizards, birds and man. It feeds as a two or three-host tick; it is found in north Africa, Sudan, Ethiopia, Eritrea, Iran, Turkey and Europ; it is the vector of Rickettsiosis, Babesiosis, Theileriosis and Crimean Congo Hemorrhagic Fever (HOOGSTRAAL, 1956, MOLTMAN *et al.*, 1983, FRIEDHOFF, 1997, KHAN *et al.*, 1997, RAZMI *et al.*, 2003, SAYIN *et al.*, 2003, AKTAS *et al.*, 2004, ESTRADA-PEÑA *et al.*, 2004 and PSAROULAKI *et al.*, 2005).

H. dromedarii infests camel as a chiefly host, but cattle, sheep, goat and horse are also infested. The nymphs feed on small burrowing animals, hares, reptiles, wild birds and rarely on lizards, and man can be attacked by nymphs. It is either two-host or three-host tick. It is distributed in Africa and the Near, Middle and Far East as far as India, Mongolia and Tibet, it transmits *Theileria*, *Anaplasma*, *Coxinella*, *Ehrlichia* and bacteria (HOOGSTRAAL, 1956, EL-KAMMAH *et al.*, 2001, APANASKEVICH, 2004, ESTRADA-PEÑA *et al.*, 2004, AHMED *et al.*, 2005, MONTASSER, 2005, GEBRE & KAAYA, 2006 and LOFTIS *et al.*, 2006).

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H. m. marginatum infests cattle, horse, goat, sheep and camel, birds are chief hosts for nymphs which also feed on hares and rabbits, it is two-host tick, it occurs in Northern Africa, Southern Europe and Steps, it is the vector of disease agents such as Babesiosis, Theileriosis, Rickettsiosis and Crimean Congo hemorrhagic fever (Hoogstraal, 1956, De La Fuente *et al.*, 2004, Estrada-Peña *et al.*, 2004, Meissner *et al.*, 2006 and Oteo *et al.*, 2006).

Inadequate literatures used light microscope in description of these tick species : *H. a. excavatum* (El Kammah, 1969 and Apanaskevich & Horak, 2005), *H. dromedarii* (Abdel-Shafy 1994) and *H. m. marginatum* (Apanaskevich, 2003). Only Apanaskevich (2002) gave a brief description for nymph of *H. dromedarii* by SEM. No previous study gave a morphological comparison between nymphs of these tick species. Therefore, this study aims to use SEM in clarification of morphological characteristics for the nymphal stage.

Material and methods

1- Specimens of *Hyalomma* nymphs. — The specimens of *Hyalomma* spp. (commonly on farm animals in Egypt) were collected as engorged females from various localities. These species were *H. anatolicum excavatum* Koch, 1844 and *H. dromedarii* Koch, 1844, from local camel, Marsa Matrouh, Egypt, while *H. marginatum marginatum* Koch, 1844, from Nikhil, Sinai, Egypt. Ticks were identified according to Hoogstraal, (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 fed on rabbits and checked daily to follow the moulting of engorged larvae to nymphs that occurred on rabbits. Newly moulted nymphs were removed and placed in water at 70 ± 10 °C, washed with normal saline 0.9% KCl several times and preserved in 70% ethanol (Famadas *et al.*, 1997).

2- Preparation of nymphs for scanning electron microscopy. — Nymphs were well cleaned by overnight immersion in water-glycerol-KCl solution at 40 °C (Homsher & 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 & Wharton, 1971). Specimens were washed in tap water again using the ultrasonic cleaner. Then they were 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/Fürstentun Liechtenstein), using liquid carbon dioxide. Specimens mounted on SEM stubs were coated with gold by using a S15OA Sputter Coater. Coated nymphs were examined by Scanning Electron Microscopy (SEM).

3- Preparation of nymphs for morphometric measurements. — Specimens, preserved in 70% alcohol were cleared in lactic acid for 24 h without heating. Internal organs were removed with fine sharp needle under a dissecting microscope after which the nymphs were washed with distilled water. These nymphs 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 IV, 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.

Character	Measurements (mm) ± SE			F. value	P. value	Sig.
	H. a. excavatum	*H. dromedarii*	*H. m. marginatum*			
Body-length	1.219 ᵃ ± 0.010	1.472 ᵇ ± 0.014	1.589 ᶜ ± 0.025	114.819	0.000	**
Idiosoma-length	0.936 ᵃ ± 0.005	1.239 ᵇ ± 0.018	1.335 ᶜ ± 0.024	144.616	0.000	**
Idiosoma-width	0.633 ᵃ ± 0.007	0.795 ᵇ ± 0.019	1.148 ᶜ ± 0.019	271.291	0.000	**
Idiosoma-length/idosoma-width	1.480 ᵇ ± 0.018	1.564 ᶜ ± 0.028	1.164 ᵃ ± 0.020	87.221	0.000	**
Scutum-length	0.531 ᵃ ± 0.010	0.573 ᵃ ± 0.008	0.717 ᵇ ± 0.026	32.987	0.000	**
Scutum-width	0.552 ᵃ ± 0.010	0.603 ᵇ ± 0.018	0.749 ᶜ ± 0.022	34.908	0.000	**
Scutum-length/Scutum-width	0.965 ± 0.025	0.955 ± 0.018	0.967 ± 0.052	0.037	0.964	NS
Palpal-length	0.270 ᵃ ± 0000	0.300 ᵇ ± 0.000	0.300 ᵇ ± 0.003	111.878	0.000	**
Length of dorsal basis capitulum	0.126 ᵃ ± 0.004	0.144 ᵇ ± 0.002	0.145 ᵇ ± 0.002	12.772	0.000	**
Length of ventral basis capitulum	0.186 ᵃ ± 0.002	0.219 ᵇ ± 0.002	0.215 ᵇ ± 0.004	30.621	0.000	**
Width of basis capitulum	0.333 ᵃ ± 0.002	0.324 ᵃ ± 0.002	0.369 ᵇ ± 0.007	30.827	0.000	**
Hypostome length	0.186 ᵃ ± 0.002	0.204 ᵇ ± 0.002	0.244 ᶜ ± 0.005	75.281	0.000	**
Hypostom-width	0.081 ᵇ ± 0.002	0.073 ᵃ ± 0.003	0.090 ᶜ ± 0.001	28.241	0.000	**

a,b,c means within the same column with different litters 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* nymphs.

RESULTS

1- BODY. — The body of *Hyalomma* nymphs (excluding capitulum) is elongate oval shape, but in *H. dromedarii*, it seems to be more elongated than other *Hyalomma* species (FIG. 1A, 2A & 3A). This finding was confirmed by the ratio of idiosomal length/idiosomal width that it was the highest in *H. dromedarii* followed by *H. a. excavatum* and *H. m. marginatum* (TABLE 1). Statistical analysis for nymphal body length, idiosomal length and idiosomal width revealed that *H. m. marginatum* had the highest measurements followed by *H. dromedarii* and then *H. a. excavatum* (TABLE 1).

Dorsally: *H. m. marginatum* has a large number of setae (67 pairs) followed by *H. a. excavatum* (55 pairs) and then *H. dromedarii* (43 pairs). Median field "the area between median groove and paramedian groove" includes 15, 10 and 9 pairs of setae for *H. m. marginatum, H. a. excavatum* and *H. dromedarii*, respectively. The respective setae of marginal field "the area between paramedian groove and lateral margin" are 52, 45 and 34 pairs of setae (FIGS. 1B, 2B, & 3B).

Ventrally: *H. a. excavatum* has a large number of setae (35 pairs) followed by *H. m. marginatum* (34 pairs) and then *H. dromedarii* (28 pairs). The ventral setal pattern or chaetotaxy for *H. a. excavatum, H. m. marginatum* and *H. dromedarii* is distributed as follows: sternal (5, 6 & 6 pairs), preanal (3, 4 & 3 pairs), anal (3 pairs for each), premarginal (9, 10 & 7 pairs) and marginal (15, 10 & 9 pairs). The position of sternal setae according to coxae distributed as follows: one seta front of each coxa I & coxa IV in all *Hyalomma* species, and coxa II in *H. a. excavatum* only; two vertical setae front of coxa III in *H. a. excavatum* and coxae II & III in *H. dromedarii*, while two horizontal setae front of coxae II & III in *H. m. marginatum* (FIGS. 4, 5 & 6).

2- SCUTUM. — All tick species have the following characters; broader than longer, cervical grooves broad and deep extended to the posterolateral margin, posterior margin rounded, eye distinct oval convex peripheral at greatest width of scutum, with distinct shallow cells on scutal surfaces. In *H. m. marginatum* antero-lateral margin is mildly convex and postero-lateral margins is straight, while both antero-lateral and postero-lateral margins are sinuous in each *H. a. excavatum* and *H. dromedarii*. Chaetotaxy differs from species to other : *H. a. excavatum* with 14 pairs of small setae: 2 posterior, 2 central, 2 anterior, 2 adjacent to eye and 6 lateral; *H. dromedarii* with 15-17 pairs of small setae: 2 posterior, 2 central, 2 anterior, 3-5 adjacent to eye and 6 lateral; *H. m. marginatum* with 14 pairs of small setae: 2 posterior, 1 central, 2 anterior, 2 adjacent to eye and 7 lateral (FIGS 7 A, B & C). Statistical analysis for scutal measurements revealed that *H. m. marginatum*

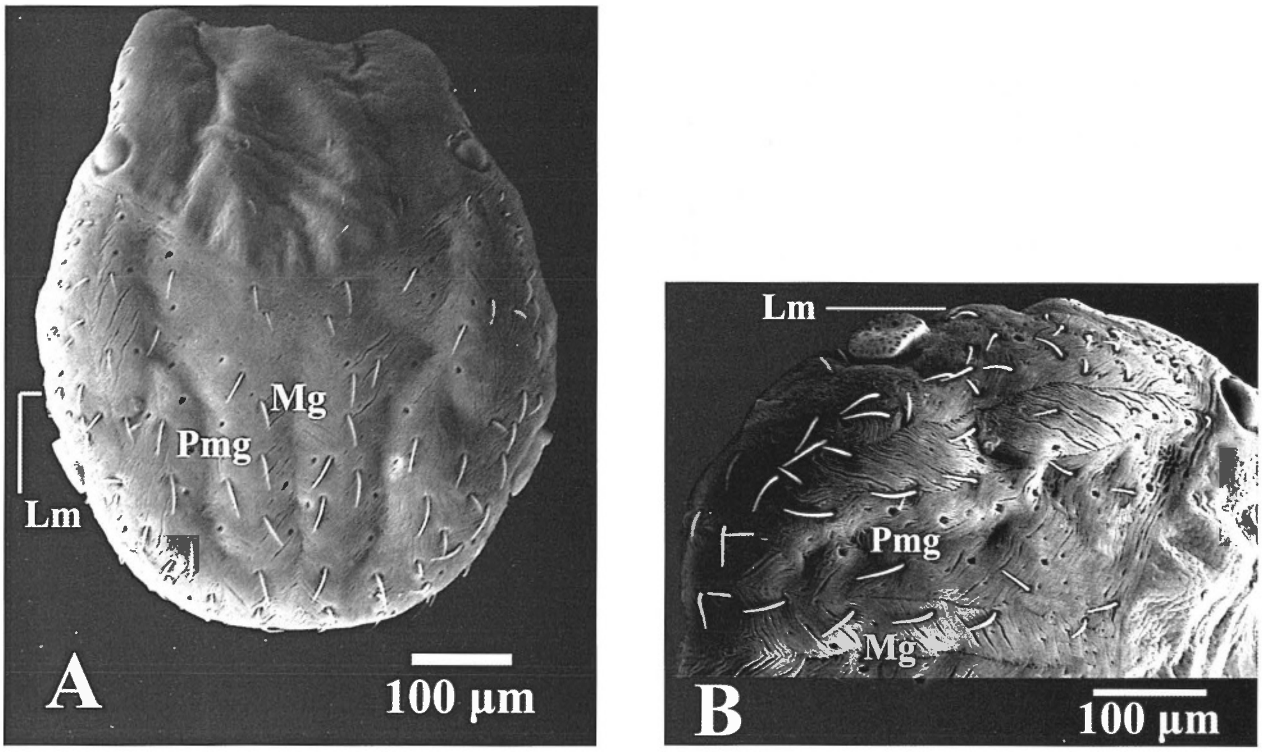


FIG. 1: Dorsal view of *H. a. excavatum*: A. — The entire idiosoma (X100), B. — The half left of idiosoma (X200), Mg. — Median groove, Pmg.- Paramedian groove, Lm. — Lateral margin.

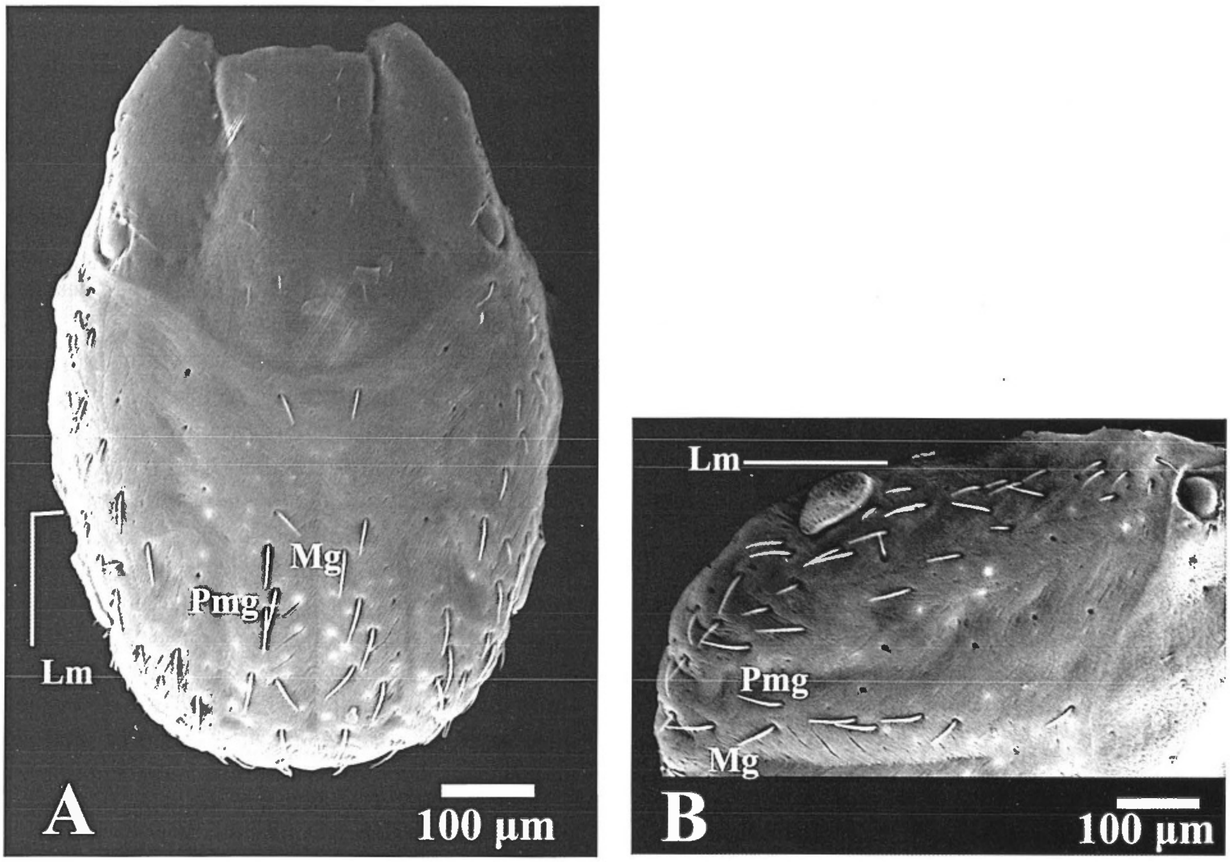


FIG. 2: Dorsal view of *H. dromedarii*: A. — The entire idiosoma (X100), B. — The half left of idiosoma (X100), Mg.- Median groove, Pmg. — Paramedian groove, Lm. — Lateral margin.

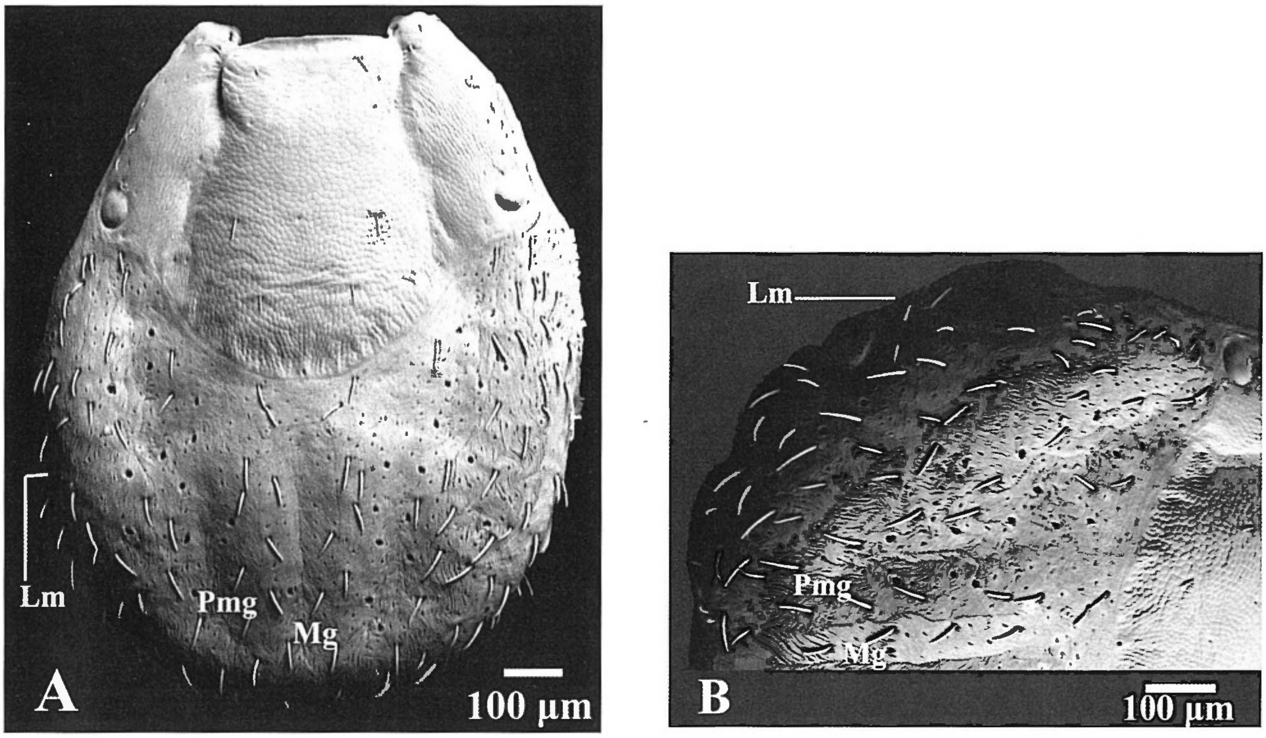


FIG. 3: Dorsal view of *H. m. marginatum*: A. — The entire idiosoma (X90), B. — The half left of idiosoma (X130), Mg. — Median groove, Pmg. — Paramedian groove, Lm. — Lateral margin.

was longer and wider than other two species. There is no significant difference between the length of *H. a. excavatum* and *H. dromedarii* while *H. dromedarii* was slightly wider than *H. a. excavatum* (TABLE 1).

3- PALPUS. — The morphological characters and chaetotaxy are similar in all tick species; external margins straight, internal margin convex, broad rounded apically, suture lines between palpal segments discernible, palpus not beyond the hypostome, with 8 setae dorsally, 7 setae ventrally and one seta apically. Palpal article IV arises from palpal article III ventrally and it carries 12 stout hairs, 8 apically and 4 basally (FIG. 8, A, B, & C and FIG. 9, A, B, C, & D). Statistical analysis for palpal length revealed *H. a. excavatum* was smaller than other two tick species which are equal in length (TABLE 1).

4- BASIS CAPITULUM. — Dorsally: the shape is triangular without setae for the three species. Posterior margin is concave in *H. a. excavatum* and *H. dromedarii,* and straight in *H. m. marginatum.* Postero-lateral margin invaginated forming pointed edge for all tick species. Anterior margins are straight in *H. a. excavatum* and *H. dromedarii* while they are sinuous in *H. m. marginatum* (FIGS. 8 A, B & C). Ventrally: tetragonal in shape, with 3 pairs of setae, 2 laterally and 1 post hypostome for all tick species (FIGS. 9 A, B & C). Statistical analysis for the dorsal and ventral length of basis capitulum revealed that *H. a. excavatum* was smaller than in the other tick species, those were approximately equal in length. *H. m. marginatum* is significantly wider than other two species, differences in width for the two other species are not significant(TABLE 1).

5- HYPOSTOME. — It is cylindrical in shape for the *Hyalomma* species. The dental formula is 2/2 for each tick species. The teeth number per file (including small basal teeth) are 13 in the outer file and 11 in the inner file for *H. a. excavatum* and *H. m. marginatum* while they are 12 teeth in the outer file and 10 teeth in the inner file in *H. dromedarii.* The large teeth of *H. dromedarii* and *H. m. marginatum* seem to be more tapering than that in *H. a. excavatum* (FIGS. 10 A, B

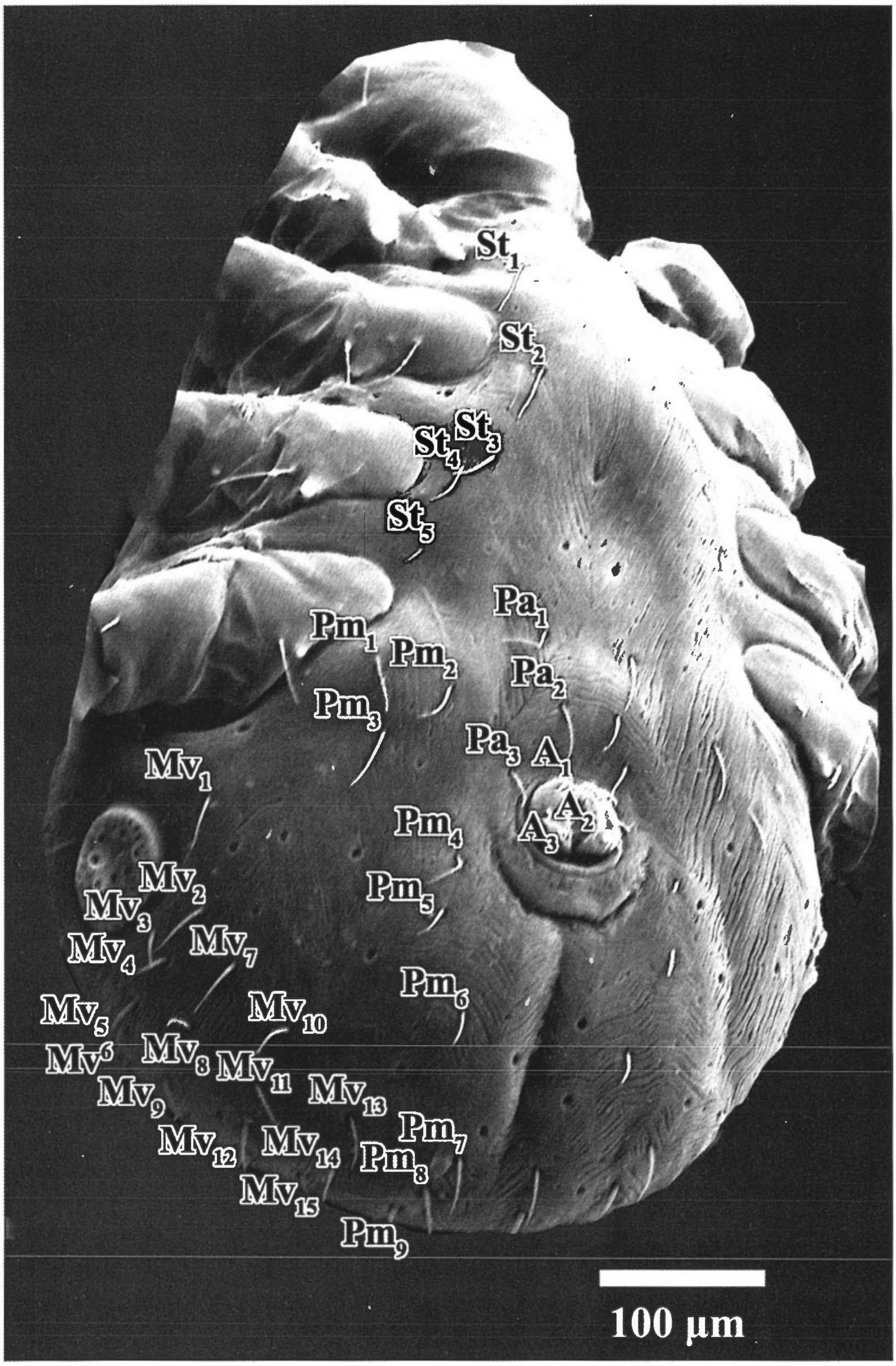


FIG. 4: *H. a. excavatum*: Body ventral view includes; St = Sternal setae (1-5), Pa = Preanal setae (1-3), A = Anal setae (1-3), Pm = Premarginal setae (1-9), Mv = Marginal ventral setae (1-15), X140.

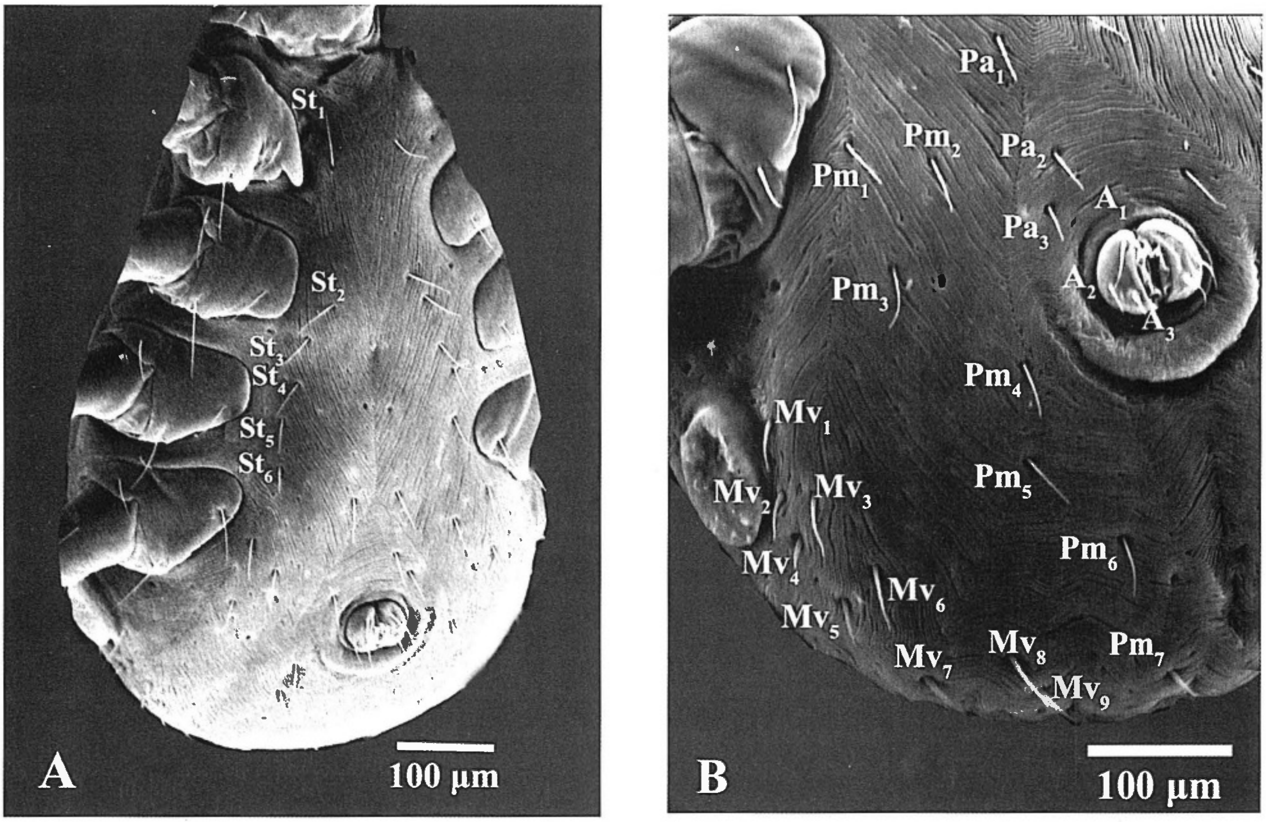


FIG. 5: *H. dromedarii*: Body ventral view includes; St = Sternal setae (1-6), Pa = Preanal setae (1-3), A = Anal setae (1-3), Pm = Premarginal setae (1-7), Mv = Marginal ventral setae (1-9), A.- X140, B.- X190.

& C). Statistical analysis for hypostome length revealed that *H. m. marginatum* was the longest followed by *H. dromedarii* and then *H. a. excavatum*, while the hypostome of the later was wider than that in *H. dromedarii* and lesser wide than that in *H. m. marginatum* (TABLE 1).

6- COXAE. — Each coxa carries 3 setae for each tick species. Coxa I carries 2 large spurs for each tick species. Coxae (II-IV) carry one small spur for each in all tick species. This spur well developed in *H. m. marginatum* and *H. a. excavatum* but in the latter, it is slightly smaller and more tapering. In *H. dromedarii* small spurs seem to be reduced or very small with blunt end (FIGS. 11 A, B & C).

7- SPIRACLES. — It is an egg shape in *H. a. excavatum* and *H. dromedarii* while it is semicircular shape in *H. m. marginatum*. The spiracle of *H. m. marginatum* is the largest followed by *H. dromedarii* and then *H. a. excavatum*. The density of spiracle pores are great in *H. dromedarii* followed by *H. a. excavatum* and then *H. m. marginatum* (FIGS 12 A, B &C).

DISCUSSION

Nymphs as larvae play an important role in distribution of ticks and transmission of disease agents. Nymphs feed on small mammals and birds which can transmit ticks from host to host, even from country to other by migratory birds, as well as it may attack human. Nymphs are considered the most dangerous stage because it can be fed on large animals like adults besides its feeding on small mammals and birds like larvae. Therefore, this study tried to give morphological differentiations between the most common *Hyalomma* species infesting farm animals in Egypt (*H. a. excavatum*, *H. dromedarii* and *H. m. marginatum*) based on nymphs by using SEM and morphometric

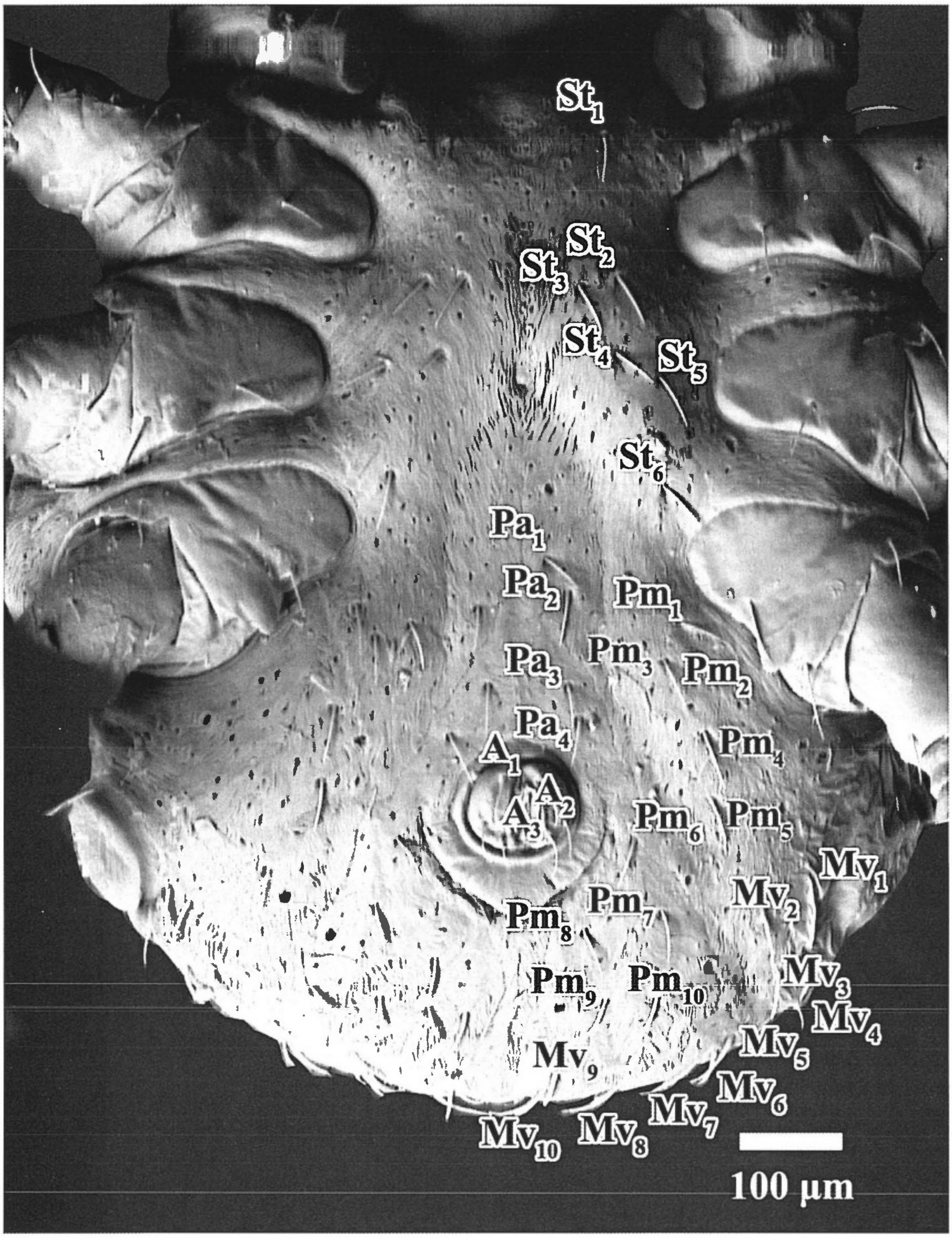


FIG. 6: *H. m. marginatum*: Body ventral view includes; St = Sternal setae (1-6), Pa = Preanal setae (1-4), A = Anal setae (1-3), Pm = Premarginal setae (1-10), Mv = Marginal ventral setae (1-10), X100.

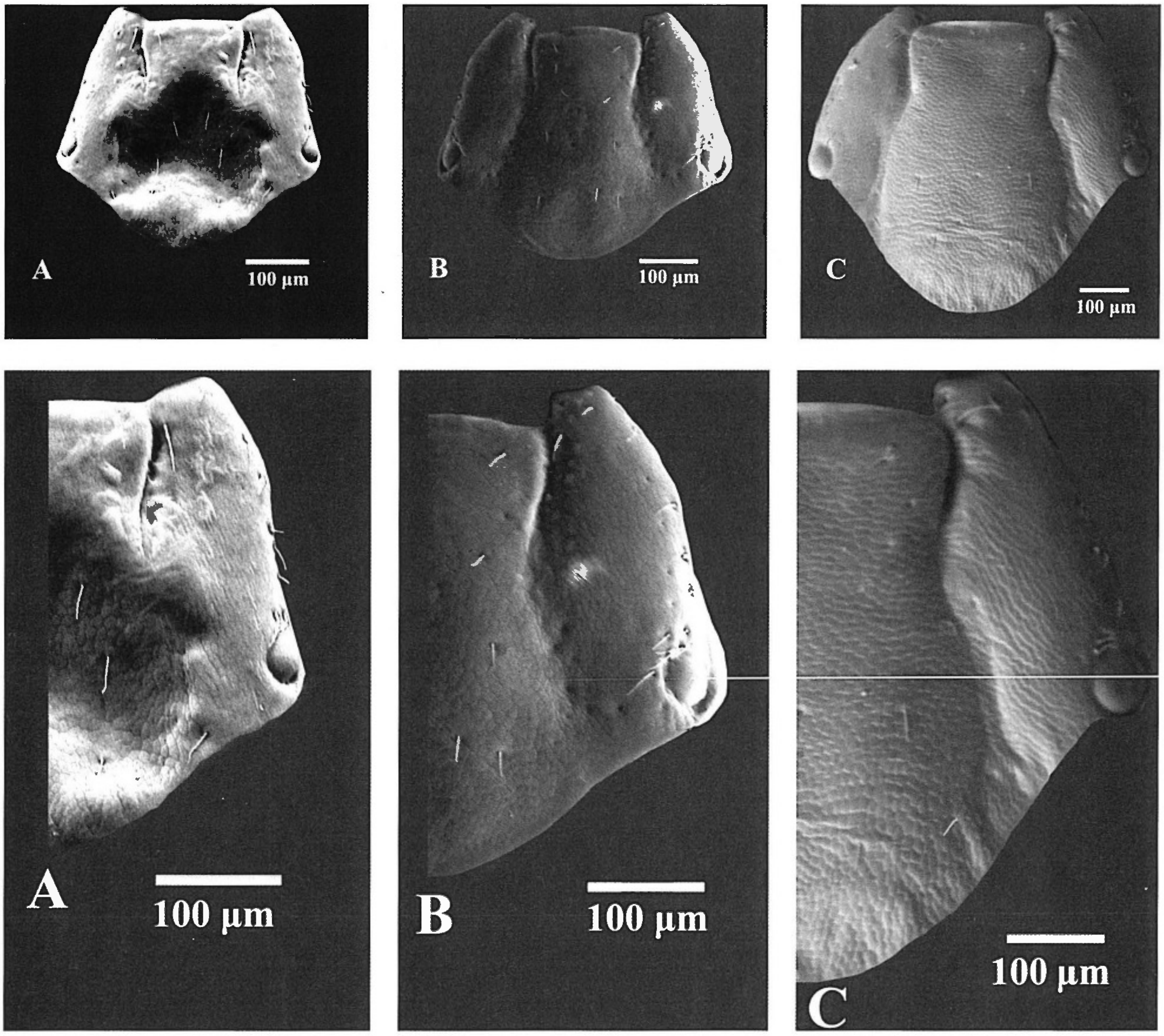


FIG. 7: Scutum view: A. — *H. a. excavatum* (X170). B. — *H. dromedarii* (X170). C. — *H. m. marginatum* (X140).

analysis. Results showed that the morphological characters useful to identify the tick-species are: the chaetotaxy of dorsal and ventral idiosoma, the chaetotaxy of scutum, the position of sternal setae especially front of coxae II and III, the shape and size of the spur on coxae II-IV, the shape of spiraculare plates and the density of spiraculare pores. Generally, *H. m. marginatum* is easily identified by the large size and by distinct morphological characters. *H. a. excavatum* is closed to *H. dromedarii* in some characters, but is easily differenced from *H. dromedarii.*

No previous study described these tick species in details by SEM except APANASKEVICH (2002) who gave a brief description for nymph of *H. dromedarii* by SEM. In general, a scarcely other previous studies described the nymphs of *Hyalomma* species by light microscope; *H. dromedarii* (ABDEL-SHAFY, 1994), *H. a. excavatum* (EL KAMMAH, 1969 and APANASKEVICH & HORAK, 2005) and *H. marginatum* (APANASKEVICH, 2003). Despite APANASKEVICH (2002) used SEM in description of *H. dromedarii* nymph, he omitted many important taxonomic characters. The measurements of nymphs recorded by him agree with

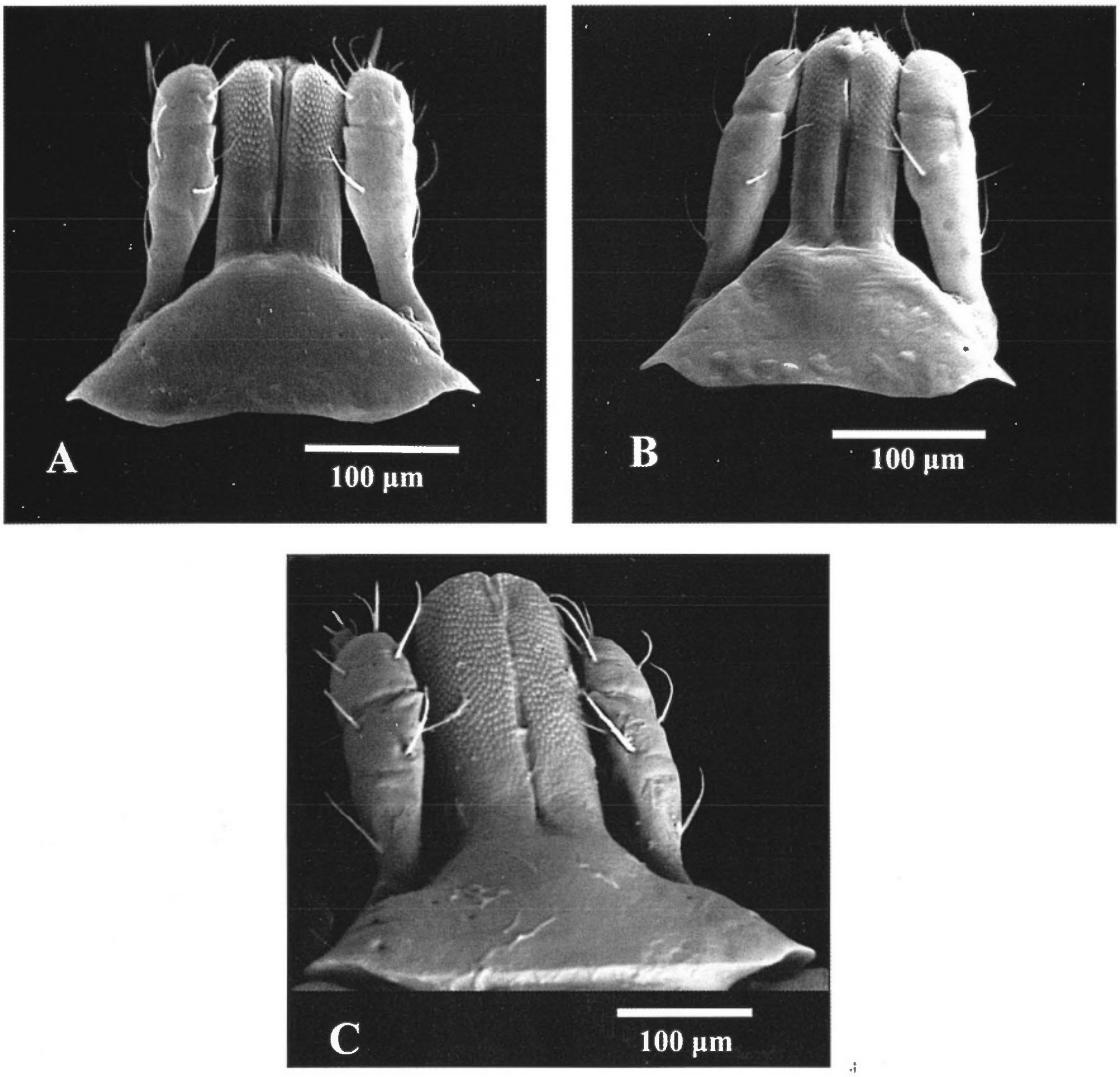


Fig: 8: Dorsal capitulm view: A. — *H. a. excavatum* (X270). B. — *H. dromedarii* (X270). C. — *H. m. marginatum* (X250).

those in the present study but contrarily of the results of APANASKEVICH, ABDEL-SHAFY (1994) referred to the scutal lateral margin of *H. dromedarii* nymph (straight, versus sinuous). This finding may be due to use of light microscope. The number of small setae on scutum of *H. dromedarii* nymph that recorded ABDEL-SHAFY (1994) agrees with that recorded in this study, while there was a discrepancy in the distribution of setae on scutum. He mentioned that central field of scutum carried 3 setae comparing with 2 setae herein and did not refer to the number of setae adjacent to the eye, this discrepancy attributed to the use of light microscope. He also found 48 dorsal and 24 ventral idiosomal setae (43 dorsal and 28 ventral observed in this study). This finding may be due to overlap the dorsal and ventral surfaces of idiosoma during the investigation by light microscope. ABDEL-SHAFY (1994) recorded 8 teeth per file in hypostome of *H. dromedarii* nymph comparing with 12 teeth in the outer file and 10 teeth in the inner file, this may be due to omitting the number of small basal teeth. Also he did not give any details about the shape of coxae especially coxae II-IV which have an important taxonimic characters.

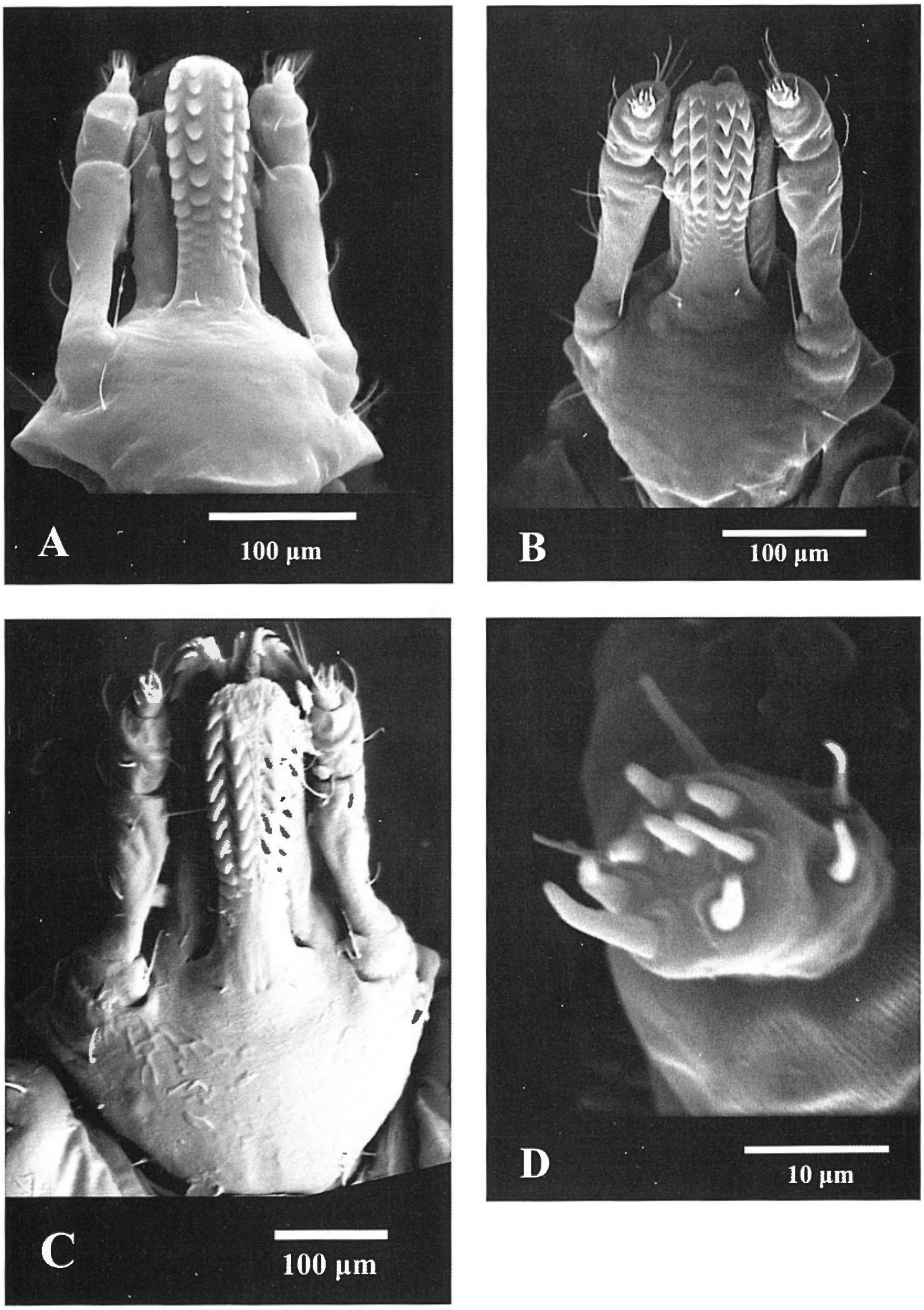


FIG. 9: Ventral capitulum view: A. — *H. a. excavatum* (X300). B. — *H. dromedarii* (X300). C. — *H. m. marginatum* (X150). D. — Palpal article IV (X3000).

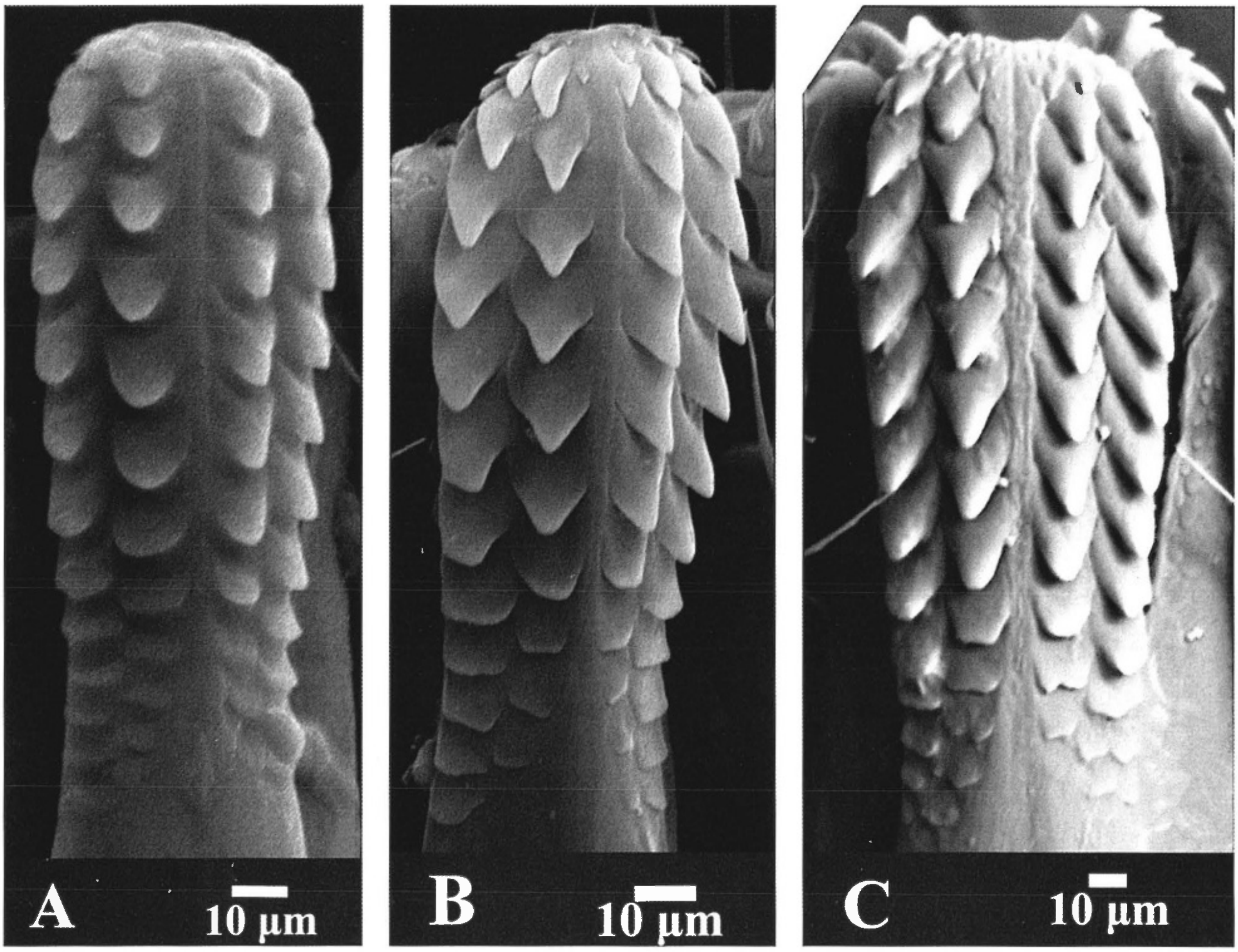


FIG. 10: Hypostome: A. — *H. a. excavatum* (X650). B. — *H. dromedarii* (X650). C. — *H. m. marginatum* (X450).

All measurements of *H. a. excavatum* nymph, those recorded by EL-KAMMAHK (1969) and APANASKEVICH & HORAK (2005) agree with those determined in this study. Authors did not provide more details for the important characters which can help in identification of this species from others. APANASKEVICH & HORAK (2005) mentioned that the postero-lateral margin of scutum of *H. a. excavatum* nymph was moderately indented but it did not see that character on SEM photo. Unfortunately, the nymph of *H. m. marginatum* was described by light microscope only by APANASKEVICH (2003) and the reprints of this study edited by Russian language. So few characters were cited from abstract and illustrated figures such as chaetotaxy of palpi and dental formula of hypostome those were agreeing with those in this results.

CONCLUSION

The morphological characters which aide to identify tick-species from others are: 1) chaetotaxy of dorsal and ventral idiosoma, 2) chaetotaxy of scutum, 3) the position of sternal setae especially front of coxae II and III, 4) the shape and size of spur on coxae II-IV and 5) the shape of spiraculare plates and the density of its pores.

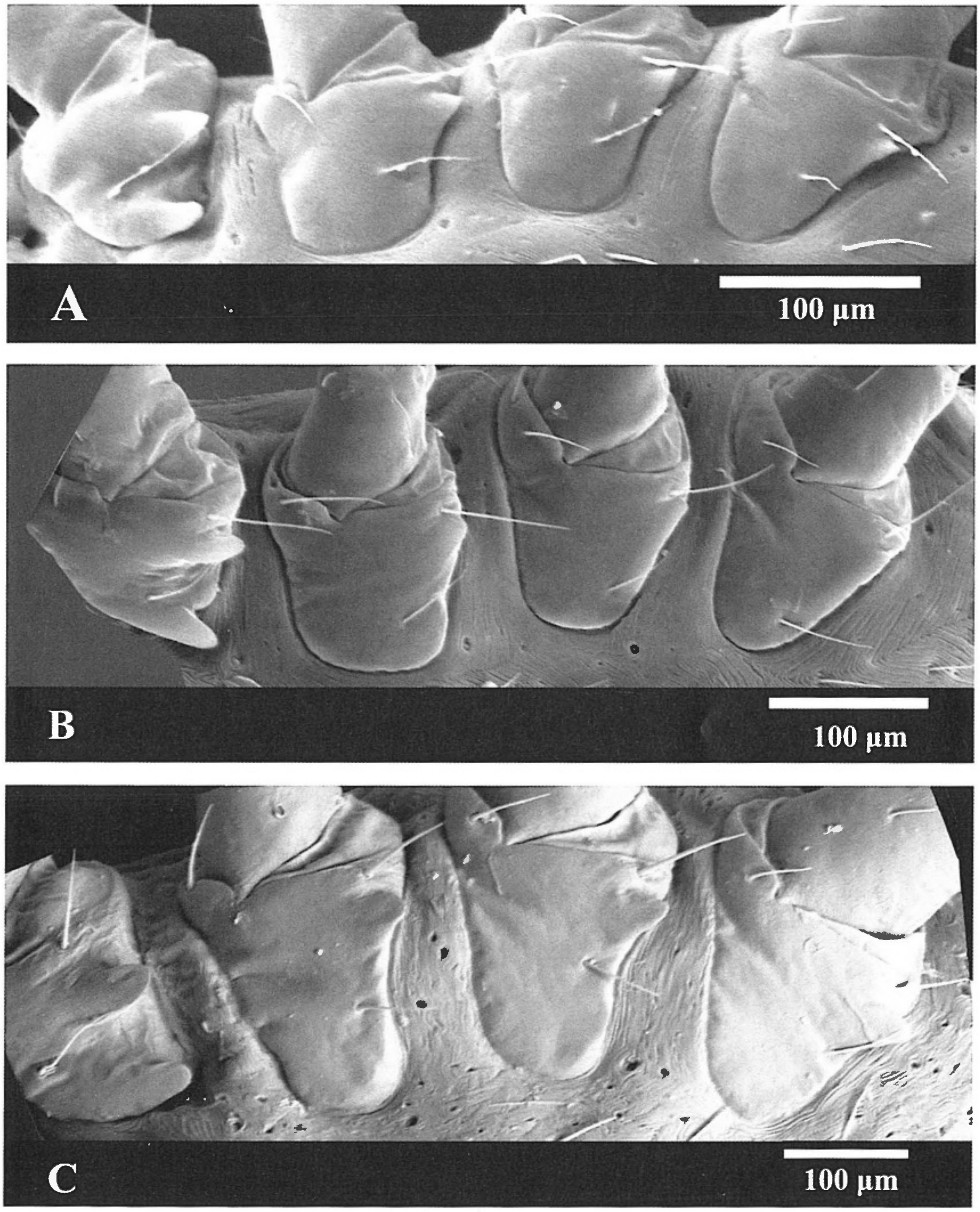
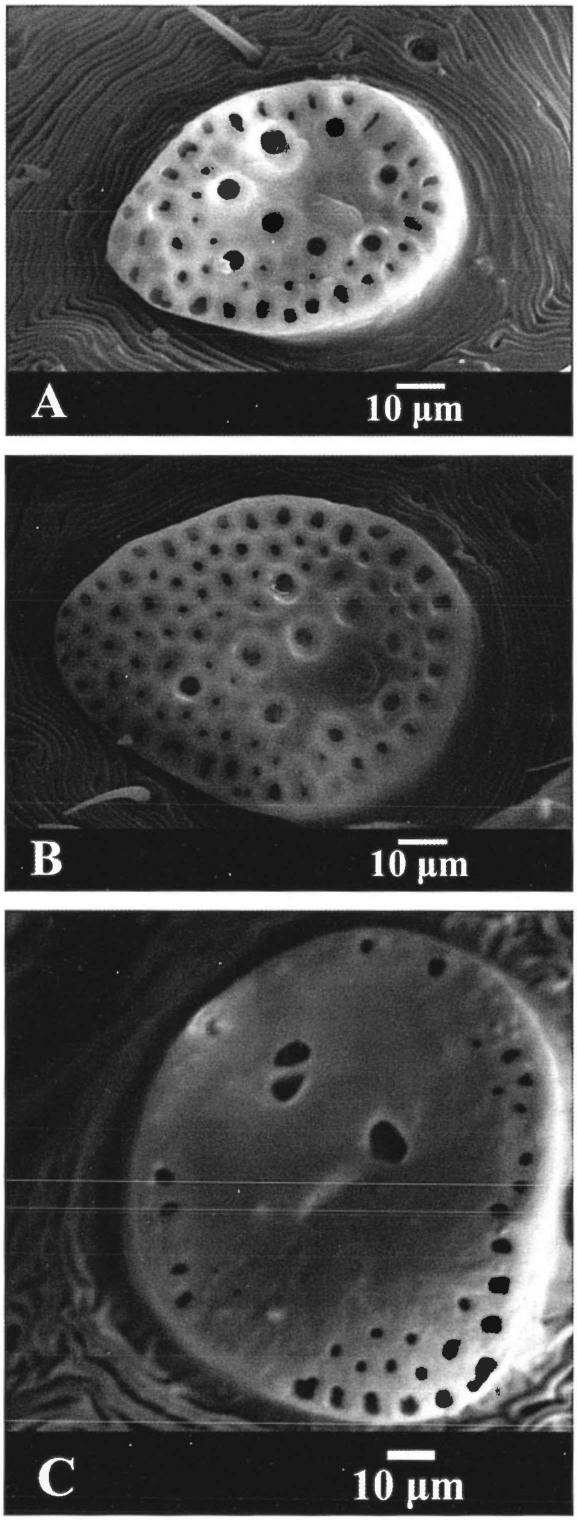


FIG. 11: Coxae: A. — *H. a. excavatum* B. — *H. dromedarii* (X400). C. — *H. m. marginatum* (X350).



Fɪɢ. 12: Spiracles: A. — *H. a. excavatum* (X1000) B. — *H. dromedarii* (X1000). C. — *H. m. marginatum* (X600).

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