

## Comparison between morphological characters and ultrastructure of *Varroa* mite from Egypt and Libya

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### ABSTRACT

Specimens of *Varroa* mite, *Varroa destructor* Anderson and Trueman were collected from Egypt and Libya to compare their morphological and morphometric characters. Females were examined using a Scanning Electron Microscope (SEM). Our morphological measurements showed that there were a lot of differences between samples of Egypt and Libya. Only two morphological characters showed significant differences among the two locations specimens' (i.e. anal plate length, anal plate length/anal plate width (ratio III) and formula of palpal chaetotaxy). These results may be due to geographical locations between Egypt and Libya. We consider samples of Egypt and Libya as morphotypes.

**Keywords:** *Varroa* mite, morphology, morphometric characters, Egypt, Libya.

### INTRODUCTION

The *Varro* mite, *Varroa jacobsoni* Oudemans (Acari: Mesostigmata: Varroidae) was first described as an ectoparasite of the Eastern honeybee, *Apis cerana* Fab., in Java, Asia. *Varroa* mite causes a serious damage by feeding on a hemolymph of larvae, pupae and adults, and by transmitting or activating viral diseases (Martin 2001; Chen et al. 2004). Honey bee keepers consider this mite to be a dangerous pest and the most damaging enemy of the honey bee around the world (Sammataro et al. 2000; Martin et al. 2012; Nazzi et al. 2012). In the last few years *Varroa* became a subject of concern to bee keepers where the majority of apiaries in the Egyptian Governorates were destroyed (Abd Al-Fattah et al. 1991).

The identity of *Varroa* mites infesting *Apis mellifera* L from 32 countries was investigated using mtDNA Co-I gene sequences and morphological characters (Anderson and Trueman 2000). All were found to be *V. destructor*, not *V. jacobsoni* as was assumed. However, only 2 of the six *V. destructor* genotypes which found on *A. cerana* were parasiting *A. mellifera*. The most common was a Korean genotype of *V. destructor*, so-called because its primary host is *A. cerana* in Korea. It was found on *A. mellifera* in Europe, the Middle East, the Americas, Africa and Asia.

*Varroa* mites have about 15 different haplotypes including (Korean, Chinese, Japanese

and Pakistan ones) (Zhou et al. 2004). The Korean haplotype is considered to be the most common one. It is possible to discriminate between *Varroa* morphotypes using morphological traits (Maggi et al. 2009).

In Egypt, *Varroa* was recorded in 1983 as *V. jacobsoni* (Wienands 1988). It was corrected later to be *V. destructor* according to Anderson and Trueman (2000) (Awad et al. 2011). Awad et al. (2010) observed genetic variability in *Varroa* belong to different localities in Egypt. Allam et al. (2014) determined the specific Egyptian *Varroa* morphological Scanning Electromicroscope as a tool to re-describe isolates of *V. destructor* from ten different locations belonging to nine different governorates.

The objective of this research was to compare the morphological and morphometric characters of *Varroa* mites collected from Egypt and Libya and to characterize and investigate the potential haplotype of *Varroa* mite.

### MATERIALS AND METHODS

Samples of *Varroa* mite's adult females (1-2 mite/colony) were collected from honeybee, *A. mellifera*, Carniolan hybrid colonies at various locations in Egypt and Libya through for two successive years 2019 and 2020. Mites were collected alive from the bodies of workers, drone bees, capped worker broods, or drone brood cells

and preserved in 70% ethanol. To confirm the specific identity, mite morphology was examined using research light microscope and the length and width of each female was measured individually. The measurements in micrometers ( $\mu\text{m}$ ) were taken for five females, obtained from each region and mounted in Hoyer's medium on glass.

Scanning Electron Microscope (SEM), was used to observe the mite characters. Living specimens of honeybees were put through a served bath of distilled water in an attempt to clean them of debris. Then they were briefly submerged in distilled water near boiling point in order to force prostration of appendages. Specimens were fixed in glutaraldehyde of 3.5% concentration for 6 hours then dehydrated in ethyl alcohol and dried using the critical point procedure. Then, they individually affixed to stubs using double-sided sticky tape, and coated with gold-palladium in a sputter coater, (microscopy was performed with JEOL GM 4200) (Fashing et al, 2000).

Scanning Electron Microscopy was performed at the Applied Centre of Entomonematodes, the Faculty of Agriculture Cairo University, Egypt. They were transferred to the low vacuum chamber of the Electron Microscope, then examined and imaged.

## RESULTS AND DISCUSSION

Morphometric characters of *Varroa* mite in Egypt and Libya are summarized in Tables (1 and 2) and Figures (1 and 2). Nomenclature of Delfinado-Baker (1974) and Delfinado-Baker and Agarwal (1987) was used. Results showed no significant differences between samples of *Varroa* mite collected from Egypt and Libya in all morphometric measurements except anal plate length and ratio III (APL/APW) (Length and width of anal plate).

Table (2) shows twenty characters distinguished and determined in *V. destructor* collected from Egypt and Libya.

### Discription of *Varroa destructor* in Egypt and Libya

#### A- Gnathosoma

The gnathosoma is situated anteroventrally and is visible through the dorsum. This character agrees with Allam et al. (2014).

#### 1- Hyposome

The surface scan of hypostome determine long striation beside the edges of hypostome and transverse striations nearly the tip of hypostome

consist of island of transverse striations in Egyptian smples (Figure1: M, N, P and R) and Libya (Figure 2 G, H, T, K). Three paris on hypostome on one line these character differs from Delfinado-Baker (1974) who found three paris of setae in one row.

#### 2- Chelicera

Bidentate (two teeth) on apical half one sharp-tipped and smaller than other blunt tipped tooth (Figure1: O in Egypt and Figure 2: P in Libya), with movable digit only (Figure1: O, Q, W in Egypt and G,T, K in Libya) and fixed digit not found dorsal seta and fissures present. These characters agree with Delfinado and Aggarwal (1987) and Allam et al. (2014).

#### 3- Palp

Apotele biforcate have one long forcate and a short one (not equal in length) (Figure1 H, N, R in Egypt) and (Figure 2 K, M, N in Libya). Number of setae on palpal trochanter, femur, genu, tibia, and tarsus 1, 2-3, 2-3, 7-9, 10-12 in Egypt and 1,1-2,2-3,6,10 in Libya, respctively (Table 2). This formula of palpal chaetotaxy differs from that of *V. jacobsoni* (Delfinado-Baker 1974), and *V. underwoodi* (Delfinado-Baker and Agarwal 1987) and formula of palpal chaetotaxy of Libya different from in Egypt by Allam et al. (2014).

#### 4- Deutosternum

The denticles agree with Delfinado-Baker and Agarwal (1987) whom reported that the deutosternum has denticles in *V. underwoodi* on the other side differes from *V. jacobsoni* (Delfinado-Baker 1974) whom reorted that the deutosternum was smooth and these characters agree with Allam et al. (2014).

#### B- Ventral shield

All ventral shields extensively developed and sclerotized with surface lightly and finely striate-reticulate this character agrees with Delfinado-Baker and Aggarwal (1987) and Allam et al. (2014).

#### 1- Tritosternum:

The basis of tritosternum is triangle. The basis of triangle contact with sternal shield of *Varroa*, the triangle extand to the hypostome. Tritosternum bipartite (Figure 1: P in Egypt and Figure 2: G, I, M), membranous, laciniae tapping and partly serrate (Figure 1 W in Egypt and Figure 2 P). Tthis description of tritosternum agrees with (Delfinado-Baker 1974; Delfinado-Baker and Aggarwal (1987); Allam et al. 2014).

#### 2- Sternal shield:

Sternal shield extending from anterior margin of coxae 2 to coxae 4, occupied the area between coxae 1 and coxa 4 behind tritosternum. from 4 to 8 pairs of setae, 4-6 pairs of lyrifissures in Egypt and 5 pairs of setae, 4-6 pairs of lyrifissures in Libyans. These numbers of setae differ from Delfinado-Baker (1974), whom reported that the number of sternal setae was 5-6 pairs and 4-5 pores in *V. jacobsoni*. Delfinado-Baker and Aggarwal (1987) reported that *V. underwoodi* has 4 pairs of setae and pores in sternal shield (Figure 1 and Figure 2: A, B). between sternal 1 (*st1*) ranged from 71-190  $\mu\text{m}$  in Egypt and in Libya ranged from (75-90). Also between sternal 2 (*st2*) ranged from 192-246  $\mu\text{m}$  in Egypt and in Libya was (196-255). Longitudinal distance between (*st1-st2*) ranged from 76-223  $\mu\text{m}$  in Egypt and in Libya was (87-236). The previous measurements agree with Egypt Allam et al. (2014).

### 3- Genital shield

Densely covered with simple setae. occupy the area between coxae 4 until anal shield on the posterior region from the ventral shields behind sternal shield, between the suture Y shape which extend laterally with genital (Figure 1 and Figure 2: A and B). In the present work, we found that the width of genital shield ranged from 656-672  $\mu\text{m}$  in the Egyptians but in Libyans ranged from 685-736 and the half number of setae ranged from 40-56 (80-112) in Egyptians but in Libya ranged from 52-62 (75-118). Also, genital plate wider than long. The anterior portion of genital shield smooth, with no setae. These descriptions agree with Delfinado-Baker and Aggarwal (1987) who stated that genital shield densely covered with setae at posterior 2/3 mostly of uniform length and smooth.

### 4- Endopodal shield

Endopodal shield well developed, half circling posterior border of coxa 4. There was suture in Y shape between endopodal plate from one side, metapodal shield on the second side and sternal and genital plate on the third side. (Figure 1: A, B, G, K in Egyptian and Figure 2: A, B, D in Libyans). The description of endopodal shield agrees with Delfinado-Baker (1974), whom described endopodal shield in characters of genus *Varroa*. But the suture in Y shape was not described in (Delfinado-Baker 1974; Delfinado-Baker and Aggarwal 1987). Endopodal plate has 8-10 setae on the posterior margin and has a little striation.

### 5- Metapodal shield

Metapodal shield broadly triangular, densely covered with simple setae (Figure 1: A, B, J, K in Egypt and Figure 2: A, B, D in Libyan). Width of metapodal shield ranged from 288-355  $\mu\text{m}$  in Egypt but in Libya was 291-345  $\mu\text{m}$ . The length of metapodal shield ranged from 672-768  $\mu\text{m}$  in Egypt but in Libya was 682-778  $\mu\text{m}$  (Table 1), these descriptions agree with (Delfinado-Baker 1974; Delfinado-Baker and Aggarwal 1987).

### 6- Anal shield

Width of anal shield ranged from 202 to 223  $\mu\text{m}$  in those of Egypt and in Libyans was 305-346  $\mu\text{m}$ . anal shield triangular, separated from genital shield, anal plate small bearing 3 setae, paranals situated on each side of anus, with one post anal, anus terminal and has valves. Anal plate has striations and the posterior edge was 8 rows from punctation (Table 1) (Figure 1: A, B and Figure 2: A, B, Q, R). This description agrees with (Delfinado-Baker 1974; Delfinado-Baker and Aggarwal 1987) that described the anal plate small, paranals situated on each side of anus.

### C- Peritreme

Visible on ventral view in a lateroventral position between the third and fourth legs. The movable emergence peritreme lies flat along the venter laterad of legs 3 and 4 but can be lowered 90° on the surface of body during respiration (Figure 1: S, T). The sclerotized peritremal groove consists of an ascending and a shorter descending arm forming a hook around the tip of the peritreme. The anterior of the groove and stigmal atrium are lined with micropapillae (microtrichiae). This description agrees with Bruce et al. (1997) whom reported that the remainder of the peritreme is a sac-like structure that is continuous with the body hemocoel, also agrees with Richard et al. (1990) whom observed that a curved peritreme which continues with a tubular diverticle that connects with the trachea. This mechanism enables the adult female to breathe inside the capped cell where the quantity of oxygen is reduced and liquid is sometimes present.

### D- Dorsal shield

Chaetotaxy Surface of dorsal shield covered with numerous finely barbed setae of varying lengths, ornamented with striation and polygonal network of simple line. (Figure 1: C, D, L and Figure 2: C, F).

Number of marginal setae (Lateral setae) ranged from 19-31 in Egypt and in Libya was 13-21. These numbers disagree with Delfinado-Baker

(1974) whom found the number 21-23 in *V. jacobsoni* and Delfinado-Baker and Agarwal (1987) whom found the number 18-19 in *V. underwoodi* these setae a narrow thickened margin broadened antero-laterally, dorsally short the tip dagger (Figure 1: A, C, D, E in Egypt and Figure 2: A, B, C, D, E).

### E- Legs

Four pairs of robust, squat legs, formed by seven segments: coxa, trochanter, femur, genu, tibia, metatarsus and tarsus (Figure 1: A, B, F, G in Egypt and A, B, E in Libya). with terminal membranous well developed ambulacrum with strong sclerotic, without claw (Figure 1: X, Y & Figure 2: O) the ambulacrum comprises an adherent sucker.

### With strong dorsal and ventral setae.

Ambulacrum well developed, membranous, with strong sclerotic, without claws (Figure 1: X, Y and Figure 2: O). These characters agree with Delfinado-Baker and Aggarwal (1987). Length of tarsus IV ranged from 106–192  $\mu\text{m}$  Legs 7 segmented with metatarsi except the first leg did not have metatarsi. Leg chaetotaxy formulas follows: (Figure 1: A, B, F in Egypt Figure 2: A, B, E in Libya) represent coxa, trochanter, femur, genu, tibia, metatarsus and tarsus.

Leg 1: 2, 4, 10, 11-12, 12, 0, 34

Leg 2: 2, 4, 5, 4, 8, 8, 13

Leg 3: 1-2, 4-5, 3-6, 11, 11, 4, 13

Leg 4: 1, 6, 5-7, 9, 10, 4, 12-13

These results agree with Delfinado-Baker (1974) whom found that the numbers of setae was the same number in almost cases and different numbers in few cases.

### Formula of chaetotaxy of legs of Libya

Leg 1: 2, 4, 10, 11-12, 12, 0, 34

Leg 2: 2, 4, 5, 4, 8, 8, 13

Leg 3: 1-2, 4-5, 3-6, 11, 11, 4, 13

Leg 4: 1, 6, 5-7, 9, 10, 4, 12-13

### CONCLUSION

Our morphological measurements showed that there were differences between samples of Egypt and Libya. Only two morphological characters showed significant differences among the two locations specimens' (i.e. anal plate length, anal plate length/anal plate width (ratio III) and formula of palpal chaetotaxy). These results may be due to geographical locations between Egypt and Libya. We considered these samples of Egypt and Libya to be morphotypes.

**Table 1.** Means and S.D. of Varroa body characteristics belong to Egypt and Libya.

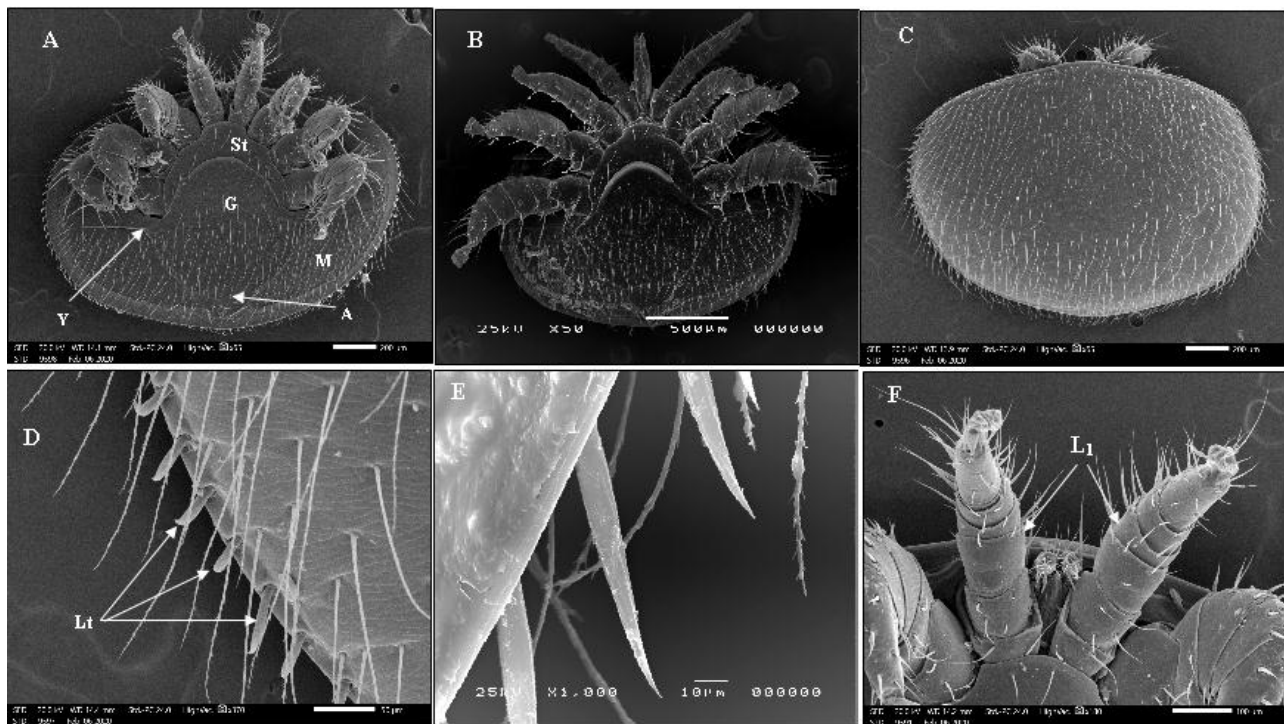
Characters	Egypt	Libya
Body length (BL)	1.07±0.01a	1.08±0.01a
Body width (BW)	1.55±0.06a	1.59±0.02a
Genital shield length (GSL)	0.50±0.01a	0.48±0.02a
Genital shield width (GSW)	0.66±0.01a	0.63±0.02a
Anal plate length (APL)	0.15±0.01a	0.13±0.01b
Anal plate width (APW)	0.23±0.00a	0.26±0.01a
Lateral plate Length (LPL)	0.65±0.01a	0.64±0.00a
Lateral plate width (LPW)	0.34±0.01a	0.33±0.01a
Ratio I (BL/BW)	0.70±0.03a	0.68±0.00a
Ratio II (GSL/GSW)	0.76±0.02a	0.76±0.04a
Ratio III (APL/APW)	0.63±0.03a	0.49±0.03b
Body size ratio (BW/BL)	1.44±0.05a	1.47±0.01a

In each row, means with the same letter are not significantly different ( $P > 0.05$ ) according to Duncan's multiple range tests.

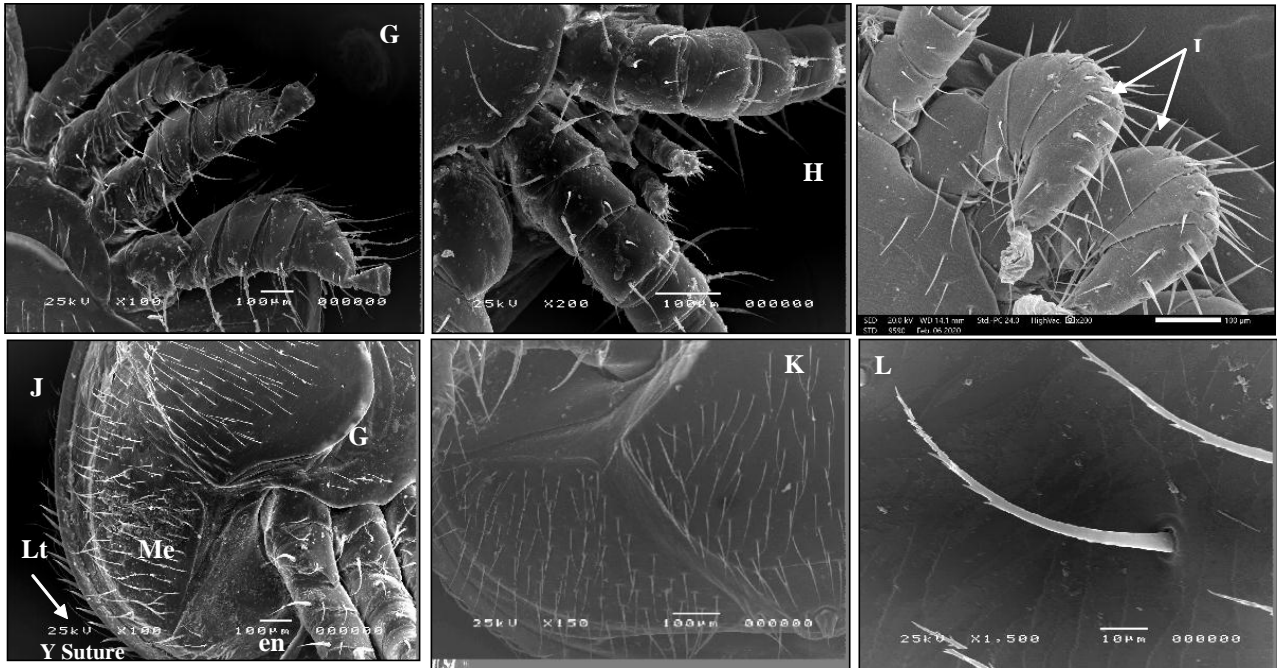
**Table 2.** Morphological characters of *V. destructor* (female) from Egypt and Libya.

Morphological characters	From Egypt	From Libya
Maximum length	4128* (1152–1728)	1468.1
Maximum width	4992 (1632–1728)	1701.67
Length of chelicera	192	190
Chelicera seta	With	With
Chelicera lyrifissures	With	With
Palpa chaetotaxy	1,2–3,2–3,7–9,10–12	1,1–2,2–3,6,10
Gnathosomal setae	3 in online	3 in online
Deutosternum	With denticles	With denticles
Endopodal plate	6–11 setae	5–12
Sternal setae + lyrifissures	4–8 pairs of setae	5 only
	4–6 pairs of pores	4–6 pairs of pores
Distance between <i>St1–St1</i>	114 (71–192)	80 (75–90)
Distance between <i>St2–St2</i>	216 (192–246)	233 (196–255)
Longitudinal distance between <i>St1–St2</i>	132 (76–223)	149 (87–236)
Genital plate Width	466.67 (656–672)	716.56 (685–736)
Count of ½ of setae on dorsal shield	153–197	158–178
Width of anal shield	214 (202–223)	313 (305–346)
Metapodal plate width	317 (288–355)	301 (291–345)
Metapodal plate length	704 (672–768)	737 (682–778)
Tarsus IV length	160 (106–192)	106 (95–134)
Number of marginal setae (lateral)	19–31 setae	13–20

\* Dimensions are in micrometers.



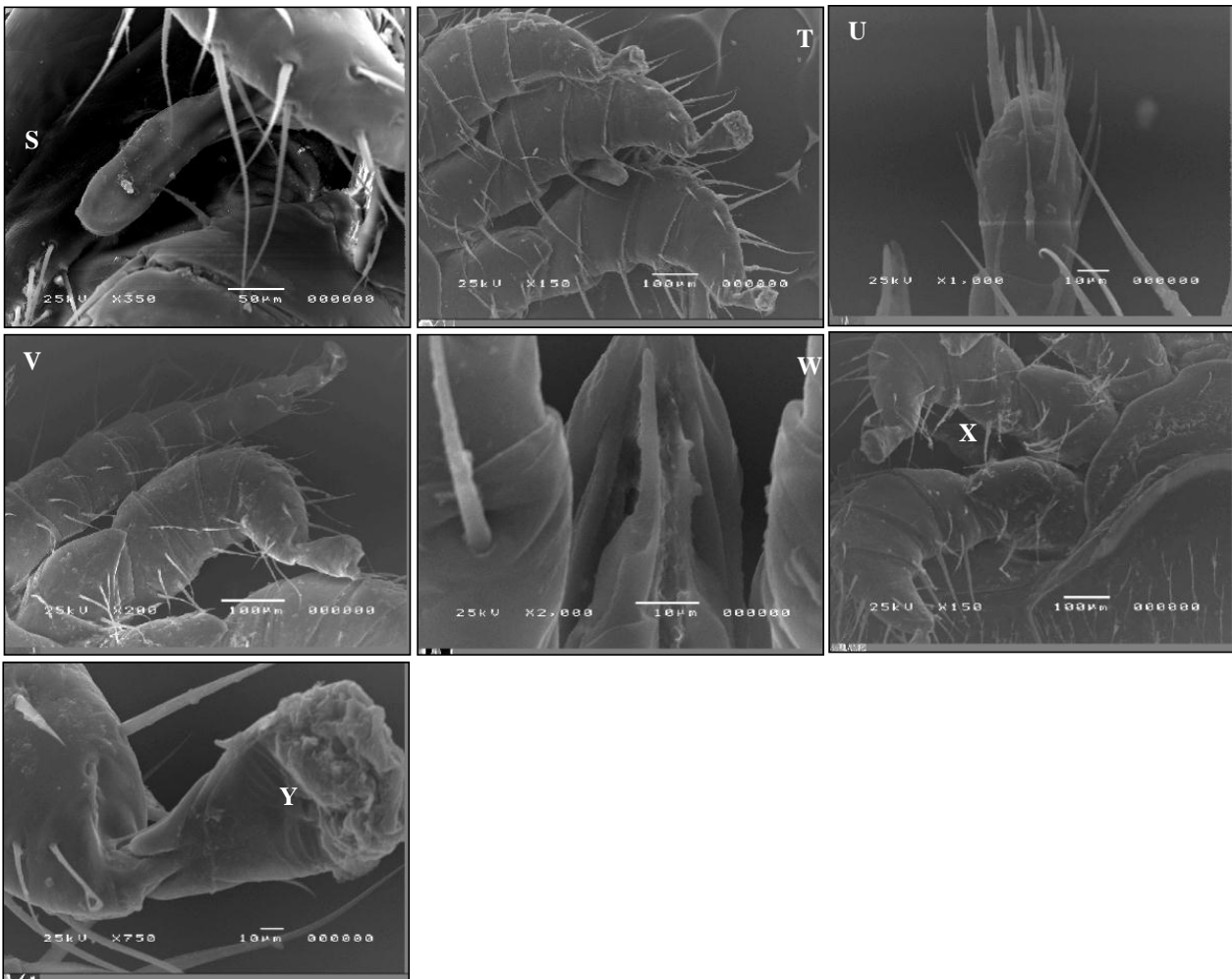
**Figure 1.** Egypt specimens, A and B: Ventral shape; St: Sternal plate; G: Genital plate; Y: suture; M: Metapodal plate; A: Anal plate; C: Dorsal shape; 3 kinds of setae; D and E: Dorsal shape and Lateral setae; F: Leg 1



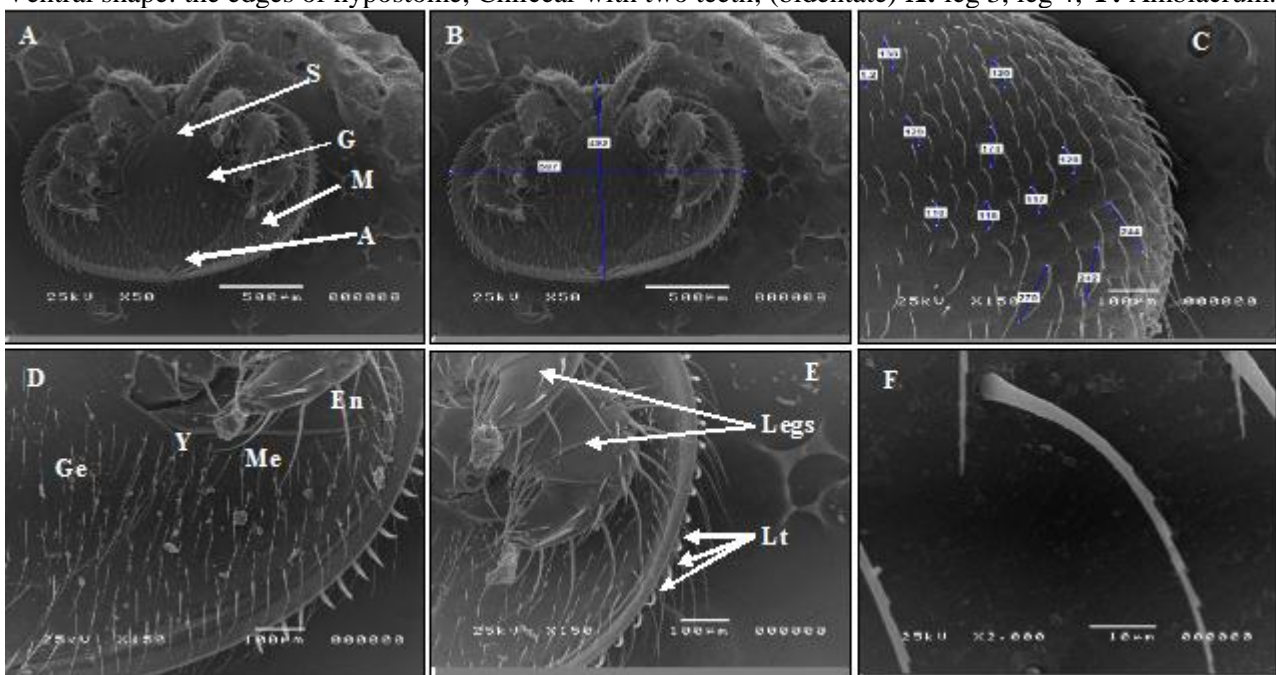
**Figure 1. Egypt specimens, G:** Legs 1,2,3,4, Amblacrum; **H:** Ventral shape: hypostome, palp, setae on palp tarsus, Leg 1; **I:** Legs 2, 3; **J and K:** Ventral shape: **en:** endopodal plate, **Me:** metapodal plat, Y suture, **Lt:** Lateral seta; **L:** Striation on dorsal shape, setae on dorsal shield



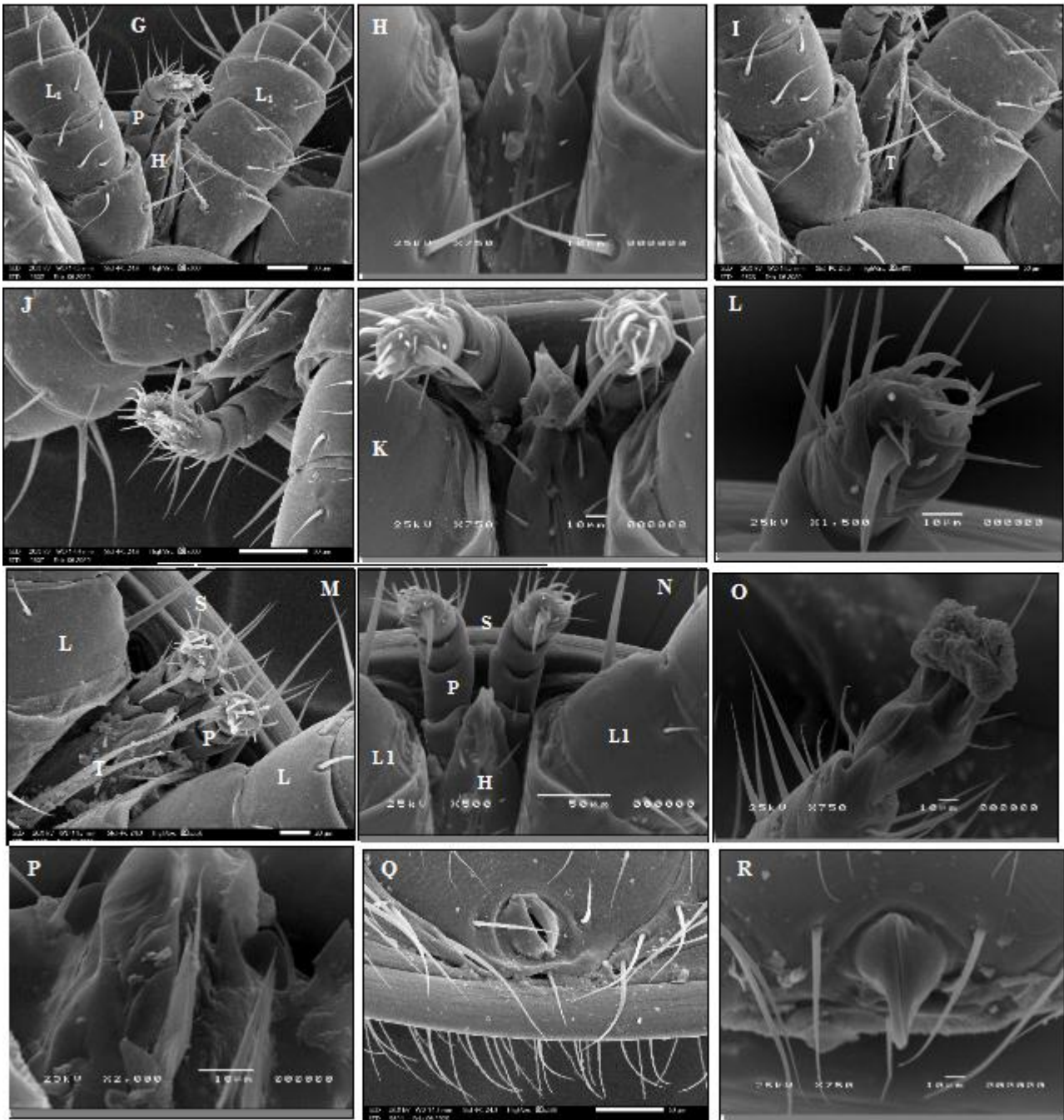
**Figure 1. Egypt specimens, M:** Ventral shape: surface of hypostome, hypostomal setae; **N:** Ventral shape: 3 setae on hypostome, apotele with bifurcate; **O:** Ventral shape: chlicera with two teeth; **P:** Ventral shape: **Q:** Ventral shape: chelicera with lyrifissure; hypostome, 3 setae on hypostome, chlicera, tritosternum; **R:** Ventral shape: the edges gnathosoma.



**Figure 1. Egypt specimens, S:** Ventral shape: pritrema between leg 3 and leg 4; **T:** Ventral shape: pritrema between leg 3 and leg4, Amblacrum; **U:** Edge of hypostome, palp; **V:** leg 1, leg 2, edge of pritrema ; **W:** Ventral shape: the edges of hypostome, Chlicear with two teeth; (bidentate) **X:** leg 3, leg 4; **Y:** Amblacrum.



**Figure 2. Libya sample, A and B:** Ventral shape – St: Sternal plate – G: Genital plate - Y suture – M: Metapodal plate - A: Anal plate; **C:** Dorsal shape - 3 kinds of setae; **D:** Ventral shape, G: Genital plate - M: Metapodal plate - En: Endopodal plate - Y suture **E:** Lateral setae, Legs 3, 4; **F:** Striation on dorsal shape, setae on dorsal shield.



**Figure 2. Libya sample, G, H, I, J, K:** Ventral shape, L1: Legs 1 with Striation, H: Hypostome with 3 setae, P: Palp with Apotele, T: Tritosternum, Lacinia and para Lacinia, Movable digit to chelicera; **L:** Palp, Ap: Aptele with bifurcate; **M:** Ventral Shape, L1: Legs 1 with Striation, P: Palp with Apotele, S: Striation on dorsal shape, T: Tritosternum; **N:** Ventral Shape, L1: Legs 1 with Striation, Palp with Apotele, H: Hypostome, **O:** Amblacrum with articulation; **P:** Ventral shape, Lacinia and para Lacinia, Movable digit to chelicera with two teeth, Lacinia and para Lacinia; **Q, R:** Ventral shape, An: Anal plate, AnV: Anal valves.



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