

## Scanning Electromicroscope as a Tool to Re-describe Isolates of *Varroa destructor* from Nine Different Egyptian Governorates

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### ABSTRACT

The new results according to Anderson & Trueman (2000) and Zaki (2009) determining the specific statuses of *Varroa* in Egypt were identical to that of the Korean genotype of *V. destructor*. The honeybee mite *Varroa destructor* specimens collected, from nine governorates representing ten regions: Arish and Rafah (North Sinai), Esmailia, Minia, Giza, Gharbia, Qalubia, New Valley (Dakhla Oasis), Alexandria and Fayoum governorates examined and measured. Observations of characters under scanning electronmicroscope were analyzed.

**Key words:** Acari, Varroidae, *Varroa jacobsoni*, *Varroa destructor*, honeybee, parasite, SEM, Egypt.

### INTRODUCTION

*Varroa* mites were firstly discovered more than 100 years ago on the Asian honey bee (*Apis cerana*) in Java, Indonesia and named *Varroa jacobsoni* (Oudemans, 1904). They were assigned to a new genus, *Varroa*, and eventually to a new family, Varroidae (Delfinado-Baker and Baker, 1974). At present, the genus contains four species.

Since the initial discovery, it has become clear that *Varroa* mites are native brood parasites of a group of cavity nesting Asian honey bees that are closely related to *A. cerana*. *Varroa* mites that have since utilized *A. mellifera* as a host are all members of *V. destructor*; the most recently described species of the genus, and are native to *A. cerana* in northeast Asia (Anderson and Trueman, 2000). It is possible that new *Varroa* species await discovery. Prolonged co-evolution of *V. destructor* and *A. mellifera* may yet see these mites also becoming genetically diverse (Oldroyd, 1999), particularly as they gradually adapt to exist on isolated populations of *A. mellifera*. However, the movement of bee stocks around the world by man and the beekeeping practice of requeening large numbers of *A. mellifera* colonies on a regular basis with queens from a common source will, to some extent, counter natural evolutionary processes that may eventually lead to *Varroa* speciation on *A. mellifera*. Various methods have been used over the years to determine variation within *Varroa*, of which the most common and simple methods which deal with measurements of mite physical characteristics (morphology). The initial discoveries of *V. jacobsoni* on *A. cerana*, *V. underwoodi* on *A. cerana* and *V. rindereri* on *A. koschevnikovi* all resulted from morphological studies. More recently, molecular methods have helped to clarify *Varroa* taxonomy and being useful for identifying genetic variation within species and even identifying cryptic species. These methods, described below, played a crucial role in the

discovery of the new species, *V. destructor*, and not being *V. jacobsoni* as previously thought (Anderson and Trueman, 2000). The taxonomy of *Varroa* on Asian honey bees can be summarized as follows by Lindquist et al., 2009: *V. jacobsoni* (Oudemans, 1904) *V. underwoodi* (Delfinado-Baker and Aggarwal, 1987) *V. rindereri* (De Guzman and Delfinado-Baker, 1996) *V. destructor* (Anderson and Trueman, 2000).

The taxonomic status of three genetically distinct *Varroa* types that infest *A. cerana* in the Philippines remains unresolved at this time (Anderson, 2000; Anderson and Trueman, 2000). Mites of just two 'haplogroups' of *V. destructor* (see section 2.4.5. 'Haplogroup and haplotype identification') have colonized *A. mellifera* globally. Of the two, those belonging to a Korea haplogroup are the most common and widespread on *A. mellifera*, being present in Europe, the Middle East, Africa, Asia, the Americas and New Zealand. At present Australia remains the only large landmass on earth on which the resident *A. mellifera* are free of *Varroa*. This mite has been known as a dangerous pest of honeybee around the world (Sammataro et al. 2000). *Varroa jacobsoni* Oud. was recorded for the first time in Egypt in 1983 (Wienands, 1988), and within the last few years it becomes a subject of concern to beekeepers where the majority of apiaries in the Egyptian governorates were destroyed (Abd Al-Fattah et al., 1991). *Varroa* causes a serious damage by feeding on hemolymph of larvae, pupae and adults, and by transmitting or activating viral diseases, (Sammataro et al., 2000; Martin, 2001). Further studies based on isozyme techniques (Issa 1989; Biasolo 1992) and random amplification of polymorphic DNA (RAPD) (Kraus & Hunt 1995; De Guzman et al., 1997) showed frequency differences among different populations of *V. jacobsoni*, suggested that the entity known as *V. jacobsoni* is more than one species. According to Anderson and Trueman (2000), the identity of *Varroa* mites

infesting *A. mellifera* in 32 countries was investigated using mtDNA Co-I gene sequences and morphological characters, all were found to be *V. destructor*, and not *V. jacobsoni* as had been assumed. However, only 2 of the six *V. destructor* genotypes found on *A. cerana* were parasiting *A. mellifera*. The most common was a Korean genotype of *V. destructor*, so-called because its primary host was *A. cerana* in Korea. It was found on *A. mellifera* in Europe, the Middle East, the Americas, Africa and Asia. Also, Awad *et al.*, 2011 found that mtDNA sequences which obtained from all 10 samples collected from *A. mellifera* in Egypt were identical to that of the Korean genotype of *V. destructor*. The purpose of this paper is to determine the specific Egyptian *Varroa* morphological characters by using Scanning electromicroscope as a tool to redescribe isolates of *Varroa destructor* from ten regions belonging to nine different Egyptian governorates.

## MATERIALS AND METHODS

Samples of adult female *Varroa* mites (1-2 mite/colony) were collected from three hybrid carniolan colonies at various locations in Egypt. These locations belong to nine governorates representing ten regions: Al-Arish and Rafah (North Sinai), New Valley (Dakhla Oasis), Qalubia, Gharbia, Esmailia, Minia, Giza, Fayoum and Alexandria governorates. Mites were collected alive from the bodies of adult 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 light microscopy and the length and width of each female were measured individually. These measurements, in micrometers ( $\mu\text{m}$ ), were taken from five females, obtained from each region and prepared in Hoyer's medium on glass microslides.

For observation of characters under scanning electron microscope, alive specimens were put through served bath of distilled water in an attempt to clean them of debris. They were, then, 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 were 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).

Also, cells of worker and drone brood were uncapped. All mite individuals, either attached to immature bee or found on the cell wall were collected with their exuvia and mounted on glass slides, using

Hoyer's medium. Slides were examined by phase-contrast microscope and identifications of the developmental stages were made using the morphological characteristics described by (Delfinado-Baker, 1974) and (Delfinado-Baker, 1984). Mounted *Varroa* stages were examined and twenty morphological characteristics were measured with a micrometer.

## RESULTS AND DISCUSSION

The characteristics of *Varroa destructor* in Egypt are summarized in table 1 and figs 2-11. Nomenclature is that of Delfinado-Baker, 1974 and Delfinado-Baker and Aggarwal, 1987.

Anderson and Trueman, 2000 reported that the identity of *Varroa* mites infesting *Apis mellifera* in 32 countries investigated using mtDNA Co-I gene sequences and morphological characters, all were found to be *V. destructor* and not *V. jacobsoni* as had been assumed. However, only 2 of the six *V. destructor* genotypes found on *A. cerana* were parasiting *A. mellifera*. The most common was the 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.

Also, when we used SEM in description of female *V. destructor* after we sure *V. destructor* by DNA sequences (Awad *et al.*, 2010 and Awad *et al.*, 2011). Twenty characters distinguished and determined in *V. destructor* in Egypt, in Table 1.

### Description of *Varroa destructor* in Egypt:

#### Gnathosoma:

The gnathosoma is situated anteroventrally and is visible through the dorsum.

#### 1- Hyposome:

Scan surface of hypostome determines long striations beside the edges of hypostome and trasverse striations near its tip consist of island transverse striations (Fig. 5c); three paris on hypostome on one line. this character differs from that of Delfinado-Baker, 1974 who found three paris of setae in one row also. Hoever, Delfinado and Aggarwal, 1987 found these setae not in line, Table 1.

#### 2- Chelicera:

Has bidentate (two teeth) on apical half one sharp-tipped and smaller than other blunt tipped tooth (Fig. 7b), (Fig. 10h, Fig. 10i) with movable digit only (Fig. 7b) and fixed digit not found dorsal seta and fissures present. These characters agree with Delfinado and Aggarwal, 1987.

#### 3- Palp of gnathosoma:

Has apotele bifurcate, one long and the other is

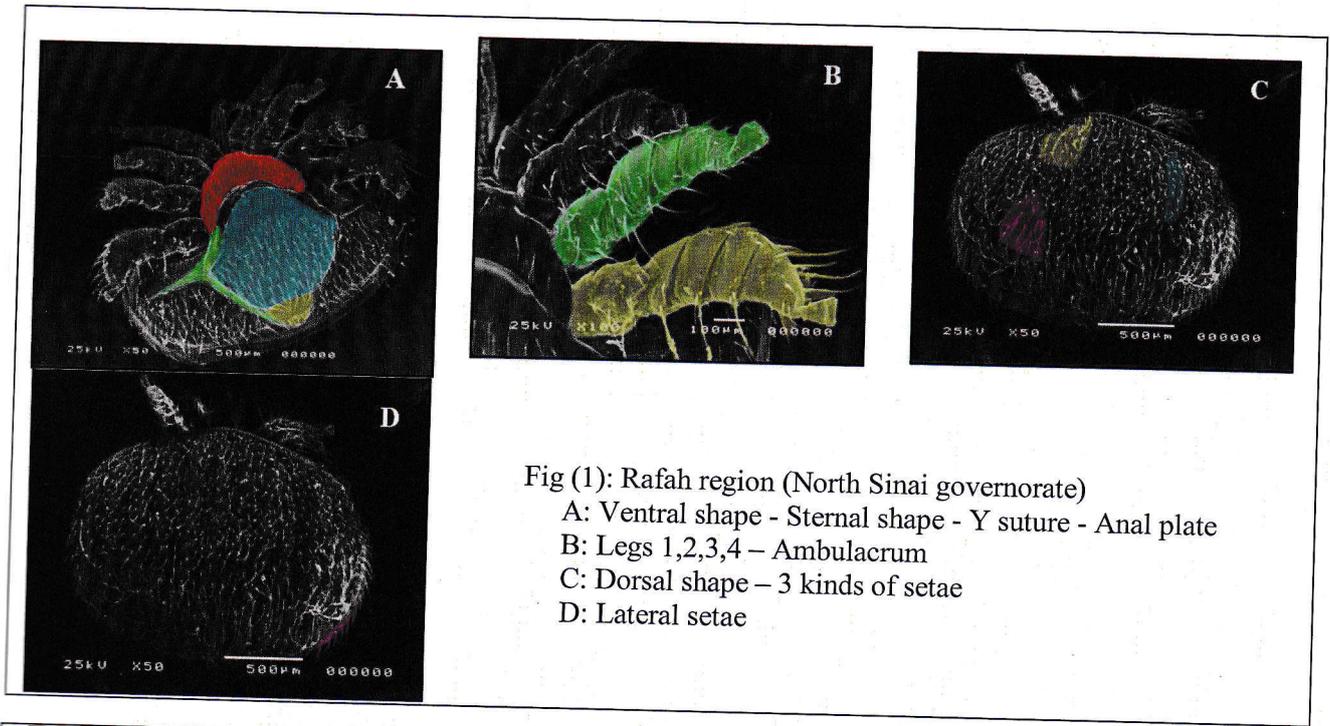


Fig (1): Rafah region (North Sinai governorate)  
 A: Ventral shape - Sternal shape - Y suture - Anal plate  
 B: Legs 1,2,3,4 – Ambulacrum  
 C: Dorsal shape – 3 kinds of setae  
 D: Lateral setae

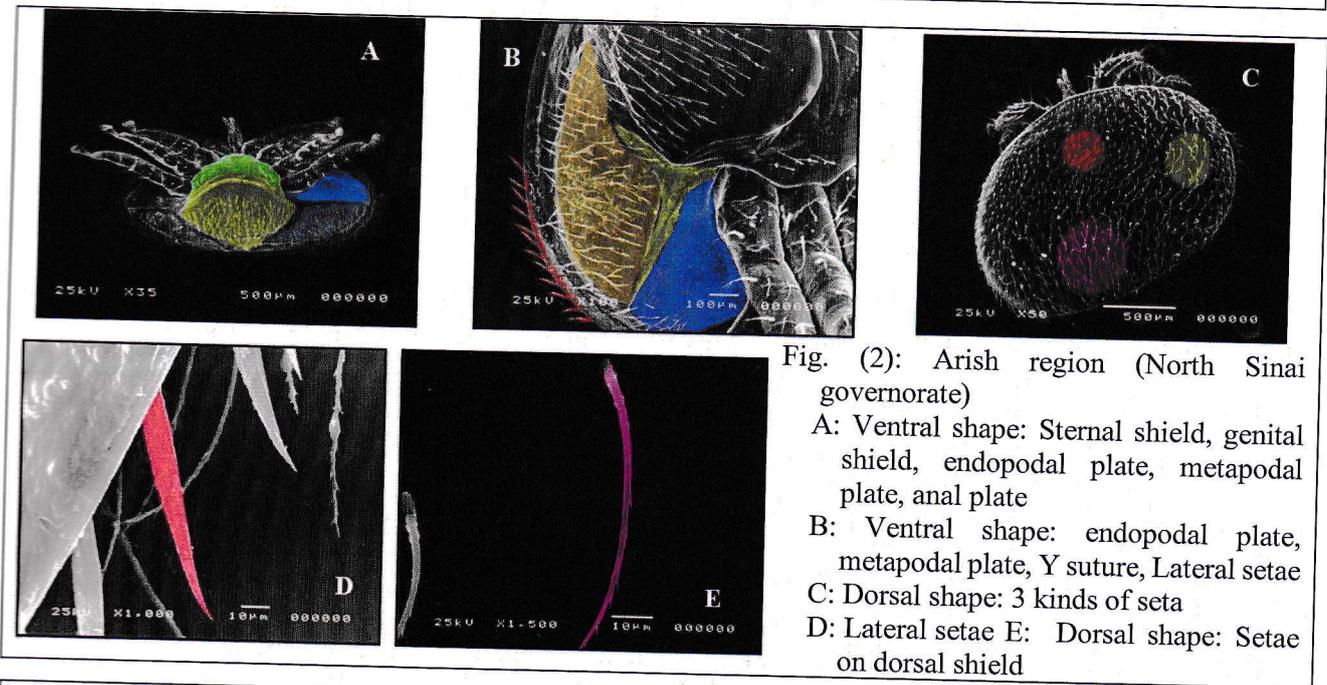


Fig. (2): Arish region (North Sinai governorate)  
 A: Ventral shape: Sternal shield, genital shield, endopodal plate, metapodal plate, anal plate  
 B: Ventral shape: endopodal plate, metapodal plate, Y suture, Lateral setae  
 C: Dorsal shape: 3 kinds of seta  
 D: Lateral setae E: Dorsal shape: Setae on dorsal shield

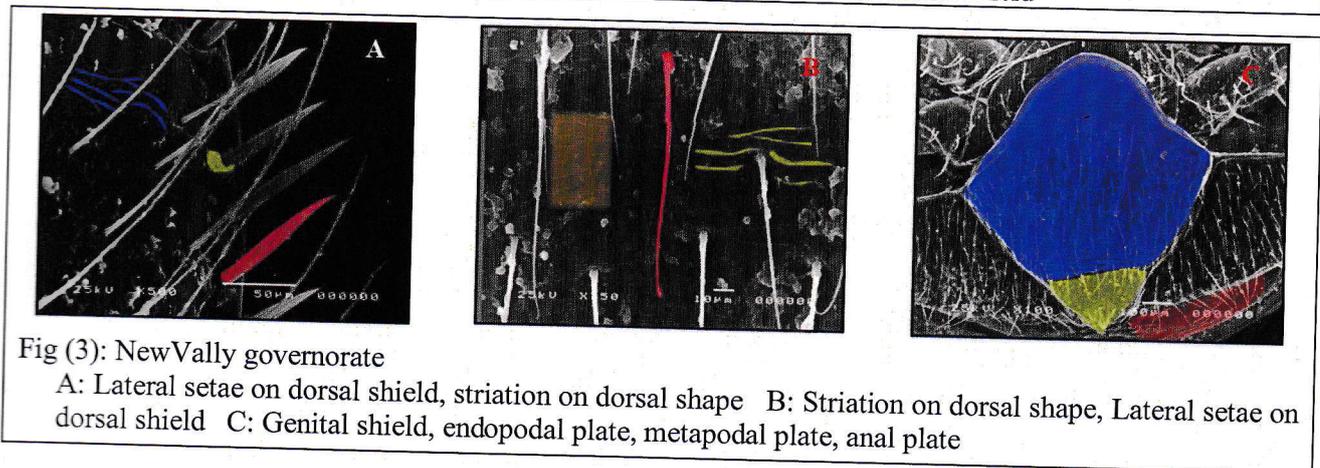


Fig (3): NewVally governorate  
 A: Lateral setae on dorsal shield, striation on dorsal shape B: Striation on dorsal shape, Lateral setae on dorsal shield C: Genital shield, endopodal plate, metapodal plate, anal plate

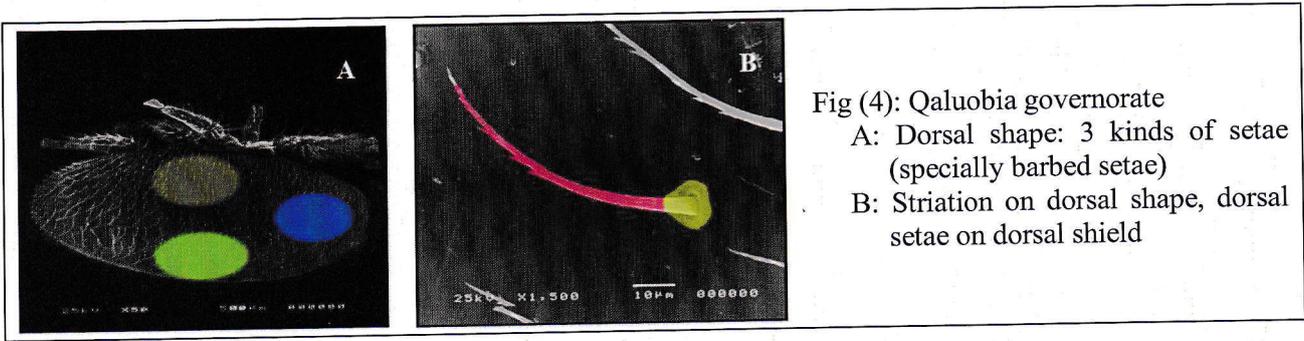


Fig (4): *Qaluobia governorate*  
 A: Dorsal shape: 3 kinds of setae (specially barbed setae)  
 B: Striation on dorsal shield, dorsal setae on dorsal shield

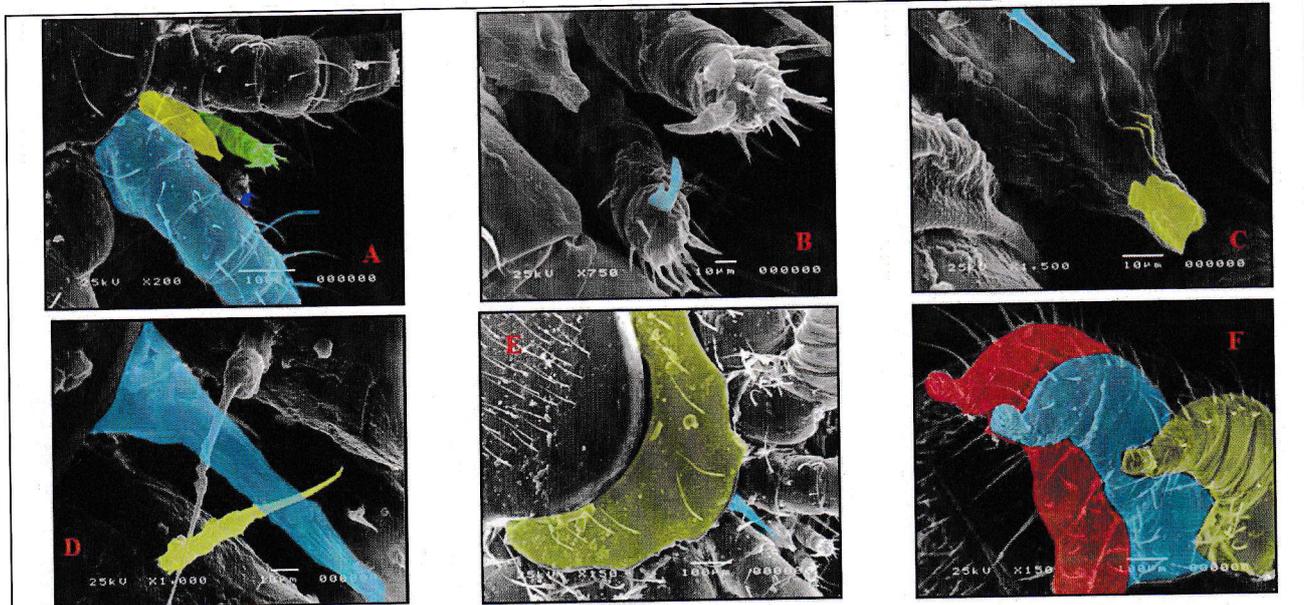


Fig (5): *Gharbia governorate*  
 A: Ventral shape: hypostome, palp, setae on palp tarsus, Leg 1  
 B: Apotele on palp  
 C: Ventral shape: surface of hypostome, hypostomal setae  
 D: Ventral shape: tritosternum shield,  
 E: Ventral shape: sternal plat  
 F: Legs 2, 3, 4

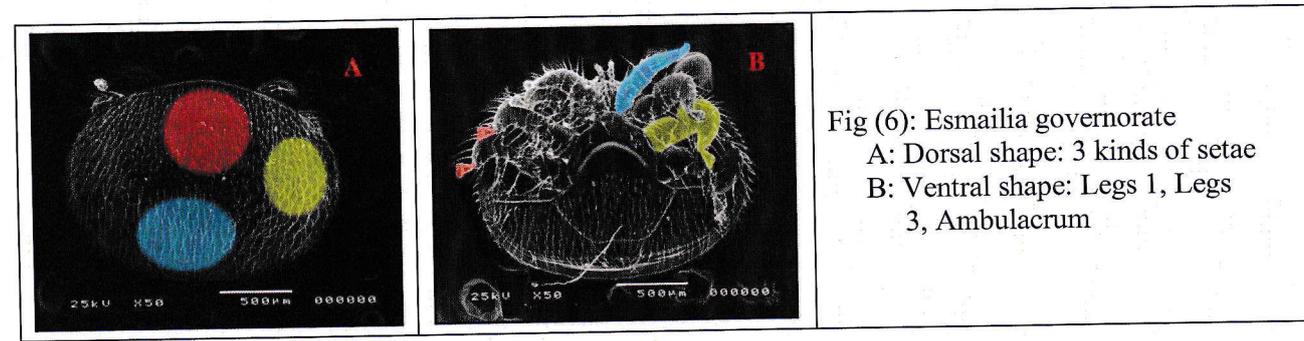


Fig (6): *Esmailia governorate*  
 A: Dorsal shape: 3 kinds of setae  
 B: Ventral shape: Legs 1, Legs 3, Ambulacrum

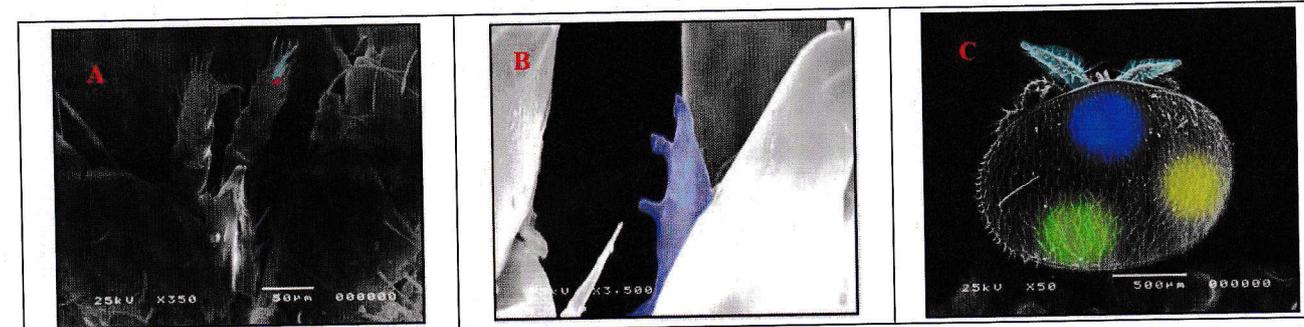


Fig (7): *Minia governorate*  
 A: Ventral shape: 3 setae on hypostome, apotele with bifurcate  
 B: Ventral shape: chlicera with two teeth  
 C: Dorsal shape: 3 kinds of setae

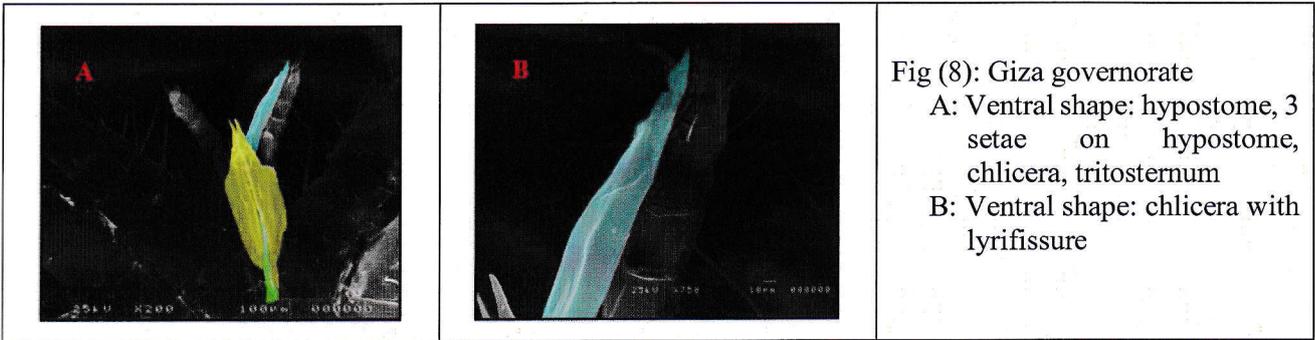


Fig (8): Giza governorate

A: Ventral shape: hypostome, 3 setae on hypostome, chlicera, tritosternum

B: Ventral shape: chlicera with lyrifissure

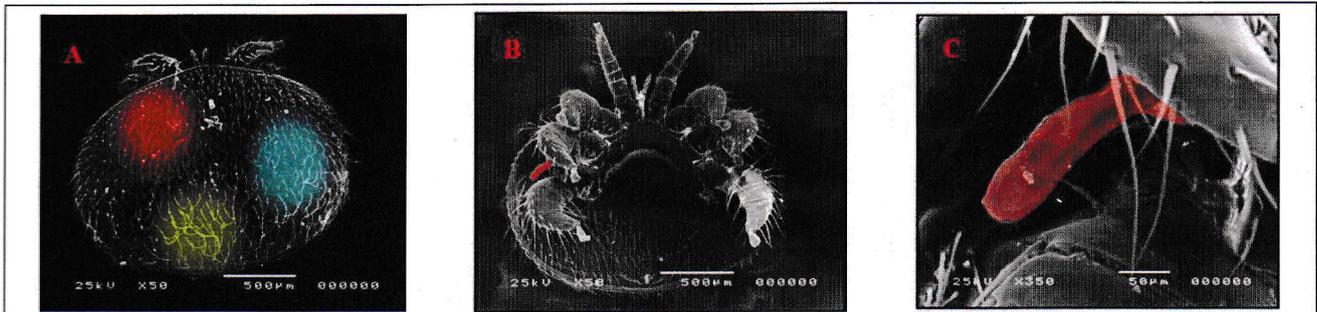


Fig (9): Fayoum governorate

A: Dorsal shape: 3 kinds of setae

B: Ventral shape: pritrema between leg 3 and leg 4

C: Ventral shape: pritrema

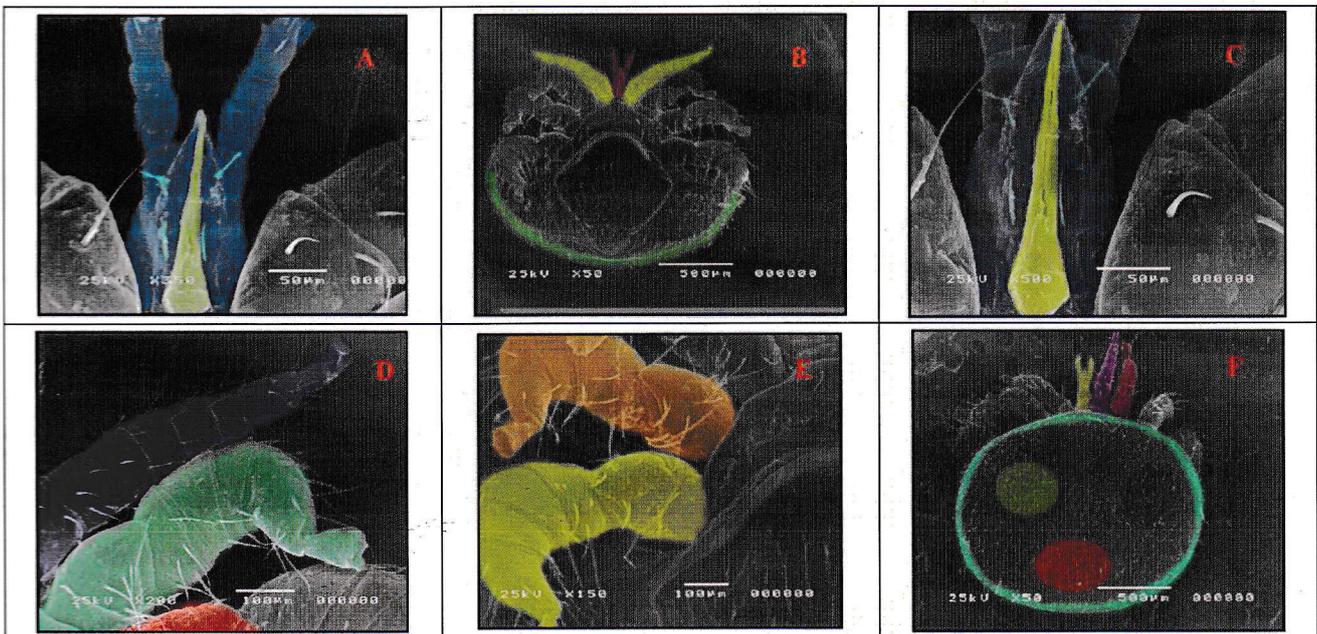


Fig. (10): Alexandria governorate

A: Ventral shape: hypostome, 3 setae on hypostome, tritosternum

B: Ventral shape: hypostome, leg 1, the edges of Varroa

C: Ventral shape: hypostome, 3 setae on hypostome, tritosternum

D: Leg 1, leg 2, edge of peritreme

E: Leg 3, leg 4

F: Dorsal shape: the edges of Varroa, leg 2, 3 kinds of setae

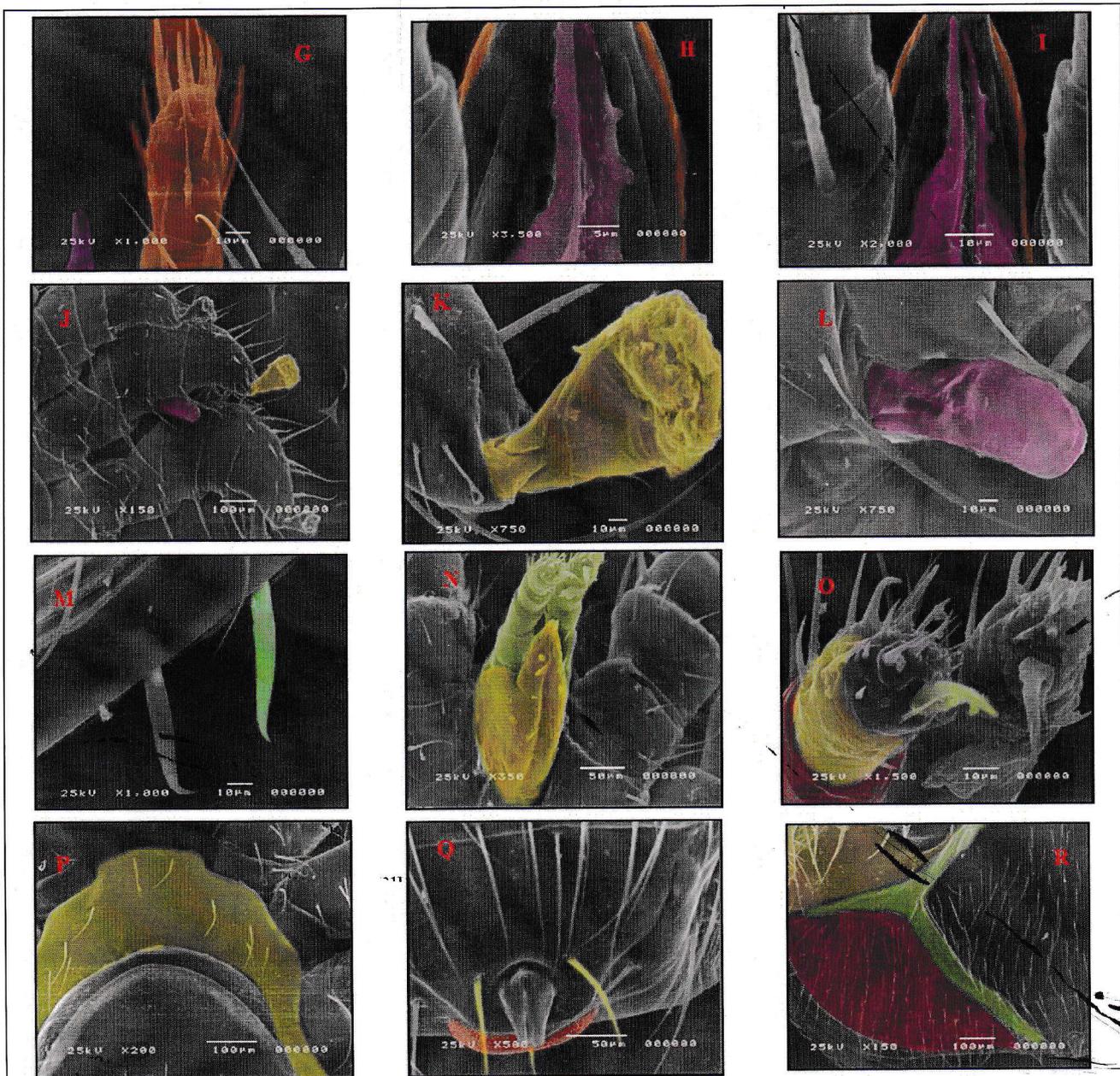


Fig (10): Alexandria-governorate

- G: Edge of hypostome, palp
- H: Ventral shape: the edges of hypostome, two teeth (bidentate)
- I: Ventral shape: the edges of hypostome, two teeth (bidentate)
- J: Ventral shape: pritrema between leg 3 and leg4 Ambulacrum
- K: Ambulacrum
- L: Edge of pritrema
- M: Lateral setae
- N: Ventral shape: the edges gnathosoma
- O: Ventral shape: apotele
- P: Ventral shape: sternal plat
- Q: Ventral shape: anal plate setae, striation on anal plat
- R: Ventral shape: endopodal plate, metapodal plate, Y seture

Table (1): Morphological characters of *V. destructor* (female) in Egypt

Morphological characters	<i>V. destructor</i> in Egypt	<i>V. jacobsoni</i>	<i>V. underwoodi</i>
Maximum length	4128* (1152-1728)	1135-1198	741-780
Maximum width	4992 (1632-1728)	568-1744	1151-1168
Length of chelicera	192	-	-
Chelicera seta	With	Without	With
Chelicera lyrifissures	With	With	With
Palpal chaetotaxy	1,2-3,2-3,7-9,10-12	1,2-3,2,7-8,12	1,3,2,10,12
Gnathosomal setae	3 in on line	Longitudinal row	Hyp.2 clearly not in line with hyp.1 and 3 (palp coxa setae)
Deutosternum	With denticles	Smooth	denticles
Endopodal	6-11 setae	5-8 setae	6-9 setae
Sternal setae + lyrifissures	4-8 pairs of setae 4-6 pairs of pores	5-6 pairs 4-5 pores	4 pairs 4 pores
Distance between <i>St1-St1</i>	114.176 (71.04-192)	-	-
Distance between <i>St2-St2</i>	216.32 (192-245.76)	-	-
Longitudinal distance between <i>St1-St2</i>	131.68 (76.32-222.72)	-	-
Width of genital plate	466.67 (656-672)	-	-
Count of 1/2 of setae on shield	40-56	-	-
Width of Anal shield	214.08 (201.6-222.72)	-	-
Width of Metapodal shield	316.8 (288-355.2)	-	-
Length of Metapodal shield	704 (672-768)	-	-
Length of Tarsus	160 (105.6-192)	-	-
Number of Marginal setae (lateral)	19-31 setae	21-23	18-19

\*Dimensions are in micrometers.

short (Fig.5b – Fig.7a – Fig.8a – Fig.10b, n, o). Numbers of setae on palpal trochanter, femur, genu, tibia, and tarsus are 1, 2-3, 2-3, 7-9, 10-12, respectively (Table 1). These formula on palpal chaetotaxy differs from *Varroa jacobsoni* (Delfinado-Baker, 1974) and palpal chaetotaxy of *Varroa underwoodi* (Delfinado-Baker and Agarwal, 1987).

#### 4- Deutosternum:

Has denticles, and this agrees with that mentioned by Delfinado-Baker and Agarwal, 1987 who reported that the deutosternum with denticles in *V. underwoodi* while differs from *V. jacobsoni*, Delfinado-Baker 1974, who reported that the deutosternum was smooth. Table 1.

#### B- Ventral shield:

All ventral shields extensively developed and sclerotized with surface lightly and finely striate-reticulate this agrees with Delfinado and Aggarwal, 1987 who determine the same description.

#### 1- Tritosternum:

The basis of tritosternum is triangle, is contacts with sternal shield of *Varroa*, the triangle extends to the hypostome. Tritosternum is bipartite (Fig. 7a), membranous, laciniae is tapering and separately serrate (Fig. 5a & d, Fig. 7a, Fig. 8a) this description of tritosternum agrees with those by Delfinado-Baker, 1974 and Delfinado-Baker and Aggarwal, 1987 who gave similar description.

#### 2- Sternal shield:

From table (1) Sternal plate is extending from anterior margin of coxae 2 to coxae 4, occupying the area between coxa 1 and coxa 4 behind tritosternum; with 4-8 pairs of setae and 4-6 pairs of pores. The numbers of setae differs from Delfinado-Baker, 1974 who reported the occurrence of 5-6 pairs sternal setae and 4-5 pores in *V. jacobsoni*. Also, Delfinado-Baker and Aggarwal, 1987 found that *V. underwoodi* has 4 pairs of setae and pores in the sternal shield (Table 1, Fig. 7a, Fig. 10p). between sternal 1 (st1) ranged from 71.04 – 190 µm, between sternal 2 (st2) ranged from 192 – 245.76 µm and longitudinal distance between (st1-st2) ranged from 76.32 – 222.72 µm.

#### 3- Genital shield:

Densely covered with simple setae. occupying the area between coxa 4 until anal plate on the posterior region from the ventral shields, behind sternal shield, between the suture Y shape which extends lateral to genital shield. (Fig.1a&b, Fig.2a&b, Fig.3c, Fig.6b, Fig.9b). The width of genital shield ranged from 656 to 672 µm. and the half number of setae ranged from 40 to 56, also genital plate is wider than long. The anterior portion of genital plate with striations and empty from setae (Fig 2b). This description agrees with that by 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 plate is well developed, half circling posterior border of coxa 4. A suture in Y shape is between endopodal plate from one side, metapodal plate on the second side and sternal and genital plate on the third side (Fig. 2b, Fig. 10r). The description of endopodal plate agrees with that by delfinado-Baker (1974) of endopodal plate in characters of the genus *Varroa*; but the suture in Y shape was not described by delfinado-Baker (1974) and delfinado-Baker and Aggarwal (1987). From Table (1) endopodal plate has 8-10 setae on the posterior margin and with a little striation.

**5- Metapodal shield:**

Metapodal shield broadly is triangular, densely covered with simple setae (Fig. 2b, Fig.3c, Fig. 10r). Width of metapodal shield ranges from 288 to 355.2  $\mu\text{m}$ , the length of metapodal shield ranges from 672 to 768  $\mu\text{m}$  (Table 1). These description agrees with that by Delfinado-Baker (1974) and delfinado-Baker and Aggarwal (1987).

**6- Anal shield:**

Width of anal shield ranges from 201.6 to 222.72  $\mu\text{m}$ ; 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. Anal plate has striations and the posterior edge has 8 rows of punctation (Table 1) (Fig. 3c, Fig. 10q). This description agrees with those of Delfinado-Baker (1974) and delfinado-Baker and Aggarwal (1987) who mentioned that anal plate is small and paranals situated on each side of anus.

**C- Peritreme:**

It is visible on ventral view in a lateroventral position between the third and fourth leg.

The movable emergent peritreme lies flat along the venter laterad of legs 3 and 4 (Fig.9b) but can be lowered 90° on the surface of body during respiration (Fig.10J, L). The sclerotized peritremal groove consists of an ascending and a shorter descending arm forming a hook around the tip of the peritreme. The interior of the groove and stigmatal atrium are lined (Fig. 9c) with micropapillae (microtrichae) (Fig.10L). This description agrees with that by Bruce *et al.*, (1997) who reported that the remainder of the peritreme is a sac-like structure that is continuous with the body hemocoel; also agrees with that by Richard *et al.*, (1990) who 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:****Dorsal chaetotaxy:**

Surface of dorsal plate covered with numerous finely barbed setae of varying lengths, ornamented with striation and polygonal network of simple line (Fig.1d, Fig. 2c&d, Fig.3b, Fig.4a&b, Fig.6a, Fig.7c, Fig.8a, Fig.10f).

**Marginal (lateral setae):**

Number of marginal setae ranges from 19 to 37; these numbers disagree with delfinado-Baker (1974) who found 21-23 setae in *Varroa jacobsoni* and delfinado-Baker and Aggarwal (1987) who found 18-19 in *V. underwoodi*. These setae are with narrow thickened margin, broadened antero-laterally, dorsally short the tip dagger (Table1, Fig. 1d, Fig. 2e, Fig. 3a, Fig. 10m).

**E- Legs:**

There are four pairs of robust, squat legs, formed by seven segments: coxa, trochanter, femur, genu, tibia, metatarsus and tarsus, with terminal membranous ambulacra without claw or apotele. The ambulacra comprises an adherent sucker.

Ambulacra are well developed, membranous, with strong sclerotic and without claws (Fig.1b, Fig.10j&k). These characters agree with those by delfinado-Baker and Aggarwal (1987). Length of tarsus ranges from 105.6 to 192. Legs 7 segmented with metatarsi except the first leg has not. Leg chaetotaxy formulas as in Table (1) and the Figures (Fig. 1a&b, Fig. 2a&b, Fig. 5a&f, Fig.6b, Fig. 7a, Fig. 8a, Fig.9b, Fig.10b-e-d-j&n) representing 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 those by Delfinado-Baker (1974) who found that the numbers of setae was the same number in almost cases but different numbers in few cases.

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