# Identification of *Varroa mite* (Acari: Varroidae) parasitizing honeybee in Egypt using DNA sequencing ,morphometric and SEM analysis

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## ABSTRACT

*Varroa mite was recorded for first time in Egypt in 1983 as V. jacobson, while another report indicated that, Varroa in Egypt is V. destructor. The purpose of this study is to provide accurate identification of Varroa mites in Egypt and to investigate which Varroa species are present on honey bees in Egypt. In this respect, the sequencing of mitochondrial cytochrome oxidase subunit gene (mt CO-I), morphometric analysis and scanning electronic microscopy were carried out. Varroa mite samples collected from ten different Egyptian localities. Based on the obtained results, all studied samples belonged to V. destructor. This is the first evidence for Varroa identification in Egypt based on mt DNA sequencing and morphometrics as well as light and scanning electronic microscopy. These results will influence quarantine protocols for bee mite, and may present new strategies for mite control in Egypt.*

***Keywords***: *genetic identification, Varroa destructor, cytochrome oxidase subunit I, mitochonderial gene, morphometric analysis.*

## INTRODUCTION

The mesostigmatid mite, *Varroa jacobsoni* Oudemans, was first described as an ectoparasite of the Eastern honey bee, *Apis cerana* Fabricius in Java in 1904 (Oudemans, 1904), although it was subsequently found to parasitise *A. cerana* throughout Asia (Koeniger *et al.*, 1983). The mite later came to the attention of bee researchers when it shifted host to the Western honey bee, *A. mellifera* Linnaeus, through bee's introduction to Asia by man (Crane, 1978 and Ruttner and Maul 1983). *Varroa jacobsoni* Oud. was recorded for the first time in Egypt at 1983 (Wienands, 1988), and within the last few years the parasite has become a subject of concern to beekeepers and has been found in the majority of the Egyptian Governorates causing economic losses to most beekeepers (Abd El-Fattah *et al.*, 1991). According to the report of Anderson and Trueman (2000) the *Varroa* in Egypt was reported as *Varroa destructor* (Korea haplotype). In order to investigate which *Varroa* species are present on honey bees in

different parts of the world, several studies were conducted based on morphlogical and molecular measurements (Zhang 2000; Anderson and Trueman 2000 and Aydin *et al.*, 2007). Various reports were introduced using morphological measurements to differentiate between *Varroa* species (Oudemans 1904; Delfinado-Baker 1984; de Guzman *et al.*, 1999; Anderson 2000). Recently, genetic analysis based on *Varroa* mitochondrial DNA have played a central role in establishing *Varroa* taxonomy and dispersal as reported by Evans and Lopez (2000) who presented the complete mitochondrial sequence of the important honey bee pest *Varroa destructor*. Mitochondrial protein-coding gene cytochrome oxidase I (COI) provided a powerful tool for phylogenetics at low taxonomic levels and studies of intraspecific variation of closely related species (Cruickshank 2002; Koeniger *et al.* 2000; Aydin *et al.*, 2007 and Haddad *et al.*, 2007). The aim of the present study was to provide accurate identification of *Varroa sp.* in Egypt using morphological and molecular genetic parameters.

## MATERIALS AND METHODS

### Collection of samples

Samples of adult female *Varroa* mites were collected from hived *A. mellifera* colonies at locations shown in Figure (1). These locations are belonging to nine governorates and represented in ten regions: Arish and Rafah (North Sinai), Esmailia, Minia, Giza, Gharbia, Qalubia, New Valley (Dakhla Oasis), Alexandria and Fayoum Governorates. In all hived colonies and in most of the hived *A. mellifera* colonies, mites were collected alive from the bodies of adult worker, drone bees, capped worker, or drone brood cells and stored immediately at -20°C for DNA extraction step and in 70% ethanol for morphological analysis.

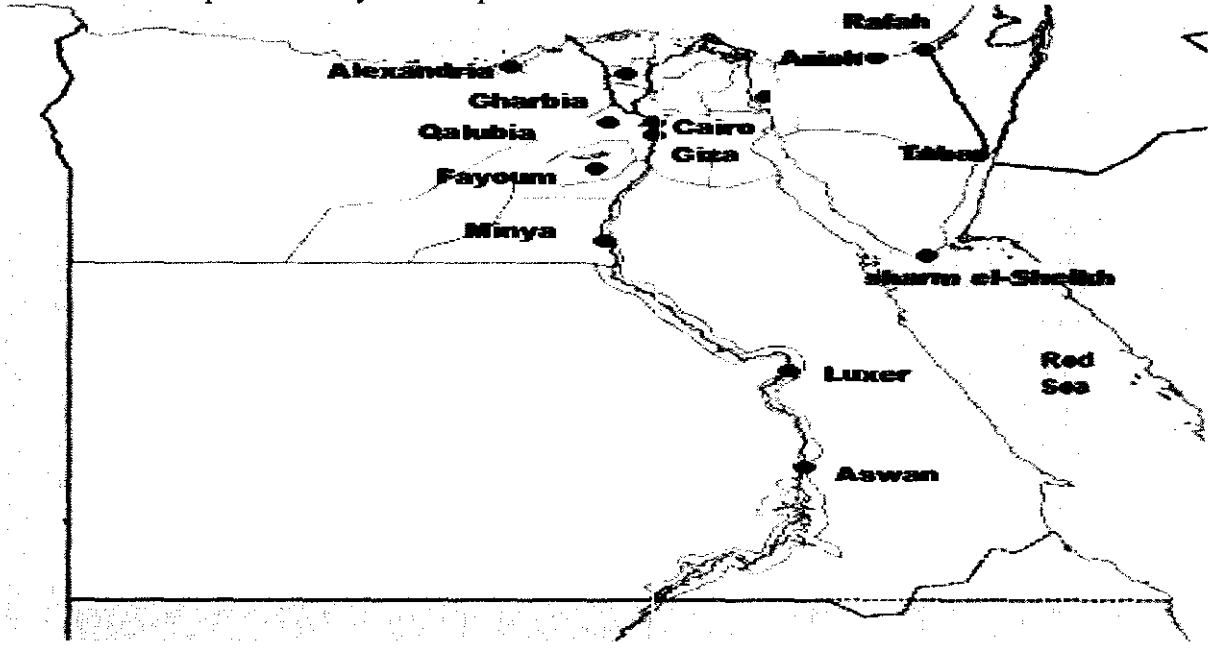


***Fig. (1):Localities from which Varroa mites were collected from A. Mellifera in Egypt.***

**Morphological analysis**

Specimens were five females collected from each of nine Governorates represented in previously mentioned ten regions. To confirm the specific identity, mite morphology was examined using both light microscopy and scanning electronic microscopy. Measurements of mite specimens were made in micrometers (μm) and were obtained from five females prepared in Hoyer's medium on glass microslides.

**Electron Microscopy Scanning**

For scanning under electron microscope, live specimens were washed 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 glutaraldhyde of 3.5% concentration for 6 hours then dehydrated in ethyl alcohol, dried using the critical point procedure, 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).

**Molecular genetic analysis**

**DNA extraction and PCR amplification of mitochonderial cytochrome oxidase I gene**

DNA was extracted according to Qiagen's DNEasy protocol for animal tissue utilizing DNA binding columns. A fragment located inside the 458 base-pair portion of the CO-I gene originally surveyed by Anderson and Trueman (2000) was amplified according to methods of Warrit (2002), using a new pair of primers (V51: 5'-GTAATTTGTATACAAAGAGGG -3' and V1400 5'-CAATATCAATAGAAGAATTAGC -3'). PCR reactions were conducted using 2x superhot PCR Master Mix (Bioron; Germany) with 10 Pmol of each V51 and V1400 primers and 50-100 ng/ μl DNA. PCR amplifications were performed in Biometra T-personal Thermal Cycler using the following PCR programme: 1 cycle at 94°C for 4 min.; 35 additional cycles consisting of (92 °C for 1 min., 54 °C for 1.5 min. and 72°C for 1.5 min.) and 72°C for 10 min. After the amplifications, the PCR reaction products were eletrophoresed on 10x14 cm 1.5% agarose gel for 30 min using Tris-borate-EDTA buffer. The gel was stained with 0.5 μg/ml of ethidium bromide.

**Sequencing of mitochonderial cytochrome oxidase I gene**

The PCR-products of each sample were purified from excess primers and nucleotides by the use of AxyPrep PCR Clean-up kit (AXYGEN Biosciences, Union City, California, USA) and directly sequenced using the same primers as described for the amplification process. The products were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ABI Applied Biosystems, Foster City, California, USA) on a 3130XL Genetic Analyzer (Applied Biosystems). The obtained sequences were analysed using Blasting program on the genbank website (www.ncbi.nlm.nih.gov.) to make relation with sequence. Each sample sequence was submitted to the genbank and has accession numbers which were listed in Table (1).

## RESULTS AND DISCUSSION

**Molecular genetic analysis**

Sequences from mtDNA and other genetic markers have great potential for clarifying the genetic structure of mites at several hierarchical levels (Navajas and Fenton, 2000 and Awad *et al.*, 2010). In the honeybee hosts of *Varroa* mite, sequences from several distinct regions of the

mitochondrial genome have been key factors in assigning differences at the species, subspecies (Sheppard and Smith, 2000), and population (Schiff and Sheppard, 1995) levels. In the present study, PCR amplification of cytochrome oxydase I gene (CO-I) followed by sequencing among ten *Varroa* mite samples were carried out. Figure (1) shows the PCR amplification of cytochrome oxydase I gene (CO-I) among ten *Varroa* mite samples. Table (1) illustrates the genebank accession number of each *Varroa* mite sample and sequence variation among ten studied isolates from different Egyptian localities has been shown. In this study, we found that international databases showed that the DNA sequence obtained from mites on *A. carnica* in Egypt was identical to the mtDNA CO-I gene sequence obtained from mites collected from the same bees. These results agree with those of Anderson and Fuchs (1998) who found that a region of the CO-I coding gene has shown sequence variation between, but not within, two different *V .jacobsoni* populations. Also, these results agree with those of Anderson and Fuchs (1998) and Anderson and Trueman (2000). They found that the DNA sequence obtained from mites on *A. cerana* in Java was identical to the mtDNA CO-I gene sequence obtained from mites collected from the same bees. These results identified that the *Varroa* mite in Egypt was only one species, i.e., *Varroa destructor*, but not *V. jacobsoni*. This result confirmed those of Anderson and Trueman (2000) who found that the Korea haplotype from *Varroa destructor* was most common, and was found infesting *A. mellifera* in Africa, Europe, the Middle East, Asia and the Americas.

**Morphological analysis**

In the respect of differentiation between *V. destructor* and *V. jacobsoni*, Anderson and Trueman (2000) showed that the two species differ mainly in mtDNA Co-I gene sequences, but it could also be differentiated by female body size: the former is larger than the latter (Table 2).The confidence interval for body length was 1121.72-1355.33 µm and for body width was 1916-2000µm. These results coincide with those of Zhang (2000) who found that 95% confidence intervals were 1132.2-1185.8 µm and 1642.3-1757.7µm body length and width, respectively. Also, Aydin *et al.*, (2007) found 1138.8±28.3 µm and 1705.1±49.4 µm. Egyptian specimens were also similar to *V. destructor* from Japan/Thailand-Vietnam in that they are both less spherical than *V. jacobsoni* (Figs. 3-5 of Anderson and Trueman 2000 and Figs. 6-7 of Egypt). *Varroa destructor* is much more widespread than *V. jacobsoni* and the Korea haplotype of *V. destructor* has the greatest geographical range among four *Varroa* species (Anderson and Trueman 2000). The mite samples collected from various regions in Egypt were identified as *V. destructor* in this study, based on morphometric measurements described by Anderson and Trueman (2000), and the measurements of these samples were similar to the data reported by them for *V. destructor* (Table 2). Specimens of *V. destructor* collected in Egypt are less spherical than *V. jacobsoni* (Anderson and Trueman, 2000 and Zhang, 2000).

***Table (1): Locations of samples and gene bank accession numbers.***

| Locations of samples | Accession number | Gene bank electronic site |
|---|---|---|
| **Arish** | HQ647221 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647221 |
| **Esamailia** | HQ647222 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647222 |
| **Minia** | HQ647223 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647223 |
| **Rafah** | HQ647224 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647224 |
| **Gharbia** | HQ647225 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647225 |
| **Giza** | HQ647226 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647226 |
| **New Valley** | HQ647227 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647227 |
| **Qalubia** | HQ647228 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647228 |
| **Alexandria** | HQ647229 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647229 |
| **Fayoum** | HQ647230 | http://www.ncbi.nlm.nih.gov/nuccore/HQ647230 |

***Table (2): Body lengths and widths (in μm) of Varroa destructor females.***

| Species | Body length | | Body width | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| V. destructor * | 1267.76 | 28.94 | 1956.15 | 29.80 |
| V. jacobsoni** | 1063.0 | 26.40 | 1506.8 | 36.00 |
| V. destructor** | 1167.3 | 26.80 | 1708.9 | 41.20 |

* Data from Egypt based on 5 females.
** Data from Anderson and Trueman (2000).

Finally, it could be concluded that, based on the obtained results from both of the molecular genetics and morphometrics, the *Varroa* mite in Egypt is only one species,i.e., *Varroa destructor*, and not *V. jacobsoni*.

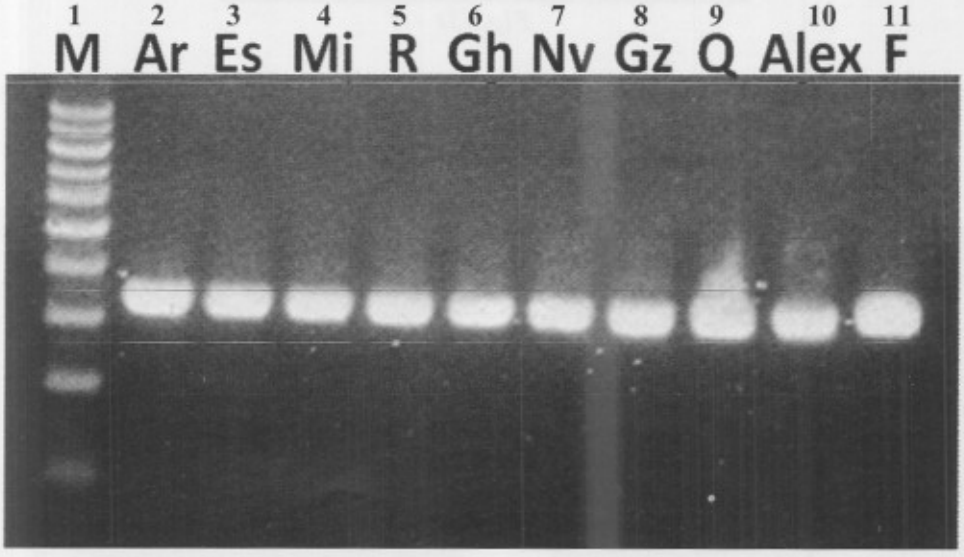


***Fig.(2): PCR amplification of cytochrome oxydase 1 gene (Co-1) among ten Varroa mite samples. Lane 1: Standard DNA Ladder 100(m); Lane2: Arish; Lane3: Esmailia; Lane4: Minia; Lane5: Rafah; Lane6: Gharbia; Lane7: New Valley; Lane8: Giza; Lane9: Qalubia; Lane10: Alexandria; Lane11: Fayoum.***

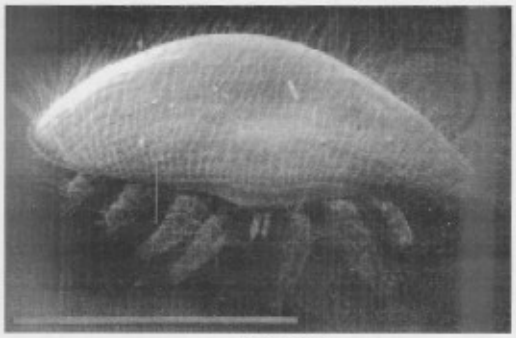

*Fig. (3)*

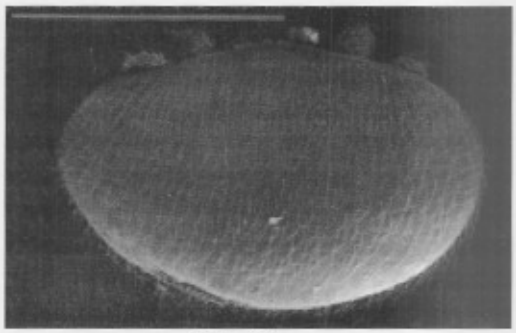

*Fig. (4)*

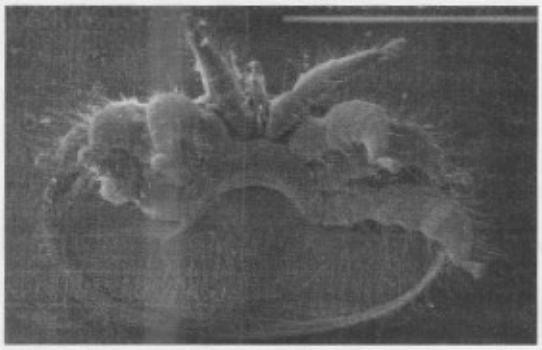

*Fig. (5)*

***Fig. (3-5): Varroa destructor Anderson and Trueman (female). 3, frontal view; 4, dorsal view; 5, ventral view. Scale bar approximately 1,000 µm.***

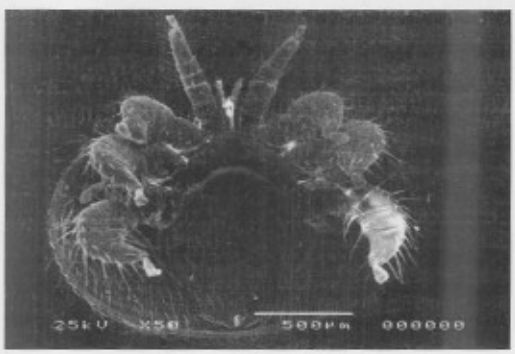


*Fig. (6)*

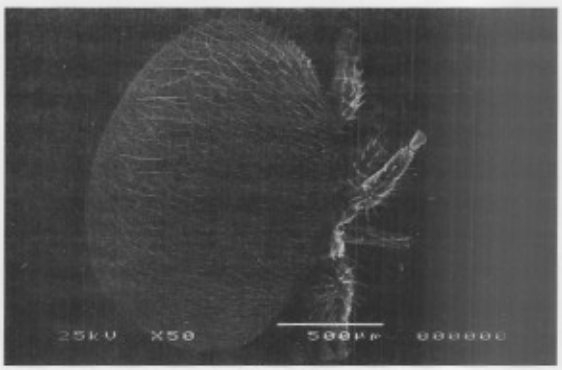


*Fig. (7)*

***Fig. (6-7):Varroa destructor of Egypt (female). 6, ventral view; 7, dorsal view.***

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## الملخص العربي

### تعريف نوع طفيل الفاروا الذي يصيب نحل العسل في مصر بإستخدام تقنيات تحليل تتابعات الدنا والقياسات المورفوميتريه والتصوير بالميكروسكوب الإلكتروني الماسح

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سجلت الفاروا لأول مرة في مصر سنة 1983 علي أنها *Varroa jacobsoni* بينما قرر Anderson & Trueman أن نوع الفاروا في مصر هي *Varroa destructor* .لذلك تهدف هذه الدراسة الى الوصول الى تعريف دقيق لنوع طفيل الفاروا الموجود علي نحل العسل الأن في مصر. إستخدمت لذلك تقنيات الوراثة الجزيئية لتحديد تتابعات جين mt CO-I والقياسات المورفومترية والتصوير بالميكروسكوب الإلكتروني الماسح على عينات الفاروا التى تم تجميعها من عدة أماكن مختلفة فى جمهورية مصر العربية. أكدت النتائج المتحصل عليها من تحليل تتابعات جين mt CO-I وصور الميكرسكوب الإلكتروني الماسح أن الفاروا في مصر هي *Varroa destructor* . وهذه النتائج يمكن أن يبني عليها بروتوكولات الحجر الزراعي بالنسبة للأكاروسات المرتبطة بنحل العسل ووضع إستراتيجية جديدة لمكافحة الأكاروس في مصر.