

MORPHOLOGICAL CHARACTERIZATION AND A MORPHOMETRY MAP FOR VARROA MITES FROM NORTHWEST OF EGYPT

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ABSTRACT. Varroa mite, *Varroa destructor*, is the most destructive factor to western honey bee colonies worldwide. In 1904, Varroa was firstly recorded on honey bees, at the beginning it was hypothesized that Varroa is one species but recently this hypothesis has been considered to be incorrect. In 1983, Varroa mite was recorded in Egypt for first time. So far, a single study was done in Egypt to confirm Varroa species to be *V. destructor* and not *Varroa jacobsoni* as it was previously thought. Still the exact haplotype of Varroa in Egypt is unknown. This study is a step towards the identification of Varroa in Egypt. Here, morphological investigations were performed on Varroa specimens belong to northwest Egypt (El-Behera governorate). Three characteristics only showed significant differences among districts, namely body width, genital shield width, and genital shield length/ genital shield width (ratio II), while the rest of characteristics did not present any significant differences. The correlations among the characteristics were very weak, except body length which correlated significantly ($P < 0.05$) with body width and

genital shield width by 0.52 and 0.42, in respect. The study presented additional confirmation that *V. destructor* is the current species infesting honey bee colonies in Egypt. Also, Varroa haplotype was identified to be the Korean one. A list of some morphological traits of Varroa mite was provided to enable further comparisons. A morphometry map for Varroa mites was also done using a geographical information system (GIS) to correlate between geographical locations and morphological characteristics. The morphometry map clearly classified studied districts, according to measured characteristics, into three classes as low, moderate and high. This study has a significant importance towards the fully understanding of Varroa populations in Egypt.

Keywords: honey bees; Varroa mite; haplotype; morphology.

INTRODUCTION

When *Varroa jacobsoni* shifted from its original host *Apis cerana* to

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infest western honey bees, *Apis mellifera*, it was initially thought that Varroa is one species. But after that Varroa has been found to be more than one species, and *Varroa destructor* is the one infesting western honey bees (Anderson and Trueman, 2000). Varroa mite, *V. destructor*, is currently considered the main problem for modern beekeeping worldwide. These mites have numerous damages to honey bees, including for example viruses transmission to infested colonies (Chen *et al.*, 2004). The death of the heavily infested colonies is the final fate of them. Thus, many materials and methods for Varroa control have been suggested and tested in the course of time as reviewed by Abou-Shaara (2014a). Varroa mites have different haplotypes, about 15 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 firstly recorded in 1983, and was considered as *V. jacobsoni* species, but after Anderson and Trueman (2000) study, Awad *et al.* (2011) have presented the first study to confirm that the species infested honey bees in Egypt is *V. destructor* and not *V. jacobsoni*. Also, Awad *et al.* (2010) have found genetic variability in Varroa belong to different localities in Egypt. However, the exact haplotype of Varroa mite has not been investigated. By 1990,

Varroa has invaded all Arabian countries (Haddad, 2011). Currently, Varroa is the major problem for beekeeping in Egypt, but most studies have been done to present potential control methods to Varroa mites (Ismail *et al.*, 2006 and Refaei, 2011). Apart from the performed studies by Awad *et al.* (2010 and 2011), there is a need to present additional confirmation about Varroa species in different parts of Egypt, and to identify the exact haplotype. Hence, morphological characterization of Varroa mites is necessary. Thus, this study is a step towards the fully understanding of Varroa infesting honey bees in Egypt. Northwest parts, namely El-Behera governorate, is covered in this study due to its relative importance to apiculture, till 1998 there was about 128000 beehives (El-Saadany, 1998) in this region and such number is in continuous increase, especially since the cultivated area has widened rapidly (Abou-Shaara, 2013a). Studying the morphological characteristics of Varroa mites from northwest part of Egypt is the objective of this study, to characterize Varroa mites and to investigate potential haplotype of Varroa.

MATERIALS AND METHODS

Sampling

Samples were collected from five apiaries existed at different districts within El-Behera governorate (30°36'36" N and 30°25'48" E). Districts were Damanhour, El-Mahmoudia, Kafer El-Dawar, El-Dalangat, and Hosh Esa. During autumn 2014, four colonies per

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each apiary were inspected and four Varroa mites per colony were used in the study, a total of 80 mites were used in the morphological characterization (16 mites per apiary). The collection of Varroa samples from honey bee colonies was done using alcohol method, sample of about 300 bees per colony was placed in jars contain 70% ethyl alcohol, and then Varroa mites were separated. Varroa samples were stored separately, samples belong to the same colony were placed in Eppendorf tube contains 75% ethyl alcohol and were stored in freezer (about -20°C) until the analysis.

Morphological characterization

Samples preparation

Varroa samples were firstly cleared in Nesbitt's solution according to the

described method by Dietemann *et al.* (2013). Subsequently, samples were mounted on glass slides using Swan's medium.

Measurements

Some characteristics were measured using micrometric slide (*Fig. 1*). Each of body length (BL) and width (BW), genital shield length (GSL) and width (GSW), anal plate length (APL) and width (APW), and lateral plate width (LPW) were measured. Some ratios were then calculated as body length/body width (ratio I), genital shield length/ genital shield width (ratio II), and anal plate length/ anal plate width (ratio III), and body size ratio (BW/BL).

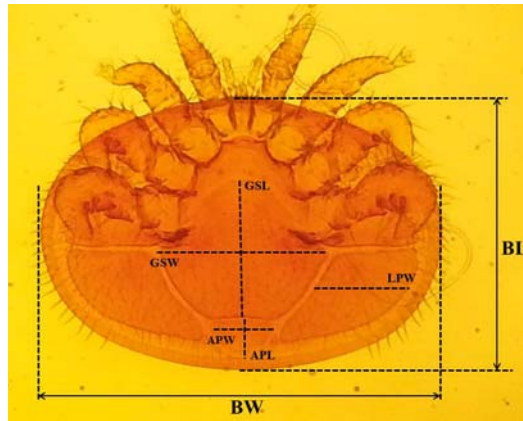


Figure 1 - Measured characteristics of Varroa mite. (BL: Body length, BW: Body width, GSL: Genital shield length, GSW: Genital shield width, APL: Anal plate length, APW: Anal plate width, LPW: Lateral plate width)

Statistical analysis

Means and standard deviations of measured characteristics and ratios were firstly calculated, and then means were compared using Duncan's multiple range test ($\alpha = 0.05$). To check the consistency degree of studied colonies, K-

means clustering was performed using colony means and based on F-statistics for characteristics showed significant differences among districts. Variations between obtained clusters were checked using discriminant analysis by Wilks's Lambda test. The possible overlapping

between resulted clusters was identified using confidence ellipse $P=0.95$ on a plot figure contains cases (colonies) and their Euclidean distance. Correlations between studied characteristics were calculated using Pearson correlation coefficient (r) at $\alpha=0.05$. The statistical analysis was performed using SYSTAT 13 and SAS 9.1.3 program (SAS, 2004).

Morphometry map

Morphometry map for Varroa mites from studied districts was done according

to the standard method described by Abou-Shaara (2013b). Morphometry map is a simple way to correlate between geographical locations and morphological characteristics. The ArcGIS 10 was used to perform the morphometry map. Raster layers of measured characteristics were firstly done, and then each layer was classified into three equal levels as (low, moderate and high). The classified layers of all characteristics were then combined at the same weight to obtain the morphometry map (Fig. 2).

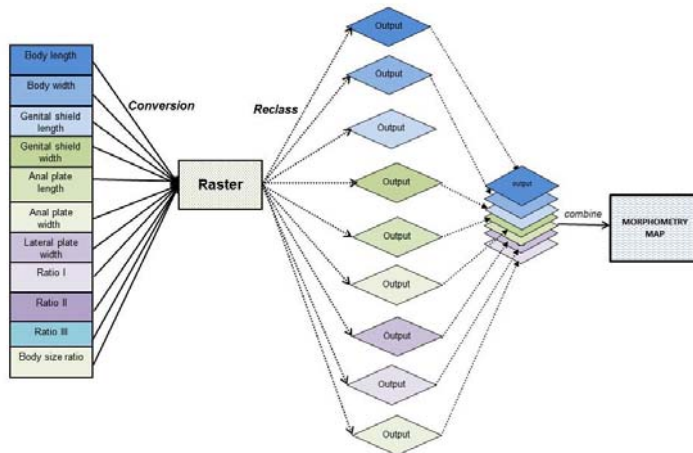


Figure 2 - Illustration of steps used to obtain the morphometry map

RESULTS

Morphological characterization

Measured characteristics varied among districts by 0.01, 0.04, 0.02, 0.03, 0.02, 0.01 and 0.01mm for BL, BW, GSL, GSW, APL, APW, and LPW, respectively, and by 0.01, 0.04, 0.03, and 0.02 for ratio I, ratio II, ratio III, and body size ratio, respectively. Three characteristics only showed significant differences among districts, namely BW, GSW

and ratio II, while the rest of characteristics did not present any significant differences (Table 1). Based on BL and BW, Varroa mites belong to Damanhour and El-Dalangat are larger than those from the other districts. The correlations among most of the characteristics were very weak, except BL, which correlated significantly ($P<0.05$) with BW and GSW by 0.52 and 0.42, in respect, as well as GSW correlated significantly ($P<0.05$) with BW by

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0.42. Varroa of El-Behera can be characterized using overall means of measured characteristics, to be with BL, BW, GSL, GSW, APL, APW, and LPW of 1.16 ± 0.03 , 1.71 ± 0.04 , 0.55 ± 0.02 , 0.71 ± 0.03 , 0.13 ± 0.02 , 0.27 ± 0.01 , and 0.36 ± 0.02 mm, respectively, and with ratio I, ratio II, ratio III, and body size ratio of 0.67 ± 0.01 , 0.77 ± 0.04 , 0.74 ± 0.08 , and 1.47 ± 0.03 , respectively.

Table 1 - Means and S.D. of Varroa body characteristics belong to five districts (Kafr El-Dawar, Damanhour, Mahmoudia, Hosh Esa and El-Dalangat). In each row, means with the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

Characteristics	District (Means $\pm$ S.D.)				
	Kafr El-Dawar	Damanhour	Mahmoudia	Hosh Esa	El-Dalangat
Body length (BL)	$1.16\pm0.03a$	$1.16\pm0.02a$	$1.16\pm0.03a$	$1.15\pm0.03a$	$1.15\pm0.02a$
Body width (BW)	$1.70\pm0.03ab$	$1.72\pm0.04ab$	$1.71\pm0.05ab$	$1.68\pm0.05b$	$1.72\pm0.03a$
Genital shield length (GSL)	$0.56\pm0.00a$	$0.56\pm0.02a$	$0.55\pm0.04a$	$0.55\pm0.02a$	$0.54\pm0.02a$
Genital shield width (GSW)	$0.71\pm0.03ab$	$0.69\pm0.01b$	$0.72\pm0.03a$	$0.71\pm0.03ab$	$0.69\pm0.02b$
Anal plate length (APL)	$0.12\pm0.02a$	$0.14\pm0.02a$	$0.13\pm0.01a$	$0.13\pm0.01a$	$0.14\pm0.02a$
Anal plate width (APW)	$0.27\pm0.01a$	$0.26\pm0.01a$	$0.27\pm0.01a$	$0.27\pm0.01a$	$0.27\pm0.01a$
Lateral plate width (LPW)	$0.36\pm0.04a$	$0.37\pm0.02a$	$0.36\pm0.02a$	$0.36\pm0.02a$	$0.37\pm0.02a$
Ratio I (BL/BW)	$0.68\pm0.02a$	$0.67\pm0.01a$	$0.67\pm0.02a$	$0.68\pm0.01a$	$0.67\pm0.01a$
Ratio II (GSL/GSW)	$0.78\pm0.03ab$	$0.80\pm0.03a$	$0.76\pm0.04b$	$0.76\pm0.03b$	$0.77\pm0.04ab$
Ratio III (APL/APW)	$0.76\pm0.14a$	$0.71\pm0.05a$	$0.75\pm0.05a$	$0.73\pm0.05a$	$0.73\pm0.05a$
Body size ratio (BW/BL)	$1.47\pm0.05a$	$1.48\pm0.03a$	$1.47\pm0.03a$	$1.47\pm0.03a$	$1.49\pm0.02a$

It was possible to divide investigated colonies into two groups (clusters) with high degree of overlapping (Fig. 3) using three characteristics; namely, BW, GSW, and ratio II (these characteristics showed significant differences among districts) in K-means clustering. Group one contains 12 colonies 50% of them are belong to El-Mahmoudia

and Hosh Esa at equal percent of 25%, and the other 50% of them are belong to Kafer El-Dawar, Damanhour and El-Dalangat at equal percent of 16.67%. Group two contains 8 colonies distributed as 25,25,12.5,12.5 and 25% to Kafer El-Dawar, Damanhour, El-Mahmoudia, Hosh Esa, and El-Dalangat, respectively.

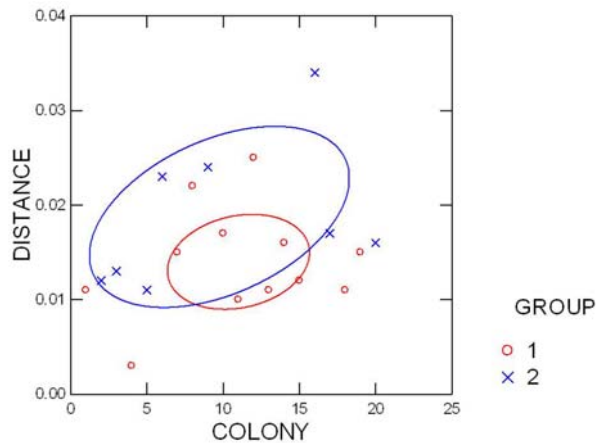


Figure 3 - Two groups (clusters) resulted from K-means clustering using means of three characteristics; BW, GSW, and ratio II. Euclidean distance resulted from K-means clustering were plotted against colony means, and 95% centroid confidence ellipses were used with resulted groups to show overlapping between them.

Morphometry map

The morphometry map, presented in *Fig. 4*, clearly classified studied districts into three classes as low, moderate and high. According to this map, Damanhour and El-Dalangat

had Varroa with highest morphological characteristics. Kafer El-Dawar and Hosh Esa showed moderate measurements, while the lowest ones were found to El-Mahmoudia district.

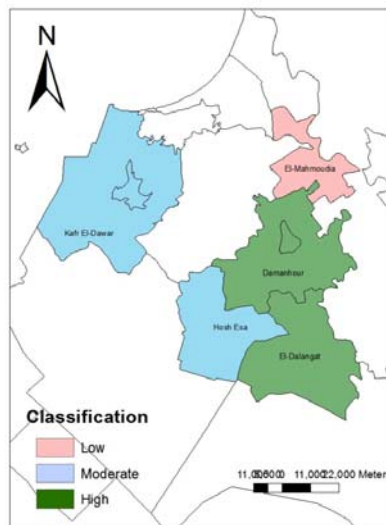


Figure 4 - Morphometry map for Varroa mites from different districts.

DISCUSSION

The obtained body size ratio BW/BL is higher than 1.4 mm, confirming that the existing species of Varroa in the examined samples is *V. destructor* (body size ratio equal or higher than 1.4 indicates *V. destructor*, in Dietemann *et al.* (2013). This study provides another evidence that the existence species of Varroa in Egypt is *V. destructor* beside the previous study done by Awad *et al.* (2011). To what extent, studied Varroa of northwest, Egypt has high degree of similarity with Varroa belong to other African countries, namely Nigeria (Rahmani *et al.*, 2006) and Tunisia (Boudagga *et al.*, 2003). Means of investigated Varroa specimens of body length and width in the present study were 1.16 and 1.71 mm, respectively. These means are somewhat in line with those measured by Akinwande *et al.* (2013) to Varroa mite in Nigeria, about 1.17 and 1.71 mm, respectively, and to Varroa mite in Tunisia, length and width were 1.16 and 1.75 mm for centre parts, and 1.20 and 1.73mm, respectively in nord parts (Boudagga *et al.*, 2003). Also, examined samples are not far from those existed in Newzealand, as measured means of body length and width were 1.15 and 1.70 mm, respectively (Zhang, 2000). But the investigated specimens are different than those found in Europe, namely Ukraine (Akimov *et al.*, 2004), they found body length and width to be 1.14 and 1.69 mm, respectively. Thus, Varroa mites of the present study

were slightly larger than those of Ukraine. Such variations could be due to host differences (i.e., different bee subspecies), as well as geographical variations (Delfinado-Baker and Houck, 1989). Also, altitude could impact Varroa morphology, mean body lengths were 1.19, 1.19 and 1.20 and mean body widths were 1.77, 1.78 and 1.78, for less than 1000 m altitude, 1000-1500 m altitude and greater than 1500m altitude, respectively (Rahmani *et al.*, 2006).

Few differences, in general, were found between Varroa belong to different districts. Migratory beekeeping practices could have a role in forming similarity between studied Varroa populations, as mixing Varroa populations could happen due to foraging activity of honey bees (Abou-Shaara, 2014b). That could indirectly explain the high degree of overlapping and the absence of significant differences between obtained Varroa groups (clusters). The relatively large Varroa mites of Damanhour and El-Dalangat, based on BL and BW, are expected to be more tolerant to acaricides due to its large body dimensions. Because positive relationship was found between body dimensions and acaricides resistance by Maggi *et al.* (2012). Such expectation needs further confirmation and detailed examinations. Also, the morphometry map confirmed that Varroa of Damanhour and El-Dalangat are larger than Varroa belong to other districts. The morphometry map can be considered as an effective way to

correlate between morphological measurements and geographical locations.

Varroa with body characteristics somewhat similar to those measured in the present study was considered to be Korean haplotype (Akimov *et al.*, 2004), similarly Varroa haplotype of investigated specimens could be considered as Korean haplotype. Especially since Korean haplotype is the common one worldwide, according to the findings of many authors (Muñoz *et al.*, 2008; Garrido *et al.*, 2003; Zhou *et al.*, 2004 and Akinwande *et al.*, 2012), including Middle East (Zhang, 2000).

CONCLUSION

The present study confirmed that *Varroa destructor* is the species infesting honey bee colonies in northwest, Egypt, and especially the Korean haplotype based on the morphological investigations. A list of some morphological characteristics of Varroa mite was provided to enable further comparisons. Creating morphometry map for Varroa mites belong to all Egyptian governorates is essential to correlate between geographical locations and morphological characteristics. Further investigations to correlated Varroa morphology with acaricide resistance are advisable to be performed, to highlight the potential role of Varroa morphology in this regard.

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