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Morphometric identification of the predominant species of *Varroa* (Parasitiformes: Varroidae) in bee colonies in Hopelchén, Campeche

Identificación morfométrica de la especie predominante de *Varroa* (Parasitiformes: Varroidae) en colonias de abejas en Hopelchén, Campeche



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ABSTRACT

Worldwide, varroasis continues to be the main sanitary problem in beekeeping production systems, causing great economic losses; currently in the Yucatan Peninsula the presence of *Varroa* has been reported, however, it is unknown which of the 4 species parasitize *Apis mellifera* bees in Campeche State. The aim of this research was to identify morphometrically the predominant species of *Varroa* (Parasitiformes: Varroidae) in bee colonies in Hopelchén, Campeche; for this purpose, 61 hives were evaluated from 5 apiaries, 200 to 300 bees were collected from each one; for the morphometric analysis, 244 mites were placed in 50% lactic acid for 2 hours at 100°C, and then the segments were measured in an ocular micrometer. The results indicated that 100% of the mites evaluated belonged to the *Varroa destructor* species, the mean K clusters indicated intraspecific differences ($P < 0.05$), observing 5 morphotypes of *V. destructor*, the variables that presented greater variability were width of the anal shield ($P = 0.001$) and width of the genital shield ($P = 0.001$). It is concluded that although 100% of the mites belonged to *V. destructor*, they showed intraspecific morphometric differences.

Keywords: haplotype, varroasis, infestation, Apis.

RESUMEN

A nivel mundial, la varroasis continúa siendo el principal problema sanitario en los sistemas de producción apícola, causando grandes pérdidas económicas; actualmente en la península de Yucatán se ha reportado la presencia de *Varroa*, sin embargo, se desconoce cuál de las 4 especies parasitan a las abejas *Apis mellifera* en el Estado de Campeche. El objetivo de esta investigación fue identificar morfométricamente la especie predominante de *Varroa* (Parasitiformes: Varroidae) en colonias de abejas en Hopelchén, Campeche; para ello de 5 apiarios se evaluaron 61 colmenas, de cada una se colectaron de 200 a 300



abejas; para el análisis morfométrico, 244 ácaros fueron colocados en ácido láctico al 50% durante 2 horas a 100°C, y posteriormente se midieron los segmentos en un micrómetro ocular. Los resultados indicaron que el 100% de los ácaros evaluados pertenecen a la especie de *Varroa destructor*, los conglomerados K medias indicaron diferencias intraespecíficas ($P < 0.05$) observándose 5 morfotipos de *V. destructor*, las variables que presentaron mayor variabilidad fueron ancho del escudo anal ($P=0.001$) y ancho del escudo genital ($P=0.001$). Se concluye que a pesar de que el 100% de los ácaros pertenecieron a *V. destructor* estos presentaron diferencias morfométricas intraespecíficas.

Palabras clave: haplotipo, varroasis, infestación, Apis.

INTRODUCTION

Varroasis is a disease caused by the *Varroa* mite, an obligate ectoparasite of honey bees ([Rosenkranz et al., 2010](#)). To date, four species of the genus *Varroa* are known, including *Varroa jacobsoni* and *Varroa underwoodi*, mites that parasitize *Apis cerana* bees and they are distributed throughout Asia, *Varroa rindereri* described in *Apis koschevnikovi* bees and distributed in Borneo, and *Varroa destructor* described in both *Apis cerana* and *Apis mellifera* bees. The presence of *V. destructor* was first recorded in the Americas in 1987 and recently through morphometric studies it has been identified in countries such as Mexico and Argentina ([De Guzman & Delfinado, 1996](#); [De Guzman & Rinderer, 1999](#); [Anderson & Trueman, 2000](#); [Anderson, 2000a](#); [Maggi et al., 2009](#); [Loeza-Concha et al., 2018](#)).

Despite the existence of four species of the genus *Varroa* only *V. destructor* is considered of economic importance, since untreated colonies can collapse due to the presence of this mite after 3 to 4 years of the initial infestation ([BüChler, 1994](#)). Mites are harmful because they generally lodge in the thorax and abdomen of drones and worker bees, besides feeding mainly on fat bodies of adult bees and the hemolymph of larvae ([Ramsey et al., 2019](#)), which causes serious damage to the health of bees affecting the immune system, reduces the growth and development of colonies ([Moreira et al., 2017](#)). In this sense, it has been observed that worker bees have a reduced lifespan, have a lower learning ability and lower rate of return to the colony ([Amdam et al., 2004](#); [Kralj et al., 2007](#)). In addition, *V. destructor* is considered a vector of several honeybee viruses ([Chen & Siede, 2007](#)) because viruses have been considered a problem for honeybee health since their emergence ([Yue & Genersch, 2005](#)).

Recent studies have shown that bee size correlates with mite size, whereby *V. jacobsoni* affects *Apis cerana*, being smaller than *V. destructor* and *A. mellifera* ([Anderson & Trueman, 2000](#)), to test this hypothesis morphometric discrimination techniques have been used by measuring body segments, which mainly use concepts of size and shape in order to know the morphological adaptations ([Delfinado & Houck, 1989](#)). In this sense authors such as [De Guzman & Delfinado-Baker \(1996\)](#), [Anderson & Trueman \(2000\)](#), [Maggi et al., \(2009\)](#), [Loeza-Concha et al., \(2018\)](#) have studied the morphometric variations of different populations of *Varroa* mites where different morphotypes have been



established in different regions of the world. In this region until now it was unknown which of the four species affects the different populations of *A. mellifera* and if there are morphological variations in *Varroa* populations that affect the different populations of *A. mellifera*. The objective of this research was to identify morphometrically the predominant species of *Varroa* (Parasitiformes: Varroidae) in bee colonies in Hopelchén, Campeche.

MATERIAL AND METHODS

Experimental area location

The research was carried out in the apiaries of the Higher Technological Institute of Hopelchén, Campeche, located at 19°76'41" north latitude and 89°86'68" west longitude at 100 m a.s.l. Two types of climates predominate in the area: warm sub-humid (awo) (w), with summer rainfall of less than 5.0 mm and warm sub-humid (aw1), with winter rainfall and precipitation between 5 and 10.2 mm. The average annual precipitation is 1,050 mm, with rainfall from May to October. The annual temperature varies between 19.5 °C and 32.5 °C, with an average of 26 °C ([Weather Spark, 2021](#)).

Sample obtaining

The research was carried out in 5 apiaries in Hopelchén municipality where 61 hives were studied with the following characteristics: five brood frames in all stages of development and 4 frames with honey and pollen. In these hives, 200 to 300 bees were collected from the third and fourth frames of the brood chamber. Bees were placed in containers with absolute alcohol until use ([Loeza-Concha et al., 2018](#)).

Varroa specimen collection

Varroa specimens were obtained using the methodology described by [De Jong et al., \(1982\)](#) with modifications ([Loeza-Concha et al., 2020](#)), which consisted of shaking plastic containers containing between 200 and 300 bees for 10 min at 60 rpm. The contents of containers were placed in a conical container with a 3 mm mesh which was filled with absolute alcohol until the bees were completely covered, then, with a glass rod the samples were shaken to detach mites from bees, so that by gravity the mites were deposited at the bottom of the cone, finally, the solution was decanted through a white cloth and mites obtained from each of the hives were stored and labeled separately in microtubes of 1.5 ml microtubes and kept refrigerated (4 °C) until use.



Mite processing

To determine the predominant *Varroa* species and morphometric variability, 244 female *Varroa* specimens were analyzed and placed in 50% lactic acid for 2 h at 100 °C; subsequently, mites were stored in 50% v/v alcohol until observation. Morphometric characters were measured using a stereo microscope with an ocular micrometer at 20X (Maggi *et al.*, 2009; Loeza-Concha *et al.*, 2018).

Morphometry

For morphometric identification of the predominant *Varroa* specimens, six variables were measured on each specimen: dorsal shield width (AED), dorsal shield length (LED), genital shield width (AEG), genital shield length (LEG), anal shield width (AEA) and anal shield length (LEA) (Figure 1) (Maggi *et al.*, 2009; Loeza-Concha *et al.*, 2018).

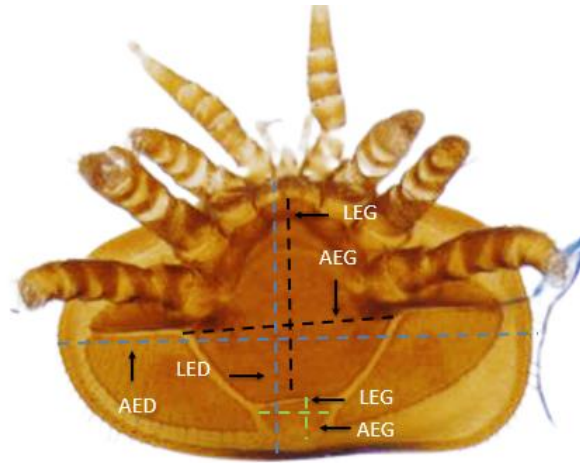


Figura 1. *Varroa* measured variables: dorsal shield width (DSW), dorsal shield length (DSL), genital shield width (GSW), genital shield length (GSL), anal shield width (ASW), and anal shield length (ASL)

Statistical analysis

To determine the morphometric differences of *Varroa* between apiaries, a comparison of means was performed with a one-factor ANOVA test; the variables that had significant differences were subjected to a second *post hoc* multiple comparison analysis using a Tukey's comparison of means ($P < 0.001$). To determine the morphotypes, a K-means cluster analysis was performed using the *Statistical Package for the Social Sciences* (SPSS) version 20.0 (IBM, 2011).



RESULTS

It was determined that 100% of the specimens evaluated in this study area belonged to the species *V. destructor*, only the variables GSW and ASW showed differences among the apiaries evaluated (Table 1).

Table 1. Mean of the studied variables (μm) belonging to the *V. destructor* populations of five evaluated apiaries

Apiarie	DSW	DSL	GSW	GSL	ASW	ASL
1	1685 ^a	1159 ^a	688 ^a	733 ^a	248 ^{abc}	199 ^a
2	1688 ^a	1153 ^a	707 ^{ab}	734 ^a	261 ^c	198 ^a
3	1691 ^a	1151 ^a	719 ^b	761 ^a	238 ^{ab}	197 ^a
4	1689 ^a	1154 ^a	711 ^{ab}	741 ^a	254 ^{bc}	206 ^a
5	1684 ^a	1146 ^a	708 ^{ab}	755 ^a	233 ^a	201 ^a
Mean	1687	1553	703	742	248	200
SEM	3.36	2.38	2.89	3.77	2.36	1.87

Dorsal shield width (DSW), dorsal shield length (DSL), genital shield width (GSW), genital shield length (GSL), anal shield width (ASW) and anal shield length (ASL). Standard error of the mean (SEM). Different literals per column indicate Tukey statistical difference with $p < 0.001$

According to the analysis of hierarchical clusters K means, it could be observed that all the variables presented discrimination for the formation of clusters among the *Varroa* populations analyzed in the five apiaries. In this sense, 5 morphotypes of *V. destructor*, which allowed us to observe that morphotypes A and E were the most widely distributed; these were differentiated because morphotype A presented smaller DSW size and larger DSL size compared to morphotype E. It was observed that morphotype B was the least distributed, presenting a larger DSW and smaller DSL compared to morphotypes A and E (Table 2).

According to the cluster analysis, it was observed that there is a morphometric variability of the mites in the 5 apiaries evaluated. In this sense, of the 5 morphotypes found, morphotypes A and E were the most widely distributed since they were found in hives of the 5 apiaries. Morphotype B was the least distributed since it was only found in hives of apiaries 1 and 5; morphotype C in hives of apiaries 1, 3, 4 and 5, morphotype A in hives of apiaries 1, 2, 4 and 5 (Table 3).



Table 2. Mean of the studied variables (μm) belonging to the 5 morphotypes of *V. destructor*

Morphotype	DSW	DSL	DSW	GSL	ASW	ASL
A	1681	1165	675	754	239	184
B	1706	1144	712	837	237	215
C	1652	1149	709	736	230	205
D	1684	1144	693	712	256	197
E	1707	1159	717	752	255	204
p<	0.0001	0.02	0.0001	0.0001	0.0001	0.002

Dorsal shield width (DSW), dorsal shield length (DSL), genital shield width (GSW), genital shield length (GSL), anal shield width (ASW) and anal shield length (ASL)

Table 1. Number of colonies belonging to each morphotype per apiary

Morphotype	A	B	C	D	E
Apiary	Number of hives colmenas /Apiary				
1	4	1	4	7	3
2	2	--	--	5	5
3	1	--	2	--	4
4	1	--	2	2	8
5	1	1	3	1	4
Total	9	2	11	15	24

According to the data obtained, when plotting the similarities between the morphotypes, a grouping in three nodes is observed, isolating morphotype B with higher values of LEG and LEA, but lower number of colonies (Figure 2, Table 2).

DISCUSSION

The results obtained indicate that the predominant species of *Varroa* in the 5 apiaries analyzed is *V. destructor* since according to [Abou-Shaara & Tabikha, \(2016\)](#). The proportion of body size is equal or greater than 1140 μm , confirming that the existing species is *V. destructor*. Our results agree with those obtained in Argentina by [Maggi et al. \(2009\)](#) where they reported 3 mite morphotypes, with a range of DSW 1696 μm to 1757 μm and DSL from 1128 μm to 1178 μm . For the case of Mexico, [Loeza-Concha et al. \(2018\)](#) found 8 morphotypes of *V. destructor* species with DSW ranges from 1582 μm to 1700 μm and DSL from 1042 μm to 1147 μm . In Japan, Thailand and Vietnam, [Anderson & Trueman, \(2000\)](#) reported the presence of *V. destructor* with a DSW of 1708 μm and a DSL of 1167 μm . In New Zealand, [Zhang, \(2000\)](#) reported that the DSL ranges of *V. destructor* were from 1132 μm to 1185 μm and the DSW 1642 μm to 1757 μm . In Poland where they studied the cell size effect so it could be demonstrated that the mite of *V. destructor* significantly reduced its size when the DSW of *V. destructor* was reduced when the *V. destructor* DSW was 1132 μm to 1757 μm . *V. destructor* mite significantly reduced its size when it was housed in small cell sizes. In this sense, [Borsuk et al. \(2012\)](#)



reported DSW and DSL of 1665 μm and 1121 μm respectively in small cell sizes and 1716 μm and 1142 μm respectively in standard cell sizes. We can mention several similar reports in Benin, Nigeria, Tunisia, Iran and Egypt (Table 4) (Rahmani *et al.*, 2006; Akinwande *et al.*, 2013; Abou-Shaara & Tabikha, 2016; Kelomey *et al.*, 2016; Yevstafieva & Nasarenko, 2018), according to the above mentioned, *V. destructor* is distributed in most of the world. This mite presents variations in size and shape within the same mite population in the different bee species it parasitizes (Akimov *et al.*, 2004). In this sense, we consider that the morphometric variability observed in this mite can be defined as an adaptation adjustment to the environment, which allows maintaining the individual fitness of the mite and the subsistence of the species (Pigliucci, 2005; Nussey *et al.*, 2007).

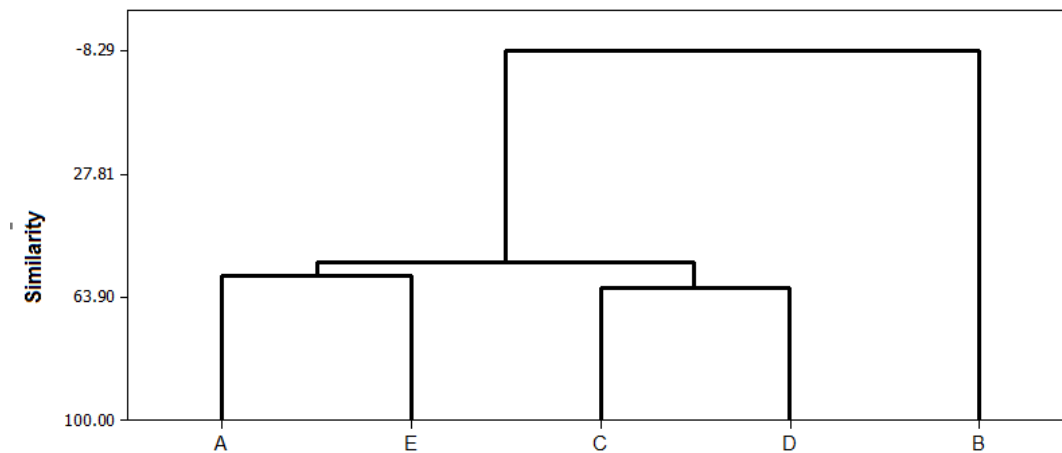


Figura 2. Grouping of morphotypes by similarity considering morphometric variables (morphotypes A, B, C, D and E)

According to the above, we consider that the variations of *Varroa* morphotypes found in the study area are mainly due to the interaction between the mite and bee species it parasitizes. Thus, we agree with Giménez *et al.* (2017) and George *et al.* (2004), who indicate that parasites tend to vary their morphotype according to their host, i.e. morphometric variability depends on the lineage of *Apis mellifera* that the mite parasitizes. The results obtained with the analysis of hierarchical clusters K means (Table 2) it could be observed that there is morphometric variability between closely related populations (Figure 1). Loeza-Concha *et al.* (2018) found 8 morphotypes in Tepic, Nayarit, Mexico, likewise, Maggi *et al.* (2009) found 7 morphotypes of *V. destructor* in colonies located in different geographical areas of Argentina, similarly, Akimov *et al.* (2004) and Dadgostar & Nozari (2018) have reported morphometric and geographical differences of *Varroa* mites in Iran and Ukraine. According to the aforementioned we differ with Rosenkranz *et al.* (2010) quienes who indicated that *Varroa* mites from different populations are physically the same; as well as with Dadgostar & Nozari (2018) indicated that geographical variations are causes of *Varroa* morphological variations. In this sense, we consider that if there are physical and morphometric differences between different *Varroa* populations



since it not only depends on geographical variation but also on colony migration, morphometric correlations between coexisting *V. destructor* populations and bee species it parasitizes and the mutations that the mite may present (Akimov *et al.*, 2004; Loeza-Concha *et al.*, 2018). These morphometric differences in arthropods and insects has been reported previously (Mozaffarian *et al.*, 2007; Lashkari *et al.*, 2015).

Table 4. Body size measurements in micrometers of *Varroa* females in the world

Taxonomic group (species)	DSL (µm)	DSW (µm)	Origin	Author
<i>V. rindereri</i>	1180	1698	Malaysia	De Guzman & Delfinado (1996)
<i>V. destructor</i>	1167	1708	Japan /Thailand/ Vietnam	Anderson & Trueman (2000)
<i>V. Jacobsoni</i>	1063	1506	Java	Anderson (2000b)
<i>V. destructor</i>	1159	1700		
<i>V. destructor</i>	1167	1708	New Zealand	Zhang (2000)
<i>V. Jacobsoni</i>	1063	1506		
<i>V. destructor</i>	1205	1738	North Tunisia	
<i>V. destructor</i>	1165	1711	Central Tunisia	Boudagga <i>et al.</i> (2003)
<i>V. destructor</i>	1197	1756	South Tunisia	
<i>V. destructor</i>	1149	1692	Ukraine	Akimov <i>et al.</i> (2004)
<i>V. destructor</i>	1197	1775	Iran Colonies less than 1000 m of altitude	
<i>V. destructor</i>	1199	1781	Iran Colonies between 1000-1500 m altitude	Rahmani, <i>et al.</i> (2006)
<i>V. destructor</i>	1200	1789	Iran Colonies at more than 1500 m altitude	
<i>V. destructor</i>	1135	1696		
<i>V. destructor</i>	1128	1711	Argentina	Maggi <i>et al.</i> (2009)
<i>V. destructor</i>	1178	1757		
<i>V. destructor</i>	1121	1665		
<i>V. destructor</i>	1142	1716	Poland	Borsuk <i>et al.</i> (2012)
<i>V. destructor</i>	1177	1718	Nigeria	Akinwande <i>et al.</i> (2013)
<i>V. destructor</i>	1115	1639	Benin	Kelomey <i>et al.</i> (2016)
<i>V. destructor</i>	1160	1710	Egypt	Abou-Shaara & Tabikha (2016)
<i>V. destructor</i>	1128	1688	Nayarit, Mexico	Loeza-Concha <i>et al.</i> (2018)
<i>V. destructor</i>	1090	1630	Ukraine	Yevstafieva & Nasarenko (2018)

Width of dorsal shield (DSW); length of dorsal shield (DSL)

Finally and according to the morphometric data obtained in this research we agree with Akimov *et al.* (2004) y Abou-Shaara & Tabikha (2016), since we consider that according to the body characteristics of *V. destructor* specimens obtained in Mexico. It is suggested that they are of the Korean haplotype, especially because the mean values of the length



and width of the dorsal shield are similar to those found in this research (1149 μm and 1692 μm). Besides that the Korean haplotype is the most common worldwide since records of its presence are found in Europe, Middle East, Africa, Asia, North America and South America (Zhang, 2000; Muñoz *et al.*, 2008; Akinwande *et al.*, 2012). The present investigation takes on greater relevance if we consider that the Japanese *Varroa* haplotype has a more restricted distribution. It is considered less virulent compared to the Korean haplotype which reproduces more easily (De Guzman & Rinderer 1999), because of this Carneiro *et al.* (2007) indicate that the reproduction rate of *V. destructor* females in young worker cells of Africanized honeybees in Brazil is currently almost double compared to the reproduction rate of twenty years ago. It is considered that *Varroa* populations of the Japanese haplotype have been replaced by the Korean haplotype, causing an increase in infestation levels in South America (Strapazzon *et al.*, 2009), which could lead to a greater loss of hives in America.

CONCLUSION

The present study confirmed that although 100% of mites belonged to *V. destructor*, they showed intraspecific morphometric differences.

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