

## **Morphometric Characterization of Parasite *Varroa* sp. of Bee *Apis Mellifera* L. in Benin**

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### **Abstract**

*Varroa* sp. is one of the most important pests of world of bees *A. mellifera* Linnaeus (Hymenoptera: Apidae). The aim of this study is to analyze the morphological variability of *Varroa* so as to identify the different morphotypes of *V. destructor*. The study was conducted in 24 localities and 24 municipalities located in three regions (North-East, North-West and the Centre) of Benin. One to two hives were randomly selected per locality for collecting samples. In total 46 *Varroa* were examined in this study. Analysis of variance components was used to determine the share of the variability of morphological characteristics of *V. destructor* based on localities and climatic zones. Numerical classification was performed to group the populations of *V. destructor* based on morphological descriptors. The results show that the variability of morphological descriptors of *V. destructor* is

usually larger at the community level than in climatic zones. In total four morphotypes of *V. destructor* are distinguished in Benin through the 4 variables used in the study.

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**Keywords:** *Varroa destructor*, morphotype, *Apis mellifera* L., Benin

## Introduction

The bee *Apis mellifera* L. is an important social insect, for his role in the pollination of crops and products generated in hives. A third of the human diet depends on plants pollinated by insects especially bees (Paraíso *et al.* 2011, Paraíso *et al.* 2013a, Akinwadé, 2013). For over a decade, weakening and colony losses due to diseases favored by environmental factors, chemical and biological are observed globally (Vidal-Naquet, 2011). The collapse of bee colonies is a phenomenon that currently affects all continents. Arthropods *V. destructor*, *Acarapis woodi*, *Aethina tumida*, *Braula coeca*; *Galleria mellonella* and *Achroea grisella* were identified as major pests of bees (Krauss and Page 1995; Finley *et al.* 1996; Wilson *et al.* 1997; Hunt, 1998; Paraíso *et al.* 2011; Paraíso *et al.* 2012 and Paraíso *et al.* 2013b). The mite *V. sp.* is the most important parasite of bees in Europe, America and Asia (Anderson and Trueman 2000). Its original host is *A. cerana* in which the latter causes almost no damage (Ball, 1994). *A. cerana* tolerate and he lives at his expense in the hive. The transition and adaptation of the parasite in the species *A. mellifera* (Anderson and Trueman 2000) sparked numerous damage observed today in this species. This is blood-sucking ectoparasites (Vidal-Naquet, 2008; De Jong, 1997). The low resistance of the bee parasite promotes its rapid spread (Peng *et al.* 1987, Le Conte, 2010), weakening and desertion of bee colonies following the transmission of many parasitic diseases (Ball, 1994). It is a mite that affects both the brood and the adult honey bees (Fries, 1993). The adult male has a whitish, spherical and measure 0.8 mm long by 0.7 mm wide. He lives within the capped brood while the female has a reddish brown color, 1.1 mm long and 1.6 mm wide.

The major problems losses of bee colonies due to *V. destructor* parasite were reported in Europe and the US by Wilson *et al.* (1997) and Sammataro *et al.* (2000). *V. destructor* is often identified late when it arrives in a region. It is a parasite very few experienced beekeepers. It was identified in the colonies of Benin by Paraíso *et al.* (2011). The aim of our study was to identify the species present in Benin and different morphotypes that exists. This study will enable a better understanding of this parasite.

## **Material and methods**

### **Study area**

The study was conducted in Benin (114,763 km<sup>2</sup>), a country in West Africa situated between meridians 0 ° 40 'and 3 ° 45' east longitude and parallels 06 ° 15 'and 12 ° 25' north latitude. The Collines Department (Zone 1) is wholly owned by the Guinean Sudanese climate zone marked by two rainy seasons that cover the periods from April to July and October to November. It is a transition zone (between South and North) of 16,900 km<sup>2</sup> extending after the plates of Abomey and Kétou until 9th parallel north. This area is fully occupied by leached tropical ferruginous soils or depleted (INRAB 1995). One also meets vertisols and waterlogged soils in the valleys of rivers and streams that cross the area. Zones 2 and 3, being in the Sudanese agro-ecological area, are between the 9th and 10th parallel north and a little beyond.

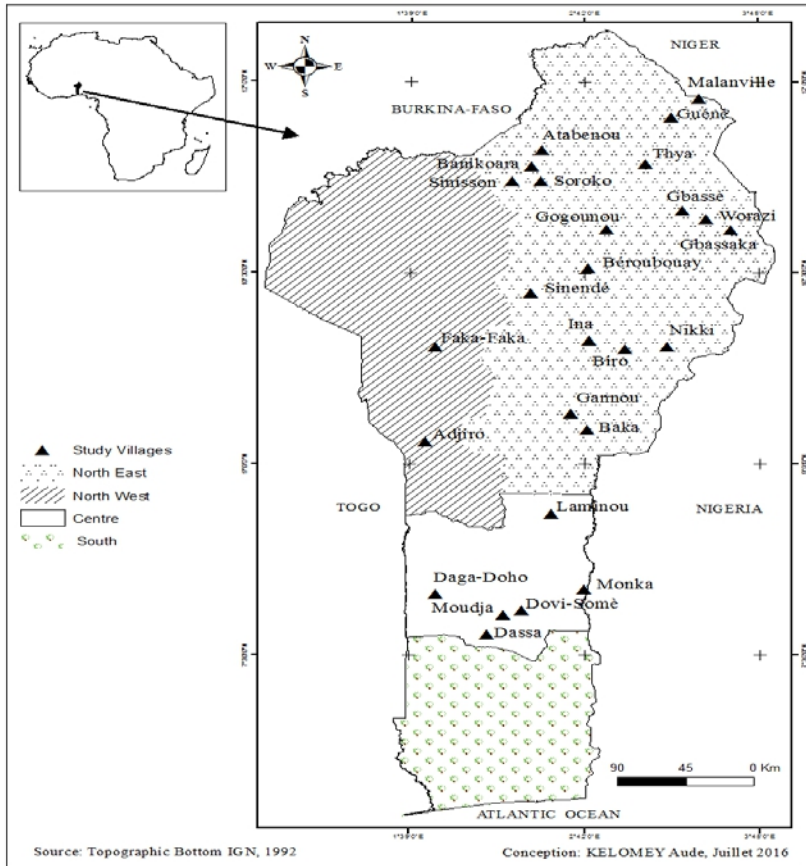
They cover some of Atacora and Donga. North East (Zone 2) is characterized by a Sudanese climate with a rainfall from April to October and a season dry season from November to March. Northwest of Benin (Zone 3) essentially has a mountain climate and has slight variations from one locality to another. Zones 2 and 3 are mainly dominated by tropical ferruginous soils with highly variable agronomic characteristics. These soils fine clay-sandy texture. We also meet lateritic soils and waterlogged soils in these areas (INRAB, 1995).

Benin's population is estimated at 9,983,884 inhabitants with a density of 57 inhabitants / km<sup>2</sup> (INSAE, 2013). The main activities of the local population are agriculture, livestock and fisheries. Some farmers in Benin associate beekeeping agriculture for a better performance given the role of bees in the pollination of crops. Nowadays, beekeeping of Benin is facing many problems including parasitic *Varroa*.

### **Sampling and data collection**

The study was conducted in 22 communes in three areas of Benin: North-West, North-East and Central area. These areas were chosen based on criteria parasite infestation rate and availability of beekeeping equipment. By town one or two localities have chosen. In total 24 localities were selected for the study. In the study area, bee colonies were visited to collect samples of *V. sp.* Total twenty-eight bee colonies were sampled in 24 localities. About a hundred bees were collected in containers containing alcohol at 96 ° and transported to the Plant Protection Laboratory of Parasitology and Pathology of bees located in Parakou for examination. Bee containers were stirred for a few minutes according to the method of Macedo *et al.* (2002) and the female mites were dislodged. The collected mites were then stored separately in Eppendorf tubes containing 96% ethanol and were then stored

at 4 ° C for subsequent morphometric analyzes. *Varroa* samples were then transported to the laboratory of IITA Biodiversity Centre morphometric studies.



**Figure 1.** Study area and geographic distribution of visited villages.

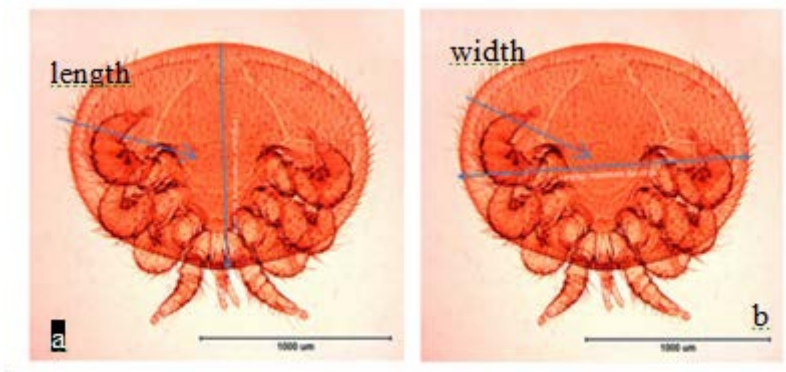
- **Preparation of mites for microscopic examination**

A total of 46 mites were measured. The preparation method used is adapted from the Laboratory of Biodiversity of the International Institute Tropical Agriculture of Center of Dr. Georg Goergen. After extracting the specimens of the harvesting liquid, they were immersed in a clean bath of 70% ethanol in order to be cleaned. After this stage, the mites were put in 10% KOH for 24 hours in order to lighten the inside of specimens for observation. Then, each specimen was cleaned under the microscope to remove the fatty mass located within the abdomen and which hinders observation. After this step, the specimens were transported in carbol Xylene for about 25 minutes for rinsing. Then, the specimens were transferred to a fuchsine solution for one hour for coloring. Finally, the specimens were placed in eye Glove for 25 min and were then mounted on microscope slides.

After having spread the specimen on a slide with its ventral side upwards, 1 or 2 drops of Canada balsam are poured over the specimen and the whole is covered with a cover glass. The slides were labeled and were placed in a drier at 40° until complete drying of the balm which will turn yellow. Then the body length, width of the body, width of the anal shield and width of the genital shield were measured on each specimen.

- **Measurements of the length and width of the mite body**

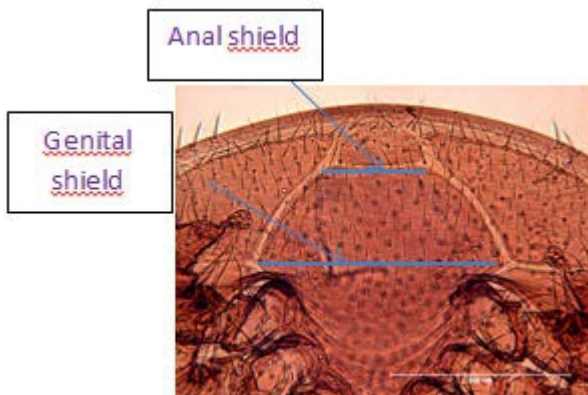
Measurements of the length and width of the body of the mites were carried out using a microscope eyepiece adapted to a computer that is equipped with a 40X magnifying power micrometer measuring software.



**Figure 2:** Measurements of length a) and width b) of *Varroa*.

- **Measurements of the width of the anal and genital shields of the mite**

Measurements of the width of anal and genital shields were taken using a microscope eyepiece adapted to a computer that is equipped with a micrometer measuring software with a 100X magnifying power.



**Figure 3:** Measurement of the width of the anal and genital shields of *Varroa*.

## Statistical analysis

An analysis of variance components was performed on all the morphological descriptors of *Varroa* in the twenty-eight people to analyze the share of the overall variability due to localities and study areas. A numerical classification was performed on the adjusted morphological descriptors, which brought together the people. In total four morphological groups mite was identified. A selection of the most discriminating morphological descriptors morphological groups of *Varroa* was made from a discriminant analysis step performed on the row-column matrix consisting of the morphological groups and morphological descriptors. Then a canonical discriminant analysis was performed on morphological descriptors *Varroa* selected to evaluate their discriminating power. Morphological characterization of the different morphological groups was carried out on the basis of discriminating descriptors after a multivariate analysis of variance (ANOVA) conducted to compare the discriminating descriptors between morphological groups of *Varroa*. Data were log-transformed, in order to homogenize their variance before being subjected to ANOVA. Statistical analyzes were performed with the R3.2.0 software (R Development Core Team, 2012. <http://www.Rproject.org/>). Principal component analysis (ACP) was also carried out with software Minitab 14.

## Results

### Variability of the morphological features of *Varroa*

The analysis of the components of the variance performed on all morphological descriptors of the varroas studied reveals a variability of the morphological descriptors which is generally larger between localities than between the areas (Table 1).

Table 1: Results of analysis of the components of the variance on the morphological descriptors of *Varroa*

Components	Body length (BL)	Body width (BW)	Width of genital shield (WGS)	Width of anal shield (WAS)
Zones	(0) 0	(0) 0	(73.76) 15,91	(0.00) 0
Localities	(2022) 100	(4083) 100	(389.75) 84,08	(94.14) 100

### Classification, discriminating powers of descriptors and morphological characterization of *Varroa*

The results of the rising hierarchical classification help to view with a height of information storage of 200 four homogeneous groups of localities (Figure 4). The total number of localities in the various groups is respectively 2; 14; 2 and 10 for groups 1; 2; 3 and 4.

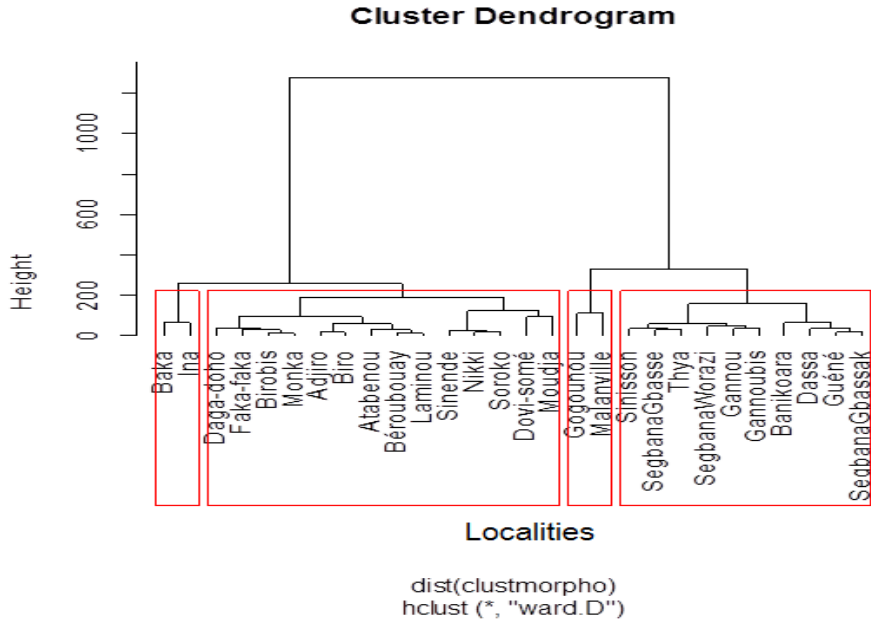


Figure 4: Dendrogram showing the grouping of varroas of localities according to their similarity

The results of the canonical discriminant analysis carried out to identify the characteristics of *Varroa* that help to discriminate the groups revealed that the first two canonical axes were generally significant ( $P < 0.05$ ) with 94.89% for the first axis and 5.02% for the second axis. Wilks test showed a significant difference between the four groups of *Varroa* (Wilks' Lambda = 0.04;  $p < 0.05$ ). The analysis of correlations between each of the two axes and morphological descriptors made it possible to deduce that the four descriptors of *Varroa* showed a positive correlation with the first axis (Table 2).

Table 2: Correlation between the original variables and the two canonical axes

Variables	Can1	Can2
Maximum Body length	<b>0.87</b>	0.31
Maximum Body width	<b>0.98</b>	0.06
Width of genital shield	<b>0.85</b>	-0.11
Width of anal shield	<b>0.70</b>	<b>-0.58</b>

The second was negatively correlated with the width of the anal shield. Thus, the four descriptors used helped to recognize the varroas of the four groups. The joint schematization of the varroas identified by their group in the first factorial plane formed by the canonical axes 1 and 2 (Figures 5.a and 5b) showed that the first axis separated groups G1 and G2 from the other two groups of *Varroa* (G3 and G4). The four descriptors used

allowed the recognition of varroas of groups G3 and G4. Indeed, the position of groups G3 and G4 on axis 1 suggested that the varroas of these groups were characterized by variable values.

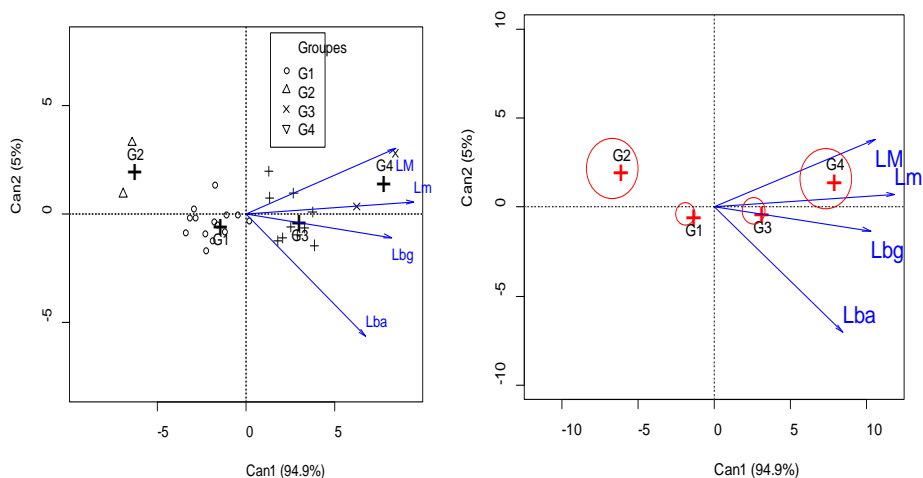


Figure 5. First factorial plane of the canonical discriminant analysis; a) joint schematization of mites and their characteristics; b) joint schematization of groups of *Varroa* and their characteristics.

**Key: LM: Maximum length of the body; the: Maximum width of the body; lbg: Length of genital shield; lba: Width of the anal shield.**

### Characteristics of groups identified

The analysis of variance showed that it has a very highly significant difference ( $p < 0.001$ ) between the four *Varroa* morphological groups regarding the four variables (Table 3).

Table 3: Quantitative description (mean  $\pm$  standard error) of measured variables associated with different groups of *Varroa*.

Variables	Group1		Group 2		Group 3		Group 4		Prob
	m	se	m	se	M	se	m	se	
Maximum Body length	1064.2	8.20	1036.6	21.71	1135.1	9.71	1224.5	21.71	***0,000
Maximum Body width	1589.9	6.68	1598.1	17.68	1681.4	7.90	1790.3	17.68	***0,000
Width of genital shield	663.4	4.56	622.3	12.07	692.0	5.40	723.1	12.07	***0,000
Width of anal shield	244.6	2.19	219.2	5.80	255.1	2.59	254.9	5.80	***0,000

m = average; se = standard error

\*  $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.001$ : Significant at 0.05\*\* : Highly significant at the 0.01 threshold\*\*\* Very highly significant at the 0.001 threshold



The principal component analysis grouped the 28 localities into various components with the first three axes explaining 97.3% of the total variation (Table 4). Principal component 1 (PC1) negatively associated with maximum body length and width accounted for 74.7% of the total variation (Table 4). PC2 negatively associated with width of genital shield accounted for 14.1% of the total variability (Table 4). The maximum body length and width of genital shield are those mostly correlated to the third axis accounting for 8.5%.

Table 4: Eigen vectors and values for the four principal component areas

	PC1	PC2	PC 3	PC 4
<b>Eigenvalue</b>	2,9873	0,203	0,3388	0,1079
<b>Proportion%</b>	0,747	0,141	0,085	0,027
<b>Cumulative%</b>	0,747	0,888	0,973	1,000
<b>Variables</b>				
LM	-0,508	0,203	0,748	
Lm	-0,554	0,125	0,002	
Lbg	-0,499	0,414	-0,650	
Lba	-0,431	-0,879	-0,133	

Groups formed in the ACP are identical to those of the canonical analysis (Figure 5).

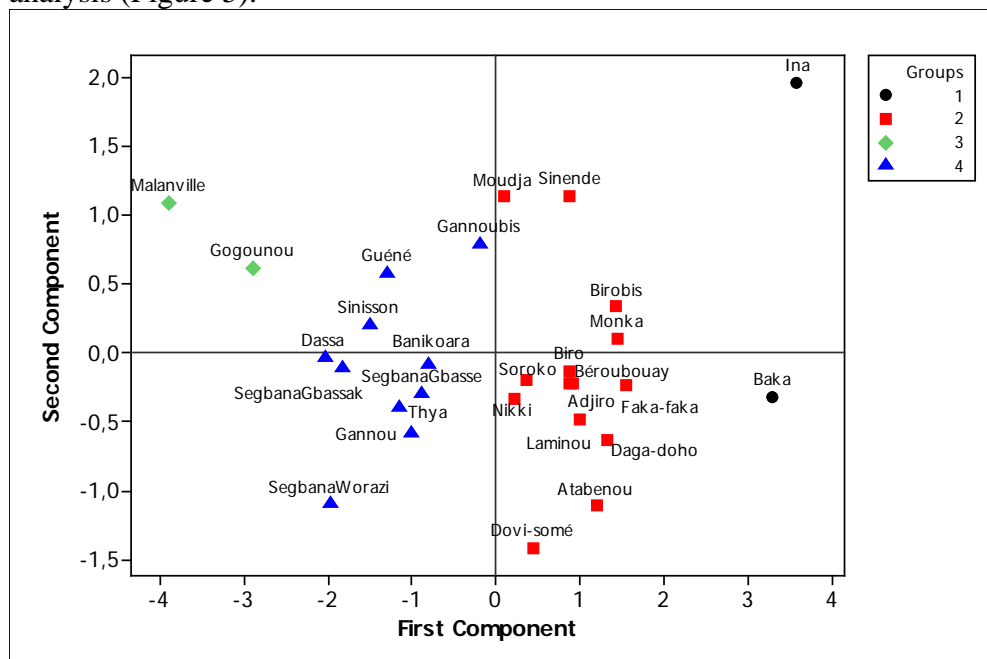


Figure 5: Principal component analysis showing the different grouping of the accessions of *Varroa sp.*

### Discussion

The objective of our study was to identify the subspecies of *Varroa*

and the different morphotypes of *Varroa* that exist in colonies of bees in Benin. This study identified the morphotypes of *Varroa* by taking morphometric measurements.

Measurements of body parameters (length and width) of the analyzed *Varroa* compared to Anderson and Trueman data (2000), Zhang (2000) and Boudagga *et al.* (2003) showed that there is a similarity between the averages for measurements of samples of *Varroa destructor* in these countries (Table 5). Morphometric characters of specimens studied corresponded to that of *Varroa destructor* and not the *Varroa*. The samples have a high phenotypic consistency and were larger in length and width of the body than *Varroa jacobsoni* confirming that all the mites specimens examined belong to the species called *Varroa destructor*.

The infestation of the parasite *Varroa* observed in all sampled hives indicates that the mite *Varroa* has invaded all the hives of Benin, which corroborates studies Paraiso *et al.* (2011). The data of De Jong (1997) show that the epidemic of *Varroa* is a global problem and that the mite *Varroa* has a strong virulence in the colonies of *Apis mellifera* L. The viral infection can cause physical abnormalities in adults such as distorted wings, reduction in the ability to fly and longevity of workers (Marcangeli, 1992, Bakonyi *et al.* 2002; Chen *et al.* 2004; Tentcheva *et al.* 2006; Cornellsen *et al.* 2011).

The results showed a morphological variability of the descriptors which is more significant between the localities than in climatic zones. This shows that the variation within population (in climate zones) was more significant for these characters than variability between populations. The very low variability of descriptors observed between the populations of the three zones is related to genotypic factors; indeed, the size (length and width) of mites varies according to the species of bees present in the zone where the sample collection is performed. Indeed, the subspecies of *Varroa* are morphologically distinct and have specific differences in their geographical distribution (Anderson and Trueman 2000). The species *Apis mellifera* L. is infested only by the subspecies *Varroa destructor* (Muñoz *et al.* 2008). *Varroa destructor* is found in Europe, Africa and the United States while *Varroa jacobsoni* is found in Asia (Solignac *et al.* 2002, 2005; Anderson and Trueman 2000). Similarly, according to the work of Delfinado-Baker (1989), the parasite *Varroa* has an average length of 1037.52 microns which is different from the average length of the parasite *Varroa destructor* which is 1106.31 µm. The average length and width of the body of parasites in the study area were 1115.1 and 1639.9 microns. According to the data of Akinwadé (2013), Boudaga *et al.* (2003), Zhang (2000) and Anderson and Trueman (2000) (Table 4), the measured mites belong to the subspecies *Varroa destructor* of Benin. The samples analyzed presented a high phenotypic consistency and were larger in the body length and width than

*Varroa jacobsoni*. However, the average values of the measurements of length and width of the body of the parasites in groups 1 and 2 consisting of samples from the Centre and Northeast are smaller than those of the literature (Anderson and Trueman, 2000). These results corroborate those of Paraiso *et al.* (2011) who obtained average values of 1mm in length to 1.75 mm in width for measurements of the samples of *Varroa* in Benin and justified this result by the adaptations of *V. destructor* to the smallest subspecies *A. m. adansonii* compared to other bees of bigger size.

The results of the rising hierarchical classification and Wilks test showed that varroas of Benin fall into four statistically distinct groups. These results indicate heterogeneity in the analyzed populations of *Varroa* and show the presence of four morphotypes of *Varroa destructor* in Benin. Moreover, all the four morphometric characters used in this study have a significant discriminating power.

Table 5: Measures (in  $\mu\text{m}$ ) of the length and width of the body (Mean  $\pm$  se) of the females *Varroa* of Benin compared to the biometry of *Varroa jacobsoni* and *Varroa destructor* referenced by Akinwadé (2013), Anderson and Trueman (2000), Zhang (2000) and Boudagga *et al.* (2003).

	Body length m $\pm$ se	Body width m $\pm$ se	No. of specimens examined
Parasites <i>Varroa</i> of Bénin	1115.1 $\pm$ 15.33	1639.9 $\pm$ 12.48	46
<i>Varroa destructor</i> of Kosere, Ogun State, Nigeria (Akinwadé 2013)	1177.96 $\pm$ 7.09	1718.12 $\pm$ 1.4	20
<i>Varroa destructor</i> (Anderson et Trueman 2000)	1167.3 $\pm$ 26.8	1708.9 $\pm$ 41.2	42
<i>Varroa jacobsoni</i> (Anderson et Trueman 2000)	1063.0 $\pm$ 26.4	1506.8 $\pm$ 36.0	73
<i>Varroa destructor</i> (Zhang 2000)	1159.0 $\pm$ 21.6	1700.0 $\pm$ 46.5	5
<i>Varroa destructor</i> (Boudagga 2003)	1204.9 $\pm$ 40.1	1738.5 $\pm$ 35.3	20
<i>Varroa destructor</i> (Boudagga, 2003)	1164.9 $\pm$ 38.46	1711.2 $\pm$ 47.44	20

## Conclusion

The parasite *V. destructor* causes significant economic losses in the beekeeping sector. Our results showed that the mite of Benin belongs to the subspecies *Varroa destructor* and fall into four statistically distinct morphotypes. We suggest that molecular studies should be conducted on varroas from Benin to determine the haplotype of *Varroa* present in Benin and the genetic diversity within morphotypes.

## Acknowledgments

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