



Studies on morphometry of Varroa species (Mesostigmata: Varroidae) collected in *Apis mellifera* and *Apis cerana* colonies of Southern India

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Abstract

Varroa mite is considered one of the most damaging pests of honey bees in India as well as worldwide. There are mainly two species of Varroa mites, *i.e.*, *Varroa destructor* and *Varroa jacobsoni*, reported from India. The present research is done to analyse the morphometric characters of Varroa mites infesting *Apis cerana* and *Apis mellifera* colonies in southern India. So far, no morphometric studies were done to identify Varroa mites from southern India. In this study, morphological investigations were done on different populations of mites collected from five districts. Seven characters namely, body width, body length, width of genital shield, length of genital shield, width of anal shield, length of anal shield and body size ratio were recorded. Mites collected from *A. mellifera* are having higher body length and body width than mites collected from *A. cerana*. The body size parameters showed a mean (\pm SD) body length ranging from 1.661 ± 0.09 to 1.710 ± 0.03 and a body width ranging from 1.113 ± 0.05 to 1.134 ± 0.02 in colonies of *A. mellifera*. A mean (\pm SD) body length of 1.430 ± 0.05 to 1.470 ± 0.02 was observed in mites collected from *A. cerana* colonies. The body size ratio (ratio of body width to body length) of all the collected specimens was greater than 1.4 in both *A. mellifera* and *A. cerana* mites.

Key words

Varroa destructor, *Varroa jacobsoni*, morphometrics, *Apis mellifera*, *Apis cerana*, body size ratio

Introduction

Most of the honey bee researchers consider the obligate ectoparasitic mite, *Varroa* spp. (Mesostigmata: Varroidae) as the most damaging enemy of the Italian bee (*Apis mellifera*). Each year a number of bee hives are destroyed directly or indirectly because of *Varroa* mite infestation, thus causing severe damage to beekeeping industry all over the world (Dietemann *et al.*, 2012). These mite parasites on larval, pupal as well as adult of honey bees causing malformations, declining adult populations, sometimes even causing loss of entire hive (Le Conte *et al.* 2010). Oudemans, (1904) reported the *Varroa* mites on *Apis cerana* in Java and Indonesia for the first time. The name given to the mite was *Varroa jacobsoni*. They were subsequently allocated to a new family, the Varroidae, and a new genus, *Varroa* (Delfinado-Baker and Baker, 1974). When the European honey bee, *A. mellifera* was introduced into Asia by man, the mite than utilized *Apis mellifera* as a preferred host. (De Jong *et al.*, 1982a). *Varroa* mite was initially believed to be a single species before Anderson and Trueman (2000) discovered that *A. mellifera* is infected by a different species, called *Varroa destructor*. It was also reported that the mite size is correlated with bee size in such a way that the smaller bee species *A. cerana* is parasitized by *Varroa jacobsoni* which is smaller than *V. destructor*. Later Anderson (2000) reported *V. destructor* present in Asia, Europe, Africa and America, is parasitizing both *A. cerana* (original host) and *A. mellifera* (new host). *Varroa jacobsoni* and *V. destructor* is reported from *A. cerana* colonies of southern India through partial sequencing of cytochrome oxidase I gene. (Bhatta *et al.*, 2019). Mathialagan *et al.* (2017) reported that *V. jacobsoni* and varroosis are common in Indian bees, *A. cerana* in most districts of Tamil Nadu. Düttmann *et al.*, 2021 used morphometric characters such as body width and length, length and width of the genital shield and of the anal shield, length of setae and chelicerae to identify four morphotypes of *Varroa destructor* at apieries of Nicaragua. The differences between hosts such as honey bee races and environmental conditions of the ectoparasite can also have an effect on morphological characters of varroa mite (Dadgostar and Nozari, 2018).

But presently, there is no study dedicated to study the morphological variations in *Varroa mites* affecting honey bees in India. Although various studies have reported the presence and infestation of these mites in India (Putatunda and Abrol, 2002; Kotwal *et al.*, 2013; Chaudhary, 2005; Puttaswamaiah and Reddy, 2011). The aim of this study is to determine the variation in morphological characters of female *Varroa* mites in southern parts of India, with the purpose of expanding ecological and epidemiologic knowledge of the parasitism caused by the mite. All measurements are given in millimetres.

Materials and methods

The study was conducted in the Apiculture unit located at Tamil Nadu Agricultural University, Coimbatore with an elevation of 409 m above sea level. Mite samples were collected from four districts of Tamil Nadu viz., Coimbatore, Tiruppur, Perumbalur, Dindigal and from one district of neighbouring state Kerala viz Palakkad. The sampling was carried out between October 2021 and April 2023. The mites were carefully removed from capped drone brood cells using a forceps and needle. Samples belonging to the same colony were placed in an eppendorf tube containing 70% ethanol and stored at 4⁰C until analysis. The morphometric characters were measured using a stereoscopic microscope (Leica 165 C stereomicroscope).

Morphometric measurements

Six variables were measured on the mites obtained from different locations (Figure 1). Body width (BW), body length (BL), width of genital shield (WGS), length of genital shield (LGS), width of anal shield (WAS), length of anal shield (LAS). Apart from this, the ratio of body width to body length (Body size ratio) is also calculated. Descriptive statistical analysis was done, and the mean and standard deviation of each variable were calculated. 95% confidence intervals were calculated for mites collected from *A. mellifera* and *A. cerana* separately. All the statistical analysis was performed with the help of IBM SPSS Statistics software (version 20.0).

Results and Discussion

Morphometric diagnosis was carried out for mites collected from eight different populations of five localities representing in Coimbatore (three populations, P1, P2 and P3), Thiruppur (two populations, P1 and P2), Dindigal, Perambalur and Palakkad districts. The mean values and standard deviations of the morphometric characters studied are shown in table 1. Mean of measured characters obtained were 1.693±0.06, 1.130±0.04, 0.715±0.03, 0.580±0.03, 0.255±0.02, 0.130±0.02 and 1.498±0.04 mm for BW, BL, WGS, LGS, WAS, LAS and BR respectively for mites collected from *A. mellifera*. In case of host *A. cerana indica*, the measured characteristics were 1.447±0.04, 0.974±0.02, 0.596±0.02, 0.503±0.02, 0.210±0.04, 0.108±0.03 and 1.447±0.04 mm for BW, BL, WGS, LGS, WAS, LAS and BR respectively. The population of mites collected from *A. mellifera* is clearly distinct from population of mites collected from *A. cerana indica*. Their body length as well as body width were observed to be smaller than that of collected from *A. mellifera*. However, their average body ratio (ratio of width to length) was not very distinct (1.498 and 1.485) and varied by

0.013. According to Anderson and Trueman (2000) the adult females of *V. jacobsoni* and *V. destructor* have similar morphological characters but latter being larger, and having a less spherical shape, can be separated by mt DNA sequencing. In the present investigation eventhough the mites collected from *A. cerana indica* clearly shows lesser body size, their body size ratio was greater than 1.4 emphasising typical ellipsoidal shape that of *Varroa destructor*.

Examination of specimens from different populations from different districts revealed that The characters varied among different districts as well as year of collection (Table 2). The size of the mite obtained from *A. cerana indica* in Coimbatore (BW=1.43 ± 0.05, BL= 0.96 ± 0.02) was the smallest among the collected specimens. This was reflected in the size of the genital shield (WGS=0.59 ± 0.02, LGS=0.49 ± 0.02) and anal shield also (WAS= 0.20 ± 0.04, LAS= 0.11 ± 0.04). Among the mites collected from *A. mellifera*, Coimbatore (P2) was the smallest with an average body length and width of 1.661 and 1.113 mm respectively. It is also noted that the mite collected during the year 2021 from the same host (either *A. mellifera* or *A. cerana indica*) has a comparatively larger size than mites collected during subsequent years, disregarding the place of collection. This may be due to climactic factors or nutritional reasons. It is observed that beekeepers are engaged in migratory beekeeping in these areas, and availability of pollen and nectar sources may contribute to these differences in morphometry. Also, a positive relationship was found between body dimensions and acaricide resistance (Maggi *et al.*, 2012) so this can be a possible reason. Such expectations, however, require in-depth analysis. The average body ratio obtained for all three populations from Coimbatore, disregarding the host is similar (1.494, 1.494 and 1.490) and is smaller than the body ratio obtained for populations from Thiruppur (1.498 and 1.509) and Palakkad district (1.512). The smallest body ratio was observed for mites collected from *A. cerana* in Perambalur district (1.481).

The mean body size of mites reported from *A. mellifera* in this research BW (body width; sometimes referred as width of dorsal shield) and BL (body length; sometimes referred as length of dorsal shield) were similar to values observed by Anderson and Trueman (2000) for *Varroa destructor* (BW= 1.708 mm ± 0.041 ; BL= 1.167 mm ± 0.026), whereas the body size of mites collected from *A. cerana* were similar to, but lower than values observed by Anderson and Trueman (2000) for *V. jacobsoni* (BW= 1.506 mm ± 0.036 ; BL= 1.063 mm ± 0.026). Previous research on the genus *Varroa* has shown that populations of *V. jacobsoni* and *V. destructor* exhibit morphological differences, with the latter species being larger in size (Delfinado-Baker and Houck, 1989; Anderson and Trueman, 2000).However,

the body size ratio obtained in all the collected specimens was greater than 1.4 mm which is a character of *V. destructor* (Definado-Baker., 1984, Dietemann *et al.* 2013). Akinwande *et al* (2013) recorded similar mean values (1.71 and 1.17 for body width and length respectively for mites in Nigeria. The examined samples from Thiruppur (P1) showed similarity to the mites collected from New Zealand, as the measured means of body width and body length showed 1.70 and 1.15 respectively (Zhang, 2000). All the parameters measured for mites from *A. mellifera* were highly similar to the mean measurement morphotypes of *V. destructor* identified from Argentina *viz*; WDS= 1.696-1.757, LDS= 1.135-1.178, WGS=0.705-0.729, LGS=0.541-0.565, WAS=0.255-0.258 and LAS=0.144-0.146 mm (Maggi *et al.*, 2009). The investigated specimens were found to have a mean body width and length lower than those recorded from Ukraine, *i.e.* BW=1.69 and BL=1.14 (Akimov *et al.*,2004). The Varroa mites collected from *A. cerana* were smaller than Varroa mites reported elsewhere so far. These differences may be due to host differences as well as variations in geography (Definado-Baker and Houck,1989).

Conclusion

All the measured characters in this experiment, except body size ratio, for the mites collected from *A. mellifera* were similar to those of *V. destructor*, and for the mites collected from *A. cerana indica*, they were in line with *V.jacobsoni*. However, the body size ratio of all the collected samples is higher than 1.4 mm, which implies that the existing species in the examined specimens is *V.destructor*. So, sequencing its mtDNA CO-I gene in the future is required for further confirmation. Moreover, the morphological study of *Varroa* mites collected from other parts of India should also be considered.

Acknowledgement

The authors would like to express their sincere gratitude to Tamil Nadu Agricultural University for the laboratory and field support throughout the course of this research. We also thank Dr. Chitra. N, Professor, Department of Agricultural Entomology, for providing microscope facilities and guidance.

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