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Current status of *Acarapis woodi* mite infestation in Africanized honey bee *Apis mellifera* in Brazil

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Acarapis woodi Rennie (Acari: Tarsonemidae) is an endoparasite that can affect the respiratory system in adults of *Apis mellifera* L. (Hymenoptera: Apidae) causing death. This parasite feeds on the host's hemolymph, and all stages of the mites' development occurs within the honey bee's large prothoracic trachea (Sammataro et al. 2013), then spreads by direct contact between honey bees (Sammataro et al. 2000).

This mite can be found in European, Asian, African, as well as North and South American (Ellis & Munn 2005) countries. However, in Brazil the current distribution of *A. woodi* is not well understood or studied, with first detection dating back to the 1970s (Nascimento et al. 1971; Wiese 1971; Flechtmann 1976), whereas the current situation is unclear (Maggi et al. 2016). Therefore, we evaluated the presence of *A. woodi* in *A. mellifera* in Brazil, to generate an understanding of the situation in this country.

We evaluated 153 colonies of *A. mellifera* from apiaries located in 15 Brazilian states between 2014 and 2016 (Fig. 1). In each colony, 30 honey bees were individually dissected using forceps under a stereoscopic microscope Olympus (Model P20, Waltham, Massachusetts, USA), with 2.5× magnification; the prothoracic trachea was examined under 5× magnification. Presence of the mite was indicated by the observation of dark points on the trachea.

Molecular analysis was performed using a pool of 30 honey bees per colony. The DNA extraction was performed in 100 µg of crushed honey bees using DNAzol (Invitrogen, Carlsbad, California, USA) with modifications, where 10 µL of proteinase K solution (Invitrogen, Carlsbad, California, USA) was added and cell lysis maintained at 37 °C for 18 h, then 2.5 µL of RNase A (Invitrogen, Carlsbad, California, USA) was added. After the DNA washes, 50 µL of ultrapure water was used for DNA elution, and evaluation of DNA quality was performed using BioPhotometer D30 (Eppendorf, Hamburg, Germany). The sample was diluted to a final concentration of 100 ng per µL for use in the PCR, and ultrapure water was used as a negative control. Supermix PCR reagent (Invitrogen, Carlsbad, California, USA) was used to perform the PCR, following the manufacturer's protocol, and *A. woodi* primers were: 5'-AAGATATTGGAACATTATATTTATTTT-3' (forward) and 5'-CAAAATCAGAATAAATGTTGAAAT A-3' (reverse) with amplicon size expected at 677 pb (Kojima et al. 2011). The following thermal cycles used were: initial denaturation-single hold at 94 °C for 2 min, followed by 35 cycles at 94 °C for 15 s (denaturation), 55 °C for 30 s (annealing), and at 72 °C for 1 min (extension). PCR product visualization was performed in 2% agarose gel.

A total of 9,180 trachea from 4,590 honey bees examined from 153 colonies in 15 Brazilian states did not show *A. woodi* infestation (Fig. 1). Also, all molecular samples tested showed negative results.

This is the first study to be performed in Brazil for *A. woodi* using 2 different techniques 47 yr after the first detection in the country (Fig. 1). Apparently this endoparasite is not adapting to tropical areas, apparently due to its preference for cold climate areas (Otis & Scott-Dupree 1992); it is known to occur in dry climates such as Africa (Pirk et al. 2016). Similar situations are being observed in other countries such as Argentina, where the mite was previously detected in 1994 (Eguaras et al. 1998), but in a more recent study the mite was not found in this area (Szawarski et al. 2017). Another example is the United States, which first detected *A. woodi* in the 1980s (Sammataro et al. 2013), but the mite was not present in honey bees surveyed in 2009 (Traynor et al. 2016). However, it has been suggested that the decimation of this mite by miticides for *Varroa destructor* mite control, may be the reason of *A. woodi* disappearance in Argentina (Szawarski et al. 2017) and the United States (Sammataro et al. 2013). However, this situation does not apply to Brazil, because *V. destructor* is not a threat to Africanized honey bees in this country. Therefore, we can conclude that *A. woodi* is not present in colonies of the Africanized honey bee *A. mellifera* in Brazil and these results contribute an update of this mite's presence in the country.

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Summary

Acarapis woodi Rennie (Acari: Tarsonemidae) is an endoparasitic mite, which can affect the respiratory system in the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) causing mortality. This mite was first recorded in Brazil in the 1970s, but its current presence is unclear. Therefore, we evaluated the presence of *A. woodi* in the Africanized honey bee *A. mellifera* in Brazil, to update the occurrence data of this parasite, 47 yr after its detection. We examined a total of 153 honey

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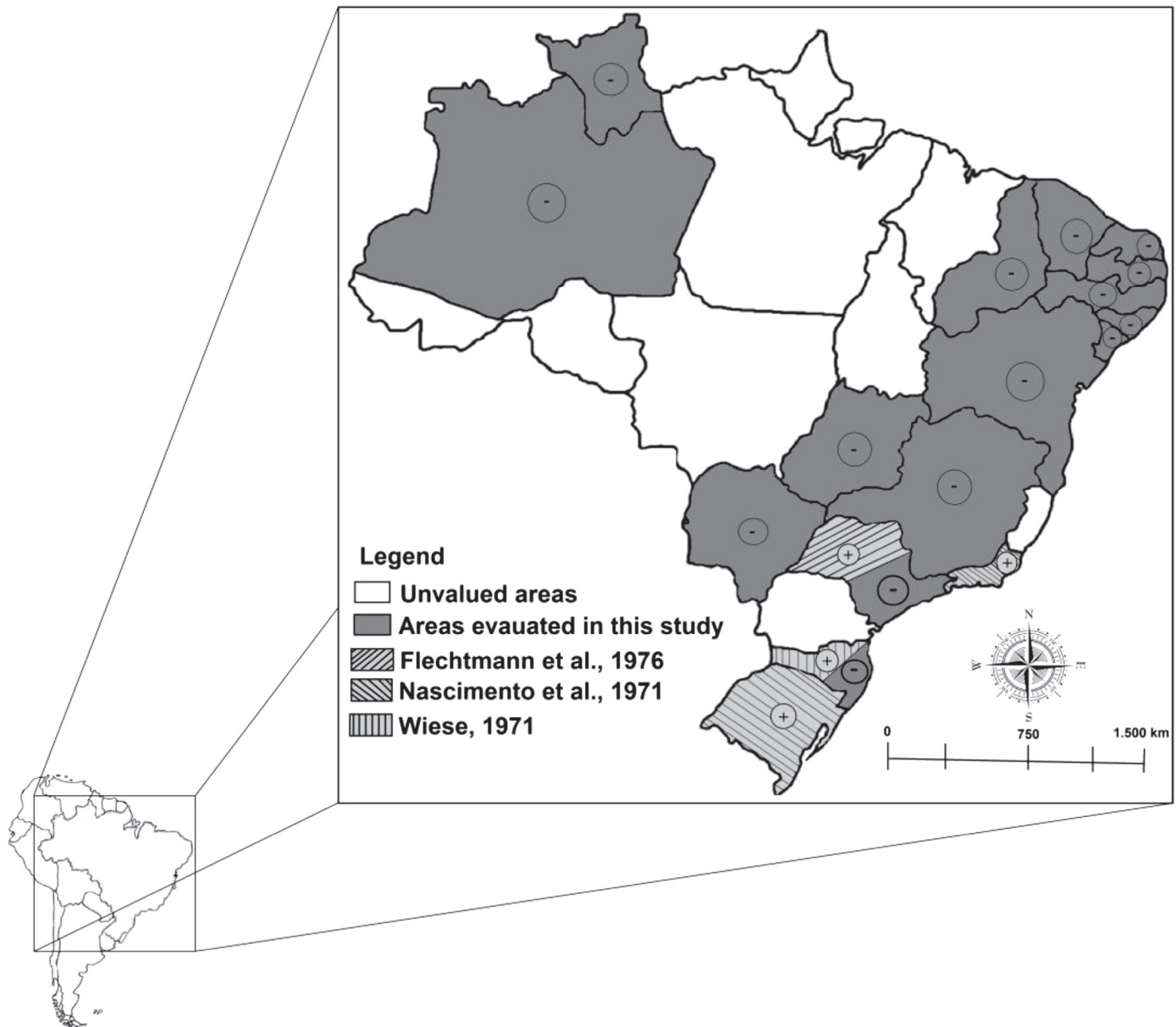


Fig. 1. States evaluated for the presence of *Acarapis woodi* in Brazil. The blank area represents the states not surveyed; dark grey areas show the states surveyed in this study which presented negative results (circles with negative sign); light grey areas show previous studies from the 1970s.

bee colonies, from 15 different states using dissection and molecular techniques. This is the first study of the detection of *A. woodi* in Brazil using molecular techniques. The results were negative for both methods employed, and we can conclude that *A. woodi* is not present in Africanized honey bee colonies in Brazil.

Keywords: Africanized honey bee; tracheal mite; bee health; endoparasite; honey bee disease

Sumario

Acarapis woodi Rennie (Acari: Tarsonemidae) é um ácaro endoparasita que pode afetar o sistema respiratório em abelhas *Apis mellifera* L. (Hymenoptera: Apidae) causando mortalidade. Esse ácaro foi registrado pela primeira vez no Brasil na década de 1970, mas atualmente não existem registros da presença desse ácaro no país. Portanto, foi

avaliada a presença *A. woodi* em abelhas africanizadas *A. mellifera* no Brasil, visando atualizar os dados de ocorrência desse parasita, 47 anos após a sua detecção. Foram examinadas 153 colônias de *A. mellifera*, em 15 diferentes estados, usando dissecação e análise molecular. Este é o primeiro estudo de detecção de *A. woodi* no Brasil utilizando análise molecular. Os resultados foram negativos para ambos os métodos empregados e podemos concluir que *A. woodi* não está presente nas colônias de abelhas africanizadas do Brasil.

Palavras Chave: abelha africanizada; ácaro traqueal; saúde das abelhas; endoparasita; doenças das abelhas

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