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MOLECULAR IDENTIFICATION AND SEQUENCING OF *VARROA DESTRUCTOR* IN WESTERN ROMANIA

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Abstract:

Varroa destructor mite (Mesostigmata - Varroidae) is one of the major problems of beekeeping worldwide, due to colony mortality at high levels of infestation and transmission off different pathogens between hives. It has been also observed to cause lower weight in infested individuals, decreased learning capacity, and shorter lifespan. The current study was undertaken to characterize morphologically and molecularly the acarian *V destructor*, identified in two regions of western Romania. The samples were collected from Arad County, from different localities, namely Nădlac, Roșia Nouă and Obârșia, and from Caraș Severin County, Corneava locality. From a total of 41 samples, collected form 8 different hives, 4 positive samples for *Varroa* spp. were identified by light microscopy and the SEM method. Molecular analysis of the Cox1 gene of the mite revealed positive results for all the isolates. The samples were sequenced using next-generation sequencing (NGS) identified as *Varroa destructor.* This study results contribute to the knowledge of the molecular epidemiology of *Varroa* mite species in western Romania.

• Introduction

The *Varroa destructor* mite (Mesostigmata - Varroidae) has been and still is a major problem in beekeeping. In addition to being a vector of viruses that can cause colony destruction, it can cause mortality at high levels of infestation. Damage depends on a number of biotic and abiotic factors, in which haplotype pattern variation is thought to be a key factor.

Varroa genus is represented by four species of obligate ectoparasitic mites of bees: *V. jacobsoni, Varroa underwoodi, Varroa rindereri* and *V. destructor*, which was misclassified as *V. jacobsoni* until it was discovered that they are separate species.

The mite, has been extensively studied since the 1980s after its introduction to Europe in *Apis mellifera* bee populations. In the last 10 years, new invasions have been observed in nonendemic areas such as Hawaii, Reunion Island, Madagascar, Mauritius Island, and also African countries such as Uganda and Ethiopia. In 2020, only Australia, a few African countries and several islands have not yet reported the presence of *V. destructor* in their *A. mellifera* populations.

The present study was undertaken to determine the diversity of *Varroa* species collected from pastoral apiaries. The identification was based on their morphological characteristics, using classical methods and scanning electron microscopy (SEM) technique. The samples were then molecularly characterized by PCR and sequencing in order to determine the species and the halotype involved in parasitism in beehives in western Romania

• Material and method

The study was conducted between October 2022 - May 2023, in two counties in western Romania, namely Arad County and Caras Severin county. The localities were from different areas of the counties, more precisely, in Arad County, they were represented by Nădlac, Roșia Nouă and Obârșia, and in Caraș Severin County, the samples were taken from Corneava. The samples were collected from different apiaries belonging to the two counties, both in their own farms and in the pastoral. Samples were taken of adult bees, combs with larval brood, drone and worker bees

The bee samples were examined under the light microscope and scan electrono-microscope for identification of specific morphological characters. Some of the samples were examined at the Scanning Electron Microscopy Laboratory (SEM) of the CLHC Timisoara. Subsequently, the samples were subjected to molecular analysis for the identification of parasite DNA. This extraction was carried out using the BIoline Tissue Protocol Kit (BIOLINE®).

The COI gene in the mitochondrial genome of *V. destructor* mite was amplified by polymerase chain reaction (PCR) using Primers:

F 5'-TACAAAGGGAGAAGAGCAGCC-3' and R 5' –GCCCCCCCCTATTCTTAATATACATAG TGAAAATG-3' described by Navajas et al. (2002). A MyTaqTM Red Mix Master Mix (BIOLINE®) was used for the reaction. The amplification program was carried out with the My Cycler thermocycler (BioRad®). This program included the steps of DNA denaturation at 95°C for 1 minute; 32 cycles of: denaturation at 95°C for 30 seconds, hybridization at 52°C for 30 seconds and extension at 72°C for 30 seconds; followed by incubation at 4°C.

Amplicon analysis and control was performed by horizontal electrophoresis in a 1.5% agarose gel and images were captured with a digital camera.

A part of the positive samples were sequenced by the biotechnology company Macrogene®. The sequences were edited and subsequently identified and compared with the sequences of both haplotypes present in Genbank® using the BLAST® tool.

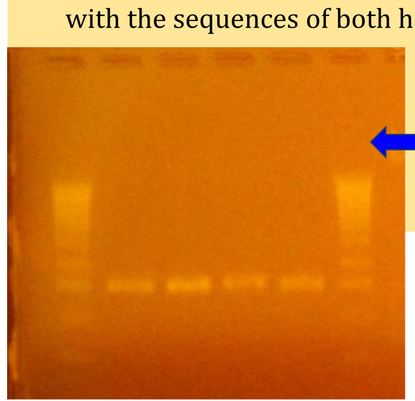


Fig. 1 Gel electrophoresis image showing positive results for Varroa spp.

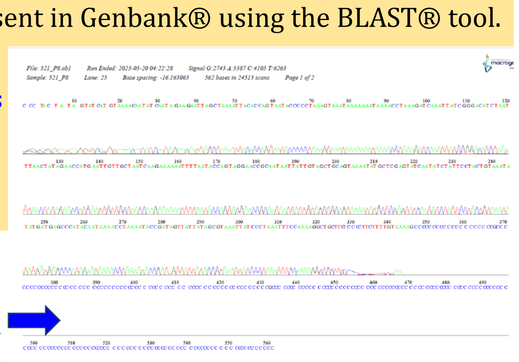


Fig. 2 Sequencing confirmation of *Varroa* spp.

• Results and discussions

Out of a total of 41 bee samples collected from 8 hives, 4 samples positive for *Varroa spp.* mites were identified by light microscopy methods representing 10% of the total samples collected.

By the polymerase chain reaction technique (PCR technique), out of a total of 4 samples from which DNA was extracted, 4 samples were confirmed positive for *Varroa* spp.

Analyzing the similarities with the GenBank isolates the most highest similarity found was with wit haplotype K

Maggi et al. (2012) also evaluated the genetic profile of the mite in 6 different provinces of Argentina, finding a 100% prevalence of the K haplotype.

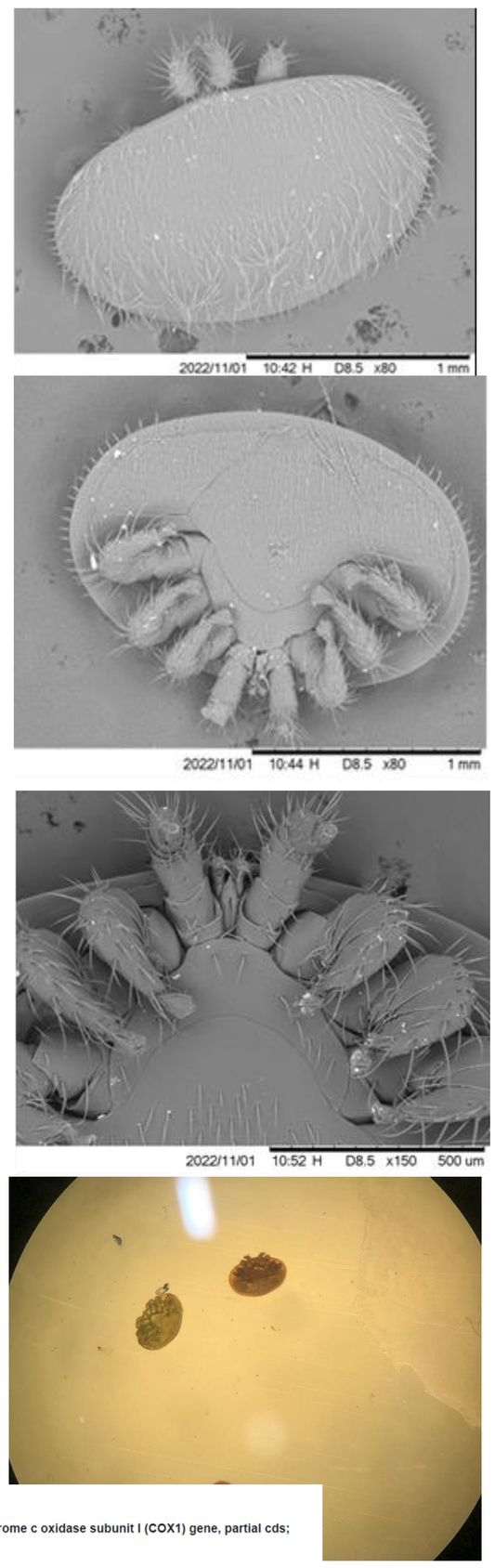
The same values were found by Anderson and Truemann (2000) in Uruguay, while Solignac et al. (2005) obtained 97.8% in Chile and, in contrast to results from other countries, only 40% prevalence of haplotype K in French Guiana. The presence of haplotype J in some regions may be mainly related to isolating factors such as geographical barriers, type of beekeeping, management offered to colonies and genetic pattern of bees.

In Europe, studies on *A. melifera* in summer/spring in hives without acaricide treatment showed the following prevalences: Bulgaria, 18-49%; southern England, 15-40% and 6-42%. In Estonia, a study was carried out in 2012-2013 on 196 hives. Kerli Mõtus et al. found that 193 of the 196 hives were invaded by the *Varroa destructor* mite, with an apparent prevalence of 98.5%. Out of 2332 colonies, 1841 showed mites in different stages of infestation. The resulting prevalence of infested colonies was 78.9%.

• Conclusions

Identification of *Varroa* spp. mites by classical methods was performed in 4 out of 41 samples evaluated showing a prevalence of 10%

Molecular identification established *Varroa destructor* as the etiological agent with haplotype K



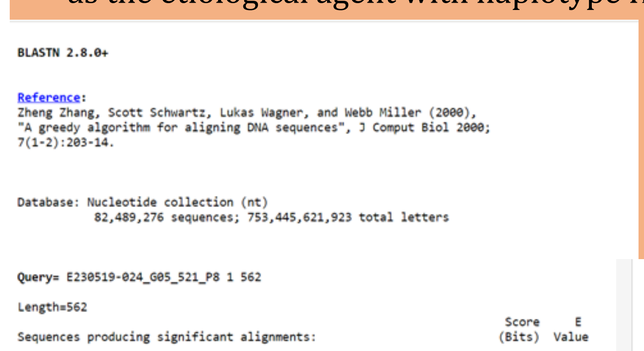


Fig. 3 Basic Local Alignment Search Tool analysis results of the obtained *Varroa destructor* sequences, showing homology against sequences producing significant alignments