

Short communication

HAPLOTYPE AFFILIATION OF *VARROA DESTRUCTOR* IN SELECTED LOCATIONS FROM POLAND AND UKRAINE

Piotr Dziechciarz¹ ORCID: 0000-0002-3013-8102Marcin Domaciuk² ORCID: 0000-0002-4294-9850Maria Iller³ ORCID: 0000-0003-0876-4542Stefan Kerek⁴Grzegorz Borsuk^{1*} ORCID: 0000-0002-8407-3383

¹Subdepartment of Apidologie, Institute of Biological Basis of Animal Production; Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Poland

²Department of Cell Biology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland, Poland

³Department of Parasitology and Invasive Diseases, University of Warmia and Mazury in Olsztyn, Poland

⁴Institute of beekeeping named after P.I. Prokopovich, National Scientific Centre, Ukraine

*corresponding author: grzegorz.borsuk@up.lublin.pl

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Abstract

The virulence patterns observed in viruses transmitted by *Varroa destructor* implied a potential association of these honey bee pests in Poland with the Korean haplotype. Nevertheless, it is noteworthy that this hypothesis lacked direct verification. Therefore, the aim of the study was to determine the haplotype affiliation of *V. destructor* mites infesting bees in Poland and Ukraine. Adult females were collected from drone brood from honey bee colonies maintained in the apiary at the University of Life Sciences in Lublin, Poland, and the apiary in Synevyr National Park in Ukraine. The haplotype affiliation was determined through the sequencing of the cytochrome oxidase subunit I gene (CO I; 929 bp) fragment. Based on the phylogenetic comparison with 84% bootstrap support, the *V. destructor* females from both locations were confirmed to represent three haplotypes: Korean 1 (AmK1-1), Korean 2 (AmK1-2), and Chinese 4 (AmK1-4). They are the most virulent types of this parasite worldwide and in Poland. Concurrently, the present study confirms that the Synevyr National Park in Ukraine is isolated from alien bee subspecies, but is not free from alien parasites.

Keywords: haplogroup, haplotype, phylogenetic analysis, *Varroa destructor*

INTRODUCTION

Currently, *Varroa destructor* mites are considered one of the main causes of Colony Collapse Disorder (CCD) and winter colony losses (Van Engelsdorp et al., 2008, 2009; Anderson et al., 2008; Cicero & Sammataro 2010; Conte et al., 2010; Guzman-Novoa et al., 2010; Noël et al., 2020). They are also biological vectors of viruses and bacteria that can damage the body integuments of bees and develop in their organisms (Oldroyd, 1999). Viruses and bacteria replicate in the salivary glands of mites (Brødsgaard et al., 2000; Ongus et al., 2004; Herrmann et al., 2005; Shen et al., 2005; Chen et al., 2006). Even a single mite is dangerous

to bees, as it can be a carrier of many potential pathogens (Chantawannakul et al., 2006). As suggested by Topolska based on the virulence of viruses transmitted by *V. destructor* females analyzed in 2001, mites parasitizing bees in Poland represent the Korean haplotype; however, this was not confirmed with the use of genetic tests.

The knowledge of all traits of *Varroa* mites is extremely important, as it may help to limit the destructive expansion of these mites in the future. Therefore, the aim of the study was to determine the haplotype affiliation of *Varroa destructor* mites occurring in Poland and Ukraine. An additional goal was to check whether the Synevyr

National Park in Ukraine (where the material was collected) is an area isolated from alien bee subspecies and is free from bee parasites.

MATERIAL AND METHODS

Collection of mites

Mites were collected from two locations: the apiary of the University of Life Sciences in Lublin, Poland (51° 13' N, 22° 38' E), and the Synevyr National Park in Ukraine (N 48° 51' N, 23° 64' E). The Synevyr National Nature Park is one of the National Parks in Ukraine; it is located in Zakarpattia Oblast in the southwest of the country. It covers an area of about 40 000 hectares. The Park contains a varied flora and fauna, including the Carpathian honeybee (*Apis mellifera carpatica*) (Safonkin et al., 2019). The Park is protected against the introduction of alien bee species. Since 2017, the Synevyr National Park has been included in the UNESCO World Heritage List as one of the massifs of beech primeval forests of the Carpathians („Synevyr National Nature Park”, 2020).

To find mature *V. destructor* females, combs with sealed drone brood were taken from each location, and two *V. destructor* females with a uniformly dark brown cuticle were selected from four colonies. The mites were placed individually in 1.5 ml Eppendorf tubes and frozen at -24°C. DNA was isolated from eight *V. destructor* females collected in Poland and Ukraine (4 mites from Poland and 4 from Ukraine).

DNA extraction and genetic analysis

After thawing, the mites were cut in half with scissors and subjected to DNA isolation. Lysis buffer (180 µL) and proteinase K (20 µL) were added to each tape segment for 24 h, and the total DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen) in accordance with the manufacturer's instructions.

The PCR analysis was performed using the Taq PCR Core Kit (Qiagen). In a total volume of 30 µL, the reaction mixture included 6 µL of the DNA sample, 1×PCR buffer, 1×Q solution, 4 mM of MgCl₂, 0.2 mM of each dNTP, 0.4 µM of each primer, and 2 U of Taq DNA polymerase. The

PCR conditions were as follows: 10 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55.8°C, and 45 s at 72°C, with final extension at 72°C for 7 min. The total DNA was analyzed by PCR using primers 10KbCOIF1 (CTTGTAATCATAAGGATATTGGAAC) and 6.5KbCOIR (AATACCAGTGGGAACCGC) as described by Navajas et al. (2010). The PCR products were electrophoresed on a 2% agarose gel and analyzed under UV light. The amplified PCR products were purified using the ExoSap-Exonuclease I and Shrimp Alkaline Phosphatase kit and sequenced with the use of Dye Terminator Cycle Sequencing Mix v3.1 in the ABI3730xl automatic DNA sequencer (Life Technologies). Gene fragment libraries were deposited in the National Center for Biotechnology Information (NCBI) database under numbers KU 953780 - KU 953787. All sequenced gene fragments were compared with sequences available in the NCBI (National Center for Biotechnology Information) database.

Statistical analysis

The genetic diversity of *V. destructor* females was calculated using the Molecular Evolutionary Genetics Analysis program, MEGA 4.0.2 (Tajima, 1989; Tamura et al., 2007). The phylogenetic analysis was performed using neighbor joining (NJ) algorithms (Tamura et al., 2007).

RESULTS AND DISCUSSION

Currently, mitochondrial DNA (mtDNA) tests distinguish two haplotypes of the *V. destructor* species parasitizing *A. mellifera*. The Korean (K) haplotype also referred to as Russian or ¼ Russian, is reported from Europe, North America, South Africa and the Middle East (Delfinado-Baker, 1988; Solognac et al., 2005; Ogihara et al., 2020). In turn, the Japanese haplotype (J) is detected in Japan, South America and Thailand. In Asia, eighteen haplotypes of *V. destructor* have been described, with eleven infesting *A. cerana* and seven parasitizing *A. mellifera* (Navajas et al., 2010; Ogihara et al., 2020). Based on the diversity of mtDNA

sequences in four fragments of cytochrome oxidase I and III (CO I, CO III), ATP synthase 6 (Atp 6), and cytochrome B (Cyt B) genes, Navajas et al. (2010) determined the phylogenetic relatedness of *V. destructor* mites infesting *A. cerana* and *A. mellifera* in Asia. Thus, the mites were divided into four separate clusters/haplogroups: Japanese, Korean, Chinese and Vietnamese.

All sequences of the cytochrome oxidase I (CO I; 929 bp) gene fragment from the *V. destructor* females collected in Poland and Ukraine were subjected to phylogenetic analysis. The comparison of the cytochrome oxidase I (CO I; 929 bp) gene fragments from the *V. destructor* females with the mite sequences available in the NCBI database showed 100% coverage and sequence identity of the cytochrome oxidase I (CO I). To illustrate the relatedness, the *V. destructor* mtDNA sequences were selected and presented on one phylogenetic tree (Fig. 1). The *V. destructor* mites originating from Poland and Ukraine were found to represent the Korean 1 AmK1 haplogroup. The phylogenetic comparison with 84% bootstrap support, i.e. the determination of the probability of the position of individual nodes in the phylogenetic tree, confirmed that the *V. destructor* females from both locations

represented three haplotypes: Korean 1 (AmK1-1), Korean 2 (AmK1-2), and Chinese 4 (AmK1-4). The sequencing of the cytochrome oxidase I (CO I; 929 bp) gene fragment allowed the precise determination of the haplogroup of the *V. destructor* females and the haplotypes of these mites collected in Poland and Ukraine (Fig. 1). The Korean (K) *Varroa* haplotype has spread worldwide and is the most virulent type of all the other haplotypes of these mites (Delfinado-Baker, 1988; De Guzman et al., 1999; Anderson & Trueman, 2000; Rosenkranz et al., 2010). Hence, *Varroa* mites are still considered the main cause of bee colony collapse (Van Englesdorp et al., 2008, 2009; Anderson et al., 2008; Cicero & Sammataro, 2010; Conte et al., 2010; Guzman-Novoa et al., 2010). This suggests that *Varroa*-vectored viruses are becoming more virulent (Traynor et al., 2020). Therefore, the *V. destructor* females collected in Poland and Ukraine can be clearly classified into the Korean 1 AmK1 haplogroup. Based on the cytochrome oxidase I (CO I; 929 bp) gene fragment used in the phylogenetic comparison (Muntaabski et al., 2020), the mites were confirmed to represent three haplotypes: Korean 1 (AmK1-1), Korean 2 (AmK1-2), and Chinese 4 (AmK1-4).

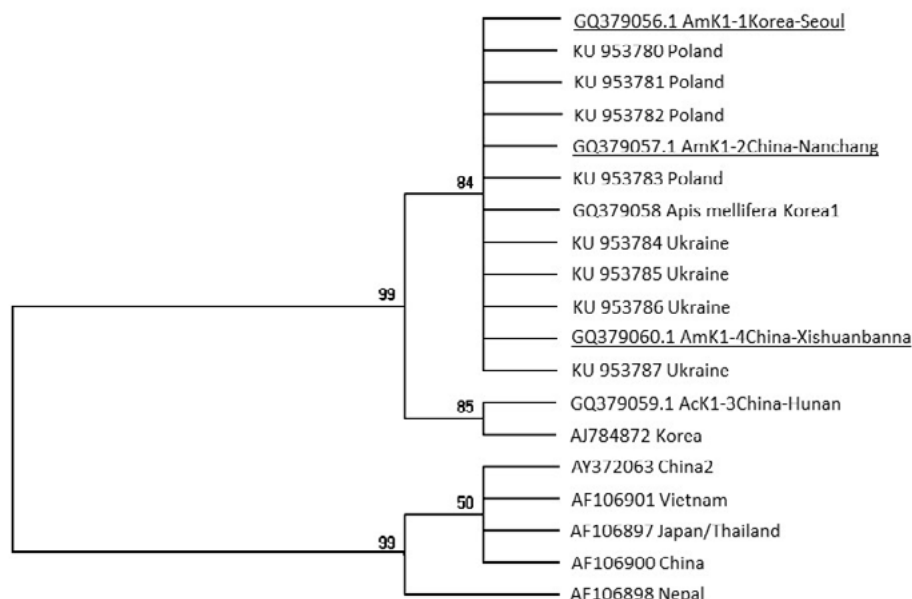


Fig. 1. Phylogenetic comparison based on sequencing of the CO I gene fragment (CO I, 929 bp) from *Varroa destructor* mites collected in Poland and Ukraine with haplotype sequences available in the NCBI database.

CONCLUSION

The genetic control of haplotype affiliation is important in the combat against *Varroa*, which is one of the most virulent types of this parasite worldwide and in Poland. The determination of the haplotype affiliation will help in the future to establish the migration transects of *Varroa* mites and the routes of their dissemination worldwide. Concurrently, the present study confirms that the Synevyr National Park in Ukraine (where the material was collected) is isolated from alien bee subspecies, but is not free from alien parasites. This is probably possible due to the uncontrolled migratory management and the introduction/substitution of alien bee colonies with alien parasites into the Park. The colonies have been removed but the parasites have spread and remained in the colonies maintained in the Synevyr National Park.

Therefore, these mites cannot be ignored (Noël et al., 2020), their presence in bee colonies should be monitored systematically, and the control of this pest should be designed precisely.

REFERENCES

- Anderson, D., East, I. J., Cox-Foster, D., Conlan, S., Holmes, E. C., Palacios, G. (2008). The latest buzz about colony collapse disorder. *Science*, 319, 724-725. <https://doi.org/10.1126/science.319.5864.724c>
- Anderson, D., Trueman, J., W. H. (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*, 24(3), 165-189. <https://doi.org/10.1023/a:1006456720416>
- Brødsgaard, C. J., Ritter, W., Hansen, H., Brødsgaard, H. F. (2000). Interactions among *Varroa jacobsoni* mites, acute paralysis virus, and *Paenobacillus larvae larvae* and their influence on mortality of larval honeybees *in vitro*. *Apidologie*, 31(4), 543-554.
- Chantawannakul, P., Ward, L., Boonham, N., Brown, M. (2006). A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in *Varroa* mites collected from a Thai honeybee (*Apis mellifera*) apiary. *Journal of Invertebrate Pathology*, 91(1), 69-73. <https://doi.org/10.1016/j.jip.2005.11.001>. Epub 2005 Dec 22.
- Chen, Y. P., Evans, J., Feldlaufer, M. (2006). Horizontal and vertical transmission of viruses in the honeybee, *Apis mellifera*. *Journal of Invertebrate Pathology*, 92, 152-159. <https://doi.org/10.1016/j>
- Cicero, J. M., & Sammartaro, D. (2010). The salivary glands of adult female *Varroa destructor* (Acari: Varroidae), an ectoparasite of the honey bee, *Apis mellifera* (Hymenoptera: Apidae). *International Journal of Acarology*, 36(5), 377-386. <https://doi.org/10.1080/01647951003757961>
- Conte, Y. L., Ellis, M., Ritter, W. (2010). *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? *Apidologie*, 41(3), 353-363. <https://doi.org/10.1051/apido/2010017>
- De Guzman, L. I., Rinderer, T. E., Stelzer, J. A. (1999). Occurrence of two genotypes of *Varroa jacobsoni* Oud. in North America. *Apidologie*, 30(1), 31-36. <https://doi.org/10.1051/apido:19990104>
- Delfinado-Baker, M. (1988). Variability and biotypes of *Varroa jacobsoni* Oudemans. *American Bee Journal*, 128, 567-568.
- Guzman-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P. G., Correa, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, 41(4), 443-450. <https://doi.org/10.1051/apido/2009076>.
- Herrmann, M., Kanbar, G., Engels, W. (2005). Survival of honey bee (*Apis mellifera*) pupae after trypan blue staining of wounds caused by *Varroa destructor* mites or artificial perforation. *Apidologie*, 36(1), 107-111. <https://doi.org/10.1051/apido:2004074>

- Muntaabski, I., Russo, R. M., Liendo, M. C., Palacio, M. A., Cladera, J. L. Lanzavecchia, S. B., Scannapieco, A. C. (2020). Genetic variation and heteroplasmy of *Varroa destructor* inferred from ND4 mtDNA sequences. *Parasitology Research*, 119, 411-421. <https://doi.org/10.1007/s00436-019-06591-5>
- Navajas, M., Anderson, D. L., de Guzman, L. I., Huang, Z. Y., Clement, J., Zhou, T., Le Conte, Y. (2010). New Asian types of *Varroa destructor*: a potential new threat for world apiculture. *Apidologie*, 41(2), 181-193. <https://doi.org/10.1051/apido/2009068>
- Noël, A., Le Conte Y., Mondet, F. (2020). *Varroa destructor*: how does it harm *Apis mellifera* honey bees and what can be done about it? *Emerging Topics in Life Sciences*, 4, 45-57. <https://doi.org/10.1042/ETLS20190125>
- Ogihara, M. H., Yoshiyama, M., Morimoto, N., Kimura, K. (2020). Dominant honeybee colony infestation by *Varroa destructor* (Acari: Varroidae) K haplotype in Japan. *Applied Entomology and Zoology*, 55, 189-197. <https://doi.org/10.1007/s13355-020-00667-w>
- Oldroyd, B. P. (1999). Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honey bees. *Trends in Ecology & Evolution*, 14(8), 312-315. [https://doi.org/10.1016/S0169-5347\(99\)01613-4](https://doi.org/10.1016/S0169-5347(99)01613-4)
- Ongus, J. R., Peters, D., Bonmatin, J. M., Bengsch, E., Vlak, J. M., van Oers, M. M. (2004). Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite *Varroa destructor*. *Journal of General Virology*, 85(12), 3747-3755. <https://doi.org/10.1099/vir.0.80470-0>
- Reich, S. E., Fuchs, S., Schulz, A., Urfer, W. (1998). Geometric approximation of the infestation of honey bee brood cells by *Varroa jacobsoni* and implications for the estimation of brood infestation, for population models and for the proportion of non-sibling matings. *Journal of Apicultural Research*, 37(2), 115-123. <https://doi.org/10.1080/00218839.1998.11100963>
- Rosenkranz, P., Aumeier, P., Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology*, 103, 96-119. <https://doi.org/10.1016/j.jip.2009.07.016>
- Safonkin, A. F., Triseleva, T. A., Bykova, T. O. (2019). Intraracial Diversity of the Carpathian Race of Honeybees *Apis mellifera carpatica*. *Biology Bulletin*, 46, 492-499. <https://doi.org/10.1134/S1062359019050091>
- Shen, M. Q., Yang, X. L., Cox-Foster D., Cui, L. W. (2005). The role of *Varroa* mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology*, 342, 141-149. <https://doi.org/10.1016/j.virol.2005.07.012>
- Solignac, M., Cornuet, J. M., Vautrin, D., Conte, Y. L., Anderson, D., Evans, J., Cros-Arteil, S., Navajas, M. (2005). The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honeybee (*Apis mellifera*), are two partly isolated clones. *Proceedings of the Royal Society Biological Sciences*, 272(1561), 411-419. <https://doi.org/10.1098/rspb.2004.2853>
- „Synevyr National Nature Park” (2020). Official Park Site. Retrieved May 31.
- Tajima, F. (1989). Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. *Genetics*, 123(1), 585-595. <https://doi.org/10.1093/genetics/123.3.585>
- Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24(8), 1596-1599. <https://doi.org/10.1093/molbev/msm092>
- Topolska, G. (2001). *Varroa destructor* (Anderson and Trueman, 2000); the change in classification within the genus *Varroa* (Oudemans, 1904). *Wiadomości Parazytologiczne*, 47(1), 151-155.
- Traynor, K. S., Mondet, F., Miranda, J. R., Techer,

- M., Kowallik V., Oddie, M. A. Y., Chantawannakul, P., McAfee, A. (2020). *Varroa destructor*: A Complex Parasite, Crippling Honey Bees Worldwide, *Trends Parasitology*, 36(7), 592-606. DOI: 10.1016/j.pt.2020.04.004
- Van Engelsdorp, D., Hayes, J., Undwerwood, R. M., Petis, J. (2008). A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PloS One*, 3(12), 4071. <https://doi.org/10.1371/journal.pone.0004071>
- Van Engelsdorp, D., Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., ... Pettis, J. S. (2009). Colony collapse disorder: a descriptive study. *PLoS One*, 4(8), 6481. <https://doi.org/10.1371/journal.pone.0006481>