Short communication

FIRST REPORT ON *TROPILAELAPS MERCEDESAE* PRESENCE IN GEORGIA: THE MITE IS HEADING WESTWARD!

Irakli Janashia[1]* ORCID: 0000-0002-4312-9133
Aleksandar Uzunov[2,3] ORCID: 0000-0003-1240-868X
Chao Chen[2,3] ORCID: 0000-0002-9582-1105
Cecilia Costa[4] ORCID: 0000-0001-9985-2729
Giovanni Cilia[4] ORCID: 0000-0002-5234-1240

[1]Institute of Entomology, Agricultural University of Georgia, Tbilisi, Georgia
[2]State Key Laboratory of Resource Insects, Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing, China
[3]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Macedonia
[4]CREA Research Centre for Agriculture and Environment, Bologna, Italy

*corresponding author: i.janashia@agruni.edu.ge
Received: 22 July 2024; accepted: 12 September 2024

Abstract

The *Tropilaelaps* spp. (Mesostigmata: Laelapidae), an ectoparasitic mite originally associated with such Asian giant honey bees as *Apis dorsata*, *A. breviligula* and *A. laboriosa*, has increasingly become a focus of global concern due to its severe effects on Western honey bee colonies (*Apis mellifera*) and its recent geographic expansion. This study documents the first reported presence of *Tropilaelaps mercedesae* in Western Georgia's Samegrelo-Zemo Svaneti region, specifically in seven honey bee colonies (*A. mellifera caucasica*) from three apiaries. We conducted brood sample inspections, DNA barcoding and morphological measurements to confirm mite identification. Our findings revealed high infestation rates of *T. mercedesae*, co-infestation with *Varroa destructor* and notable mite reproductive success. These results underscore the threat posed by *T. mercedesae* to Georgian apiculture and highlight the potential for further spread across Europe. Immediate action and vigilant monitoring by national and international authorities are crucial to mitigate the impact on beekeeping and agriculture.

Keywords: beekeeping, ectoparasitic mite, Georgia, honey bees, invasive species, *Tropilaelaps*

INTRODUCTION

The ectoparasitic mite *Tropilaelaps* spp. (Mesostigmata: Laelapidae) was originally hosted by the Asian giant honey bees *Apis dorsata*, *A. breviligula,* and *A. laboriosa* (Anderson & Roberts, 2013; Chantawannakul et al., 2018). It has been gaining the attention of beekeepers and researchers worldwide because of the well-documented devastating effects on Western honey bee colonies (*Apis mellifera*) when not controlled (Anderson & Roberts, 2013) and recent invasions into new territories. The species *T. mercedesae* was recently reported to have spread to Central Asia, Uzbekistan (Namin et al., 2024) and even more worryingly to have reached the European continent in southwestern Russia's Krasnodar and Rostov regions (Brandorf et al., 2024). Given that the life cycle, biology, reproduction and pathology of *Tropilaelaps* spp. are comparable to those of *Varroa destructor* (de Guzman et al., 2017), the expected scenario of west and southward spread is greatly alarming for beekeepers and authorities in Europe. This study aims to address this emerging threat by documenting the first reported case of *Tropilaelaps mercedesae* infestation in Georgia.

MATERIALS AND METHODS

In summer 2024, following an anecdotal verbal report (personal communication) and suspicions from a beekeeper in the region of Samegrelo-Zemo Svaneti in Georgia, seven (N=7) honey bee colonies (*A. mellifera caucasica*) from three

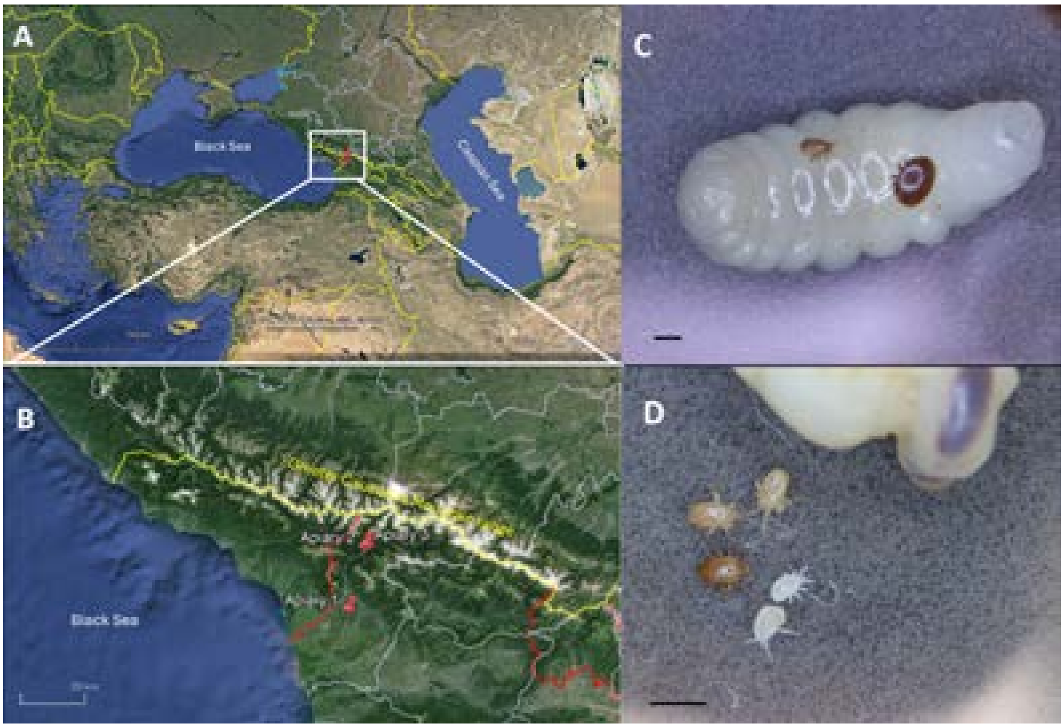

Fig. 1. A regional perspective of the sampled apiaries (A), apiary locations in Georgia (B), pupa from the collected brood samples with *T. mercedesae* and *V. destructor* adult females; bar=1 mm (C), *T. mercedesae* foundress (dark brown) with progeny in different stages bar=1 mm (D).

(N=3) apiaries (Fig. 1) were sampled for sealed worker brood to check for the presence of *Tropilaelaps* spp. A section of the brood area (5x6 cm) containing pupae cells was cut from the comb, placed in plastic zip bags and transported to the laboratory within two hours. The sealed brood samples were frozen and kept at -20°C to prevent accidental mite dispersal and to immobilise the mites for efficient and precise counting, as *Tropilaelaps* spp. mites are known to move very fast. With the use of binocular-type stereo microscope Carl Zeiss Stemi 508 magnification x6.3, at least 100 sealed worker brood cells per brood sample were opened and individually inspected for the assessment of mite presence, reproductive status of the foundress mites and recapping rate for both ectoparasitic mites species, *Tropilaelaps* spp. and *V. destructor* (Tab. 1). In addition, ten mites were measured with the use of an ocular micrometre. Mites collected during the inspections were stored in 99% ethanol. A pool of eight mites was used for DNA barcoding, amplifying the cytochrome c oxidase subunit I (COI) region of mtDNA (Folmer et al., 1994). The mites were frozen with the use of liquid nitrogen and crushed to a fine powder. The powder was resuspended into 150 µl of phosphate-buffered saline (PBS) and DNA was extracted with the use of the commercial kit Quick DNA Microprep Plus Kit (Zymo Research, Irvine, CA, USA) as previously reported (Tiritelli et al., 2024). The COI region was amplified with the use of HotStarTaq Polymerase (Qiagen, Hilden, Germany), and the PCR assay was performed on Applied Biosystems®2720 Thermal Cycler (ThermoFisher Scientific) according to Selis et al. (2024). The obtained amplicons were visualized on a 1.5% agarose gel, purified with the use of ExoSAP-IT Express (ThermoFisher Scientific, Waltham, MA, USA) and sequenced through Sanger methodology with SeqStudio™ (ThermoFisher Scientific) (Cilia et al., 2022).

RESULTS AND DISCUSSION

The obtained sequences (563bp) confirmed that the samples belong to *Tropilaelaps mercedesae*, and BLAST analysis showed high similarity (96.80%) with specimens from Russia (Accession Numbers OR965215.1), Thailand (KY865193.1), China (NC_082931.1) and India (OK188792.1) and 96.61% from Uzbekistan (OR165785).

Table 1.

Results of inspection of brood samples (N=7) from three (N=3) apiaries: single and multispecies infestation level by *T. mercedesae* or/and *V. destructor* in individual cells, rate of mites in reproductive status and recapping rate for both ectoparasitic mite species

Sample	**Apiary (altitude)**	**Inspected sealed worker brood cells**	***T. mercedesae* infestation in % (% reproductive*)**	***V. destructor* infestation in % (% reproductive*)**	**Co-infestation in % (% reproductive *, V = *V. destructor*, T = *T. mercedesae*)**	**Total infestation (%)**	**Recapping % (V = *V. destructor*, T = *T. mercedesae*, C = combined)**
1	1 (278)	100	19 (89.5)	14 (78.6)	1 (0)	32	6 (V - 2 / T - 4)
2		100	24 (95.8)	13 (100.0)	5 (V - 100 / T - 80)	32	5 (V - 1 / T - 2 / C -2)
3		100	14 (100.0)	5 (80.0)	1 (V - 100 / T - 100)	17	0 (0.0)
4		100	18 (100.0)	9 (100.0)	5 (V - 100 / T - 100)	22	2 (T -1 / C - 1)
5		100	10 (100.0)	5 (100.0)	0 (0.0)	15	0 (0.0)
6	2 (500)	300	1 (100.0)	0 (0.0)	0 (0.0)	1	0 (0.0)
7	3 (550)	100	7 (100)	14 (85.6)	0 (0.0)	20	1 (T - 1)

*Reproductive status of the foundress mites. Percentage of infested cell/foundress with offspring.

The sequence was uploaded to Genbank: PQ049741.

Six of the seven colonies harboured both ectoparasitic mite species. In the nine hundred (N=900) inspected sealed worker brood cells, the total sample infestation level ranged from 1 to 32% (Tab. 1). We found a higher infestation by *T. mercedesae,* with an average of 13.3% (1-24%) than by *V. destructor,* with an average of 8.6% (0-14%), in agreement with the study by Buawangpong et al. (2015) in Thailand. A similar trend was observed for mite reproduction with 97.9% of the *T. mercedesae*-infested cells found to have offspring and a slightly lower reproduction rate (90.7%) for *V. destructor.*

Co-infestation of individual bee pupae was observed in four of the seven brood samples and ranged from 0 to 5%, higher than in reports from Thailand (<0.1%) but lower than that observed in South Korea (26%) (Buawangpong et al., 2015; de Guzman et al., 2017). We also assessed the recapping rate, which ranged from 0 to 6%, with no clear differences between the cells infested with a single species or co-infested. Hygienic behaviour of *Apis* spp. as a mechanism of reducing infestation by *Tropilaelaps* spp. has been reported, but no study has specifically measured the recapping rate in naturally infested cells, though uncapped ("bald") brood has been described in the presence of *T. mercedesae* (de Guzman et al., 2017). In addition, the morphometric analysis of the mites shows that the mites' length was 0.91±0.01 mm (mean±SE) and width 0.49±0.01 mm (mean±SE). This size is in agreement with the "summer mites" described in the study by Brandorf et al. (2024).

Overall, our results show that *T. mercedesae* successfully, and with high reproductive rates, infests honey bee colonies (*A. m. caucasica*) in Georgia, where the mite is reported for the first time.

The presence of *T. mercedesae* in the South Caucasus is not surprising, due to recent reports confirming the presence of the mite in the North Caucasus (Brandorf et al., 2024). If beekeeping migration routes and operations are ignored, it is probable that *T. mercedesae* spread through the subtropical zone of the Black Sea coast via the region of Abkhazia. An invasion directly from the northern Caucasus is less likely, as the Greater Caucasian mountain chain, with peaks over 5,000 meters altitude, is a considerable geographic obstacle for the honey bees. Another invasion route could be via northern Iran (Shahrouzi, accessed 24 July 2024) but other sources denied *Tropilaelaps* spp. presence in Iran (Moshaverinia et al., 2013) and, to our knowledge, there have been no reports of its presence, even anecdotal, in South Georgia, Armenia and Azerbaijan. Instead, Namin et al. (2024) confirmed *T. mercedesae* in Uzbekistan from where two routes around the Caspian Sea could be feasible. In any case, an introduction via traded honey bee materials, such as bee swarms and packages, nuclei or queens with attendant bees, using various transportation means, is a possible scenario. Considering the anecdotal reports as well as our results, according to which all sampled colonies were infested, some with high mite loads, *T. mercedesae* seems to have been present in northwest Georgia at least since the last season.

The intensive migratory operations in Georgia may contribute to the rapid dispersion of the mites in different regions. Its diverse climatic zones, which include subtropical areas, are likely to contribute to the establishment of *Tropilaelaps* mite populations at least in these regions. Due to climate change and the rise in average annual temperatures, which prolongs the brood-rearing season almost all year round in many regions, it is also probable that the mite will establish populations in other zones. Finally, besides the natural dispersal of honey bees, the cross-border cooperation and possible exchange of queens between the Georgian and Turkish beekeepers open the possibility for penetration of *Tropilaelaps* spp. in Turkey, where the mite is not yet reported. Considering *T. mercedesae*'s pathology and epidemiology (de Guzman et al., 2017; Truong et al., 2023), and the capability of co-infestation with *V. destructor*, European beekeeping is evidently under significant threat and risk. Therefore, we urge and alert the national and international competent authorities to be vigilant and further monitor honey bee colonies to slow down invasion by *T. mercedesae* and to be prepared with effective eradication measures. History is repeating itself, and unlike the case of *V. destructor*, now the European community should be prepared and counteract. Training of beekeepers, extensionists and veterinarian services is required for an efficient and timely response to mitigate damages to the environment and the agricultural production that the spread of this ectoparasitic mite may cause.

ACKNOWLEDGEMENT

We thank Audrey Meazza (trainee at CREA from the University of Purpan) for her support with the measurements.

AUTHOR CONTRIBUTION

AU developed the study's conception and design. Fieldwork and laboratory analysis were performed by IJ, and mtDNA assignment was done by GC. IJ and AU share first authorship, with AU writing the first draft of the manuscript. All authors commented on previous versions and read and approved the final manuscript.

FUNDING

The mtDNA analysis assignment was funded by project GENAPIS.IT.2 funded by Italian Ministry of Agriculture under REG. UE 2021/2115 (CAMPAGNA 2024 N. 43705030161). All other costs and expenses were privately covered by the authors.

DATA AVAILABILITY

Sequences were submitted to Genbank under the accession number PQ049741.

Code availability
Not applicable

DECLARATIONS

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.
Consent to participate Not applicable.
Consent for publication Not applicable.
Conflict of interest The authors declare no competing interests.

REFERENCES

Anderson, D L., & Morgan, M J. (2007). Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (Acari: Laelapidae): new and re-defined species. *Experimental and Applied Acarology, 43*(1), 1-24. DOI: 10.1007/s10493-007-9103-0

Anderson, D. L., & Roberts, J. M. K. (2013). Standard methods for *Tropilaelaps* mite research. In: The COLOSS BEEBOOK, Volume II: Standard methods for *Apis mellifera* pest and pathogen research, Dietemann V., Ellis J.D., Neumann P., eds. *Journal of Apicultural Research, 52*(4), 1–16. https://doi.org/10.3896/IBRA.1.52.4.21

Brandorf A., Ivoilova, M. M., Yañez, O., Neumann, P., Soroker, V. (2024). First report of established mite populations, *Tropilaelaps mercedesae*, in Europe. *Journal of Apicultural Research*, 1-3 DOI: 10.1080/00218839.2024.2343976

Buawangpong, N., de Guzman, L. I., Khongphinitbunjong, K., Frake, A. M., Burgett, M., Chantawannakul, P. (2015). Prevalence and reproduction of *Tropilaelaps mercedesae* and *Varroa destructor* in concurrently infested *Apis mellifera* colonies. *Apidologie, 46*, 779-786. DOI: 10.1007/s13592-015-0368-8

Chantawannakul, P., Ramsey, S., Vanengelsdorp, D., Khongphinitbunjong, K., Phokasem, P. (2018). *Tropilaelaps* mite: An emerging threat to European honey bee. *Current Opinion in Insect Science, 26*, 69-75. https://doi.org/10.1016/j.cois.2018.01.012

Cilia, G., Flaminio, S., Quaranta, M. (2022). A novel and non-invasive method for DNA extraction from dry bee specimens. *Scientific Reports, 12*, 11679. https://doi.org/10.1038/s41598-022-15595-8

de Guzman LI, Williams GR, Khongphinitbunjong K, Chantawannakul P. (2017). Ecology, life history, and management of *Tropilaelaps* mites. *Journal of Economic Entomology, 110*(2), 319-332. DOI: 10.1093/jee/tow304. PMID: 28334185

Folmer, O., Black, M., Hoeh W., Lutz R., Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299

Moshaverinia, A., Abedi, V., Safaei, H. (2013). Mite infestation of honey bee (*Apis mellifera*) in apiaries of North East of Iran. *Scientia Parasitologica, 14*(1), 31-35.

Namin, M. S., Joharchi, O., Aryal, S., Thapa, R., Kwon, S.-H., Kakhramanov, B. A., Jung, C. (2024). Exploring genetic variation and phylogenetic patterns of *Tropilaelaps mercedesae* (Mesostigmata: Laelapidae) populations in Asia. *Frontiers in Ecology and Evolution, 12*. 1-8. https://doi.org/10.3389/fevo.2024.1275995

Shahrouzi, R. Natural and chemical control of *Varroa destructor* and *Tropilaelaps mercedesae* in Afghanistan. Retrived July 22, 2024, from https://www.apiservices.biz/documents/articles-en/natural_chemical_control_%20of_varroa.pdf

Tiritelli, R., Flaminio, S., Zavatta, L., Ranalli, R., Giovanetti, M., Grasso, ... Cilia, G. (2024). Ecological and social factors influence interspecific pathogens occurrence among bees. *Scientific Reports, 14*(1), 5136.

Truong, A. T., Yoo, M. S., Yun, B. R., Kang, J. E., Noh, J., Hwang, T. J., ... Cho, Y. S. (2023). Prevalence and pathogen detection of *Varroa* and *Tropilaelaps* mites in *Apis mellifera* (Hymenoptera, Apidae) apiaries in South Korea. *Journal of Apicultural Research, 62*(4), 804-812. DOI:10.1080/002188392021.2013425