Original Article

FIRST EVIDENCE OF PRESENCE OF *VARROA UNDERWOODI* MITES ON NATIVE *APIS CERANA* COLONIES IN PRIMORSKY TERRITORY OF RUSSIA BASED ON *COX1* GENE

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Abstract

The species of genus *Varroa* mites parasitize on the honey bees of genus *Apis*. Unlike the well-studied *V. destructor* and *V. jacobsoni* mites, *V. underwoodi* remain less known. According to English language publications, the proven *V. underwoodi* distribution area of *A. cerana* colonies covers Nepal, South Korea, Indonesia, Papua New Guinea, Vietnam and China, but not Russia even though it had been described morphometrically in Russian language publications in Russia's Primorsky Territory. According to Vavilov's law (1920) of a homologous series, all the species of *V. underwoodi, V. destructor* and *V. jacobsoni* have the ability to spill over onto new hosts. Thus, *V. underwoodi* is a potential parasite of *A. mellifera* that should be carefully studied. In this study, *V. underwoodi* mites in colonies of honey bee subspecies *A. c. ussuriensis* native to Russia's Primorsky Territory are first proven using both morphometry and mitochondrial *COX1* gene sequencing. The genetic divergence and p-distances between *V. underwoodi* and other *Varroa* species ranged from 7 to 10% and from 0.072 to 0.099, respectively, which matched the intraspecific level of differences. Two identical northernmost *V. underwoodi* samples from Russia's Primorsky Territory and China's Jilin province with GenBank accession number MH205176 were assigned as *COX1* haplotype China 1 MH205176. The first discovery of *V. underwoodi* in the Primorsky Territory in northern Asia outlined the northern border of its range.

Keywords: *Apis cerana*, *COX1* gene, haplotype, Primorsky Territory of Russia, range, *Varroa underwoodi*

INTRODUCTION

In modern beekeeping, honey bee colonies are moved over long distances to improve crop pollination and increase the honey harvest. Honey bees are introduced to ecosystems beyond their natural distribution range and frequently exposed to new pathogens and parasites that they have never encountered before, including ectoparasitic mites of the genus *Varroa* (Varroidae) represented by at least four species (Anderson & Trueman, 2000; Rosenkranz et al., 2010). The first species, *Varroa jacobsoni* Oudemans 1904, originally parasitized on

A. cerana on Java island of Indonesia (Oudemans, 1904) for the first time spilled over to *Apis nigrocincta* in Indonesia (Hadisoesilo & Otis, 1998; Anderson & Trueman, 2000) and to *Apis mellifera* in Papua New Guinea (Roberts et al., 2015). The second species, *Varroa destructor* Anderson & Trueman 2000, originally parasitized on *Apis cerana* in China, Japan, Korea, and Thailand for the first time spilled over to *Apis mellifera* in Japan (Beaurepaire et al., 2015). The third species, *Varroa underwoodi* Delfinado-Baker & Aggarwal, 1987, originally parasitized on *Apis cerana* in Nepal, for the first time spilled over to *A. nigrocincta* in Indonesia (Delfinado-Baker & Aggarwal, 1987; Anderson et al., 1997; Kuznetsov, 2005). The fourth species, *Varroa rindereri* de Guzman & Delfinado-Baker, 1996, originally parasitized on *Apis koschevnikovi* on the Borneo island of Malaysia (de Guzman & Delfinado-Baker, 1996) does not have any spillover events to other host species yet.

The honey bee *A. mellifera* is predominantly parasitized by *V. destructor* worldwide (Traynor et al., 2020), and by *V. jacobsoni* in Papua New Guinea (Roberts et al., 2015). Several mitochondrial *V. destructor* haplotypes have been described, but only two of them - Korean (K) and Japanese (J) are capable of reproducing in *A. mellifera* colonies (Anderson, 2000; Anderson & Trueman, 2000; Muñoz et al., 2008). Up to now, two *Varroa* species - *V. rindereri* and *V. underwoodi* have been poorly studied (Oudemans, 1904; Delfinado-Baker & Aggarwal, 1987; de Guzman & Delfinado-Baker, 1996; Anderson et al., 1997; Rath, 1999; Anderson & Trueman, 2000; Wang et al., 2019).

The distribution area of *V. underwoodi* is constantly expanding and spans populations of *A. cerana* in Nepal (Delfinado-Baker & Aggarwal, 1987), South Korea (Woo, 1992; Chantawannakul et al., 2016), Indonesia (Anderson et al., 1997; Chantawannakul et al., 2016), Papua New Guinea (Lee, 1995; Anderson et al., 1997; Chantawannakul et al., 2016), Vietnam (de Guzman & Rinderer, 1999; Chantawannakul et al., 2016) and China (de Guzman Rinderer, & 1999; Huang, 2004; Chantawannakul et al., 2016; Wang et al., 2019). Russia was not listed as a proven distribution area for *V. underwoodi* according to English language publications (Chantawannakul et al., 2016; Wang et al., 2019), even though Russian language publications had been previously described morphometrically from feral *A. cerana* population in the Primorsky Territory (Kuznetsov, 2005; Kuznetsov & Lelej, 2005).

Along with the expansion of the area of *V. underwoodi*, the number of its host species has increased: *A. cerana* in Nepal (Delfinado-Baker & Aggarwal, 1987), *Apis nuluensis* in Malaysia (Delfinado-Baker & Aggarwal, 1987; de Guzman et al., 1996; Anderson et al., 1997), *A. nigrocincta* in Indonesia (Anderson et al., 1997; Hadisoesilo, 1997), *A. mellifera* in Papua New Guinea (Lee, 1995; Anderson et al., 1997; de Guzman & Rinderer, 1999). Although evidence for *V. underwoodi* reproduction was only found in *A. cerana*, the frequent reports on this mite species appearing in the colonies of other species suggested abundant opportunities for cross-species transmission. *V. underwoodi* is especially dangerous to *A. mellifera* colonies because they are kept near *A. cerana* colonies in most Asian countries (Zheng et al., 2011, 2018; Chantawannakul et al., 2016; Wang et al., 2019; Roberts et al., 2020).

Varroa species haplotypes have different virulence for host species and only K and J of the *V. destructor* six haplotypes are capable of reproducing on *A. mellifera* (Anderson, 2000; Anderson & Trueman, 2000; Muñoz et al., 2008). Similarly, a high level of genetic diversity of *V. underwoodi* (Navajas et al., 2010; Roberts et al., 2015; Wang et al., 2019) allows several haplotypes to form with various virulences for host species, including honey bee *A. mellifera*. Besides, the manifestation of similar traits in related species is supported by the law of homologous series (Vavilov, 1920).

Thus, honey bee *A. mellifera* is a potential host species for *V. underwoodi* in its further evolution (Anderson, 2000; Anderson & Trueman, 2000; Muñoz et al., 2008). The potential factors for *V. underwoodi* to become a parasite of honey bee *A. mellifera* must be investigated to mitigate their further negative effects and prevent future invasions (Thompson, 1994; de Guzman &

Rinderer, 1999; Kolar & Lodge, 2001; Woolhouse et al., 2005). In this study, we used morphometry and mitochondrial *COX1* gene sequences to prove the presence of *V. underwoodi* on the northernmost feral *A. cerana* subspecies *Apis cerana ussuriensis* inhabiting Russia's Primorsky Territory.

MATERIAL AND METHODS

The adult *Varroa underwoodi* mites were collected in 70% ethanol during summer 2004 from brood cells of two managed *Apis cerana ussuriensis* colonies in the village Romashka of the Khasansky district, the Primorsky Territory (43.5N, 131.3E). The reproduction ability of *V. underwoodi* in *A. cerana* colonies was assumed when 5-6 adult females and 2-3 nymphs were found together in drone brood cells. All collected mites were subsequently stored in 70% ethanol at -20°C until needed for further analyses. *V. underwoodi* mites were exclusively found in *A. cerana* colonies, where the colony infestation rate was 50%. The drone brood infestation rate was 2.8% in June, 35% in July, 58% in August. The worker brood infestation rate was 1%.

For a preliminary confirmation of species identity, the morphometrics and size of adult *V. underwoodi* female mites (N=10) were compared with previous reports (Delfinado-Baker & Aggarwal, 1987; Woo, 1992; Anderson et al., 1997; Huang, 2004; Wang et al., 2019). The sampled *V. underwoodi* mites were dried with ethanol at room temperature for one minute. The size of the dorsal shield with lateral setae was used to characterize *V. underwoodi* (Delfinado-Baker & Aggarwal, 1987; Woo, 1992; Anderson et al., 1997; Huang, 2004; Wang et al., 2019). The morphometry of each individual was measured with an EOS Kiss X7 digital microscope (Canon, Japan) with the lens MP-E 65mm f/2.8 1-5x Macro Photo (Canon, Japan) under 150x magnification according to the manufacturer's instruction.

The total DNA of *A. c. ussuriensis* was extracted from three mites per colony according to Qiagen DNEasy protocol for animal tissue (Qiagen, Valencia, Ca.). A mitochondrial *COX1* gene sequence was used to identify mite species as *V. underwoodi* and to identify its particular mtDNA haplotype. PCR amplified the *V. underwoodi COX1* gene according to Wang et al. (2019) using a pair of primers (COX1_821_F: 5'-GGAGTAGGTACAGGTTGAACGG-3' and COX1_821_R: 5'-ACAACCCCAGCAATAATAG-CAA-3') with 821 bp product (Wang et al., 2019). The PCR-amplified fragments were sequenced with Sanger's methods for all *V. underwoodi* samples, with the use of a pair of primers (F-V51: 5'-GTAATTTGTATACAAAGAGGG- 3' and R-V1400: 5'-CAATATCAATAGAAGAATTAGC- 3') (Warrit et al., 2004).

All PCR products were purified with the QIAquick PCR Purification Kit (250) (QIAGEN, Hilden, Germany) according to the instructions of the manufacturer. The nucleotide sequences of the *COX1* gene of *V. underwoodi* samples were determined through the sequencing of the PCR products using the Sanger dideoxy method (Sanger et al., 1977) on the ABI 3730xl (Applied Biosystems, Foster City, CA, USA) with the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's instructions. All PCR products were sequenced from both strands. The 458 bp sequence of the mitochondrial *COX1* gene of *V. underwoodi* was uploaded into the DDBJ/GenBank database with accession number LC532104.

The *V. underwoodi COX1* gene sequences - MH205173 (Hangzhou, China), MH205174 (Jinhua, China), MH205175 (Nanchang, China), MH205176 (Jilin, China), MH205177 (Maoming, China)), *V. destructor* (KJ403739, KJ507740, KJ403742, KJ403744 (Riyadh, Saudi Arabia)), and *V. jacobsoni* - (MF462134 (Moresby, Papua New Guinea), AF010479 (Canberra, Australia) from GenBank were compared with *V. underwoodi* from Russia's Primorsky Territory. The samples, which were closely related to species *V. destructor* and *V. jacobsoni* were used for outgroup comparison.

The genetic divergences among *V. underwoodi*, *V. destructor,* and *V. jacobsoni COX1* gene sequences were estimated using p-distance (proportion of nucleotide sites at which two

sequences differ) with CLUSTALW alignment as implemented in MEGA 10.0.5 (Kumar et al., 2018). A neighbor-joining (NJ) phylogenetic tree based on the p-distances of the *COX1* gene sequences with 2000 bootstrap replications was constructed with CLC Genomics Workbench 20 (Qiagen Inc., Mississauga, ON, Canada). The statistical analysis and the analysis of molecular variance (AMOVA) were performed with the use of ARLEQUIN 3.5.2 (Excoffier & Lischer, 2010), STATISTICA 8.0 (StatSoft, OK, USA), and EXCEL 2010 (Microsoft, CA, USA).

RESULTS

The taxonomic affiliation of *Varroa* mite samples was provided using morphometry measurements and mitochondrial *COX1* gene sequence analysis. The colour of the ellipsoidal body of *V. underwoodi* females was chestnut brown (Fig. 1). The dorsal shield surface was lightly striated and reticulated with tightly covered setae, which are approximately the same length and a little spiny. The setae on each lateral edge gradually increased in length posteriorly, with the last three pairs decreasing in size again. The body length of adult female *V. underwoodi* was 767.50±20.5 μm (mean±SD), and the width was 1,300.50±20.5 μm (n=10). For comparison, the body lengths and widths of adult female *V. underwoodi* were the following: *A. cerana* - 700-752 μm and 1,089-1,157 μm (n=15); *A. mellifera* - 700-735 μm x 1,090-1,120 μm (n=6); *A. cerana* in Irian Jaya - 690-730 μm x 1,050-1,130 μm (n=5); *A. cerana* in Sulawesi and Java - 720-780 μm x 1,050-1,080 μm (n=2); *A. nigrocincta* in Sulawesi - 740-760 μm x 1,120-1,220 μm (n=5) (Anderson et al., 1997); *A. cerana* in Nepal - 741-780 μm x 1,151-1,168 μm (n=2) (Delfinado-Baker & Aggarwal, 1987); *A. cerana* in South Korea - 703-784 μm x 1,135-1,324 μm (n=2) (Woo, 1992). Due to the congruence of morphology parameters to the previously published morphology of *V. underwoodi* (Delfinado-Baker & Aggarwal 1987; Anderson et al., 1997; Huang 2004; Wang et al., 2019), the *Varroa* mite samples were assumed to belong to the species *V. underwoodi*.

The *COX1* gene sequences of six *V. underwoodi* samples from both *A. c. ussuriensis* colonies #2 and #5 were identical to one another and to MH205176 (Jilin, China) (Wang et al., 2019). We called both of them a haplotype *China 1 MH205176*. The pairwise differences among *V. underwoodi*, *V. destructor,* and *V. jacobsoni* were counted based on the *COX1* gene sequences polymorphism. The pairwise number of nucleotide and amino acid differences, p-distances, and percent of genetic divergence based on mitochondrial *COX1* gene sequences is presented in Tab. 1.

COX1 gene sequences of mite species differ significantly ($p \leq 0.05$). It confirms with a 95% probability that *COX1* sequences of *V. underwoodi*,

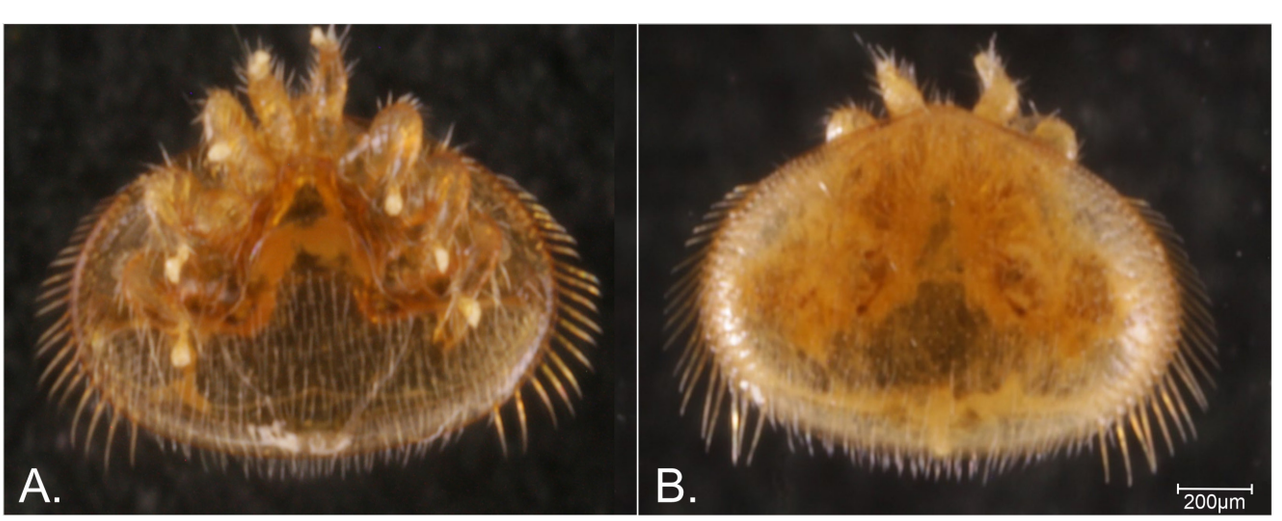

Fig. 1. The ventral (A) and dorsal (B) surfaces of the adult female mite *Varroa underwoodi* were sampled from the Primorsky Territory, Russia. Pictures were taken by Junichi Takahashi.

Table 1.

Pairwise p-distance and genetic divergence below diagonal, nucleotide and amino acid differences above the diagonal among *V. underwoodi*, *V. destructor*, and *V. jacobsoni* based on *COX1* gene sequence of mtDNA

Species		*V. underwoodi*, N = 6	*V. destructor*, N = 4	*V. jacobsoni*, N = 2
		Number of nucleotide differences/number of amino acid differences		
V. underwoodi	p-distance		45 / 36	44 / 34
V. destructor	/ genetic	* 0.099 / 10%		33 / 26
V. jacobsoni	divergence	* 0.097 / 10%	* 0.072 / 7%	

Notes: * statistically significant differences (p≤0.05)

V. destructor, and *V. jacobsoni* are quite distinct species. Of the three species, *V. destructor,* and *V. jacobsoni* are closest with a 7% value of genetic divergence. The species *V. underwoodi* differs from both mite species equally with a 10% value of genetic divergence.

The pairwise genetic divergences and sequence differences of nucleotides and amino acids among each sample of *V. underwoodi* (N=6), *V. destructor* (N=6), and *V. jacobsoni* were counted based on a comparison of the mitochondrial *COX1* gene sequences. The pairwise number of nucleotide and amino acid differences, p-distances, and percent of genetic divergence are presented in the table (Tab. 2).

In this study, there was a variation in the genetic divergence (0% to 2%), p-distance (0.000 to 0.022), number of nucleotide differences (0 to 10), and number of amino acid differences (0 to 8) in the *V. underwoodi* samples. Most of them differed from all other *V. underwoodi* samples MH205177 from China, Maoming. There were no differences between MH205176 from Jilin, China, and LC532104 from Primorsky Territory,

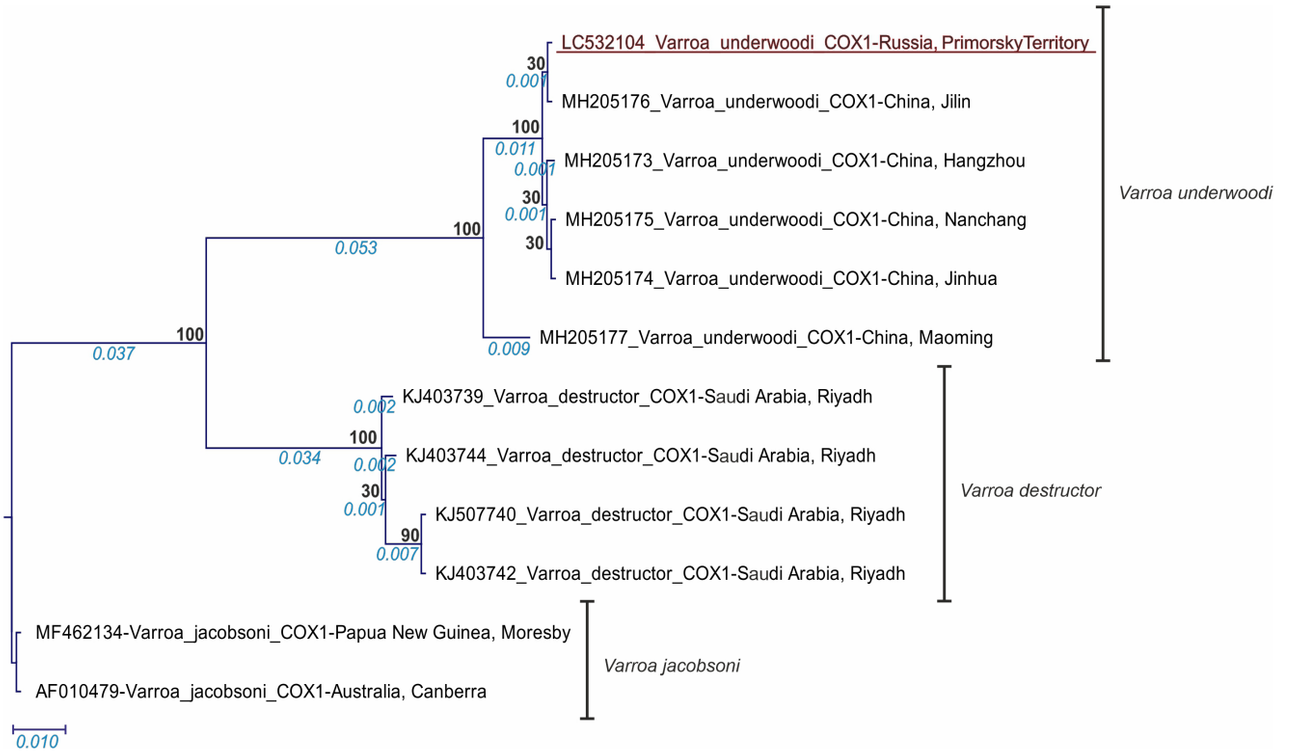

Fig. 2. A neighbor-joining (NJ) phylogenetic tree constructed in CLC Genomics Workbench 20 using p-distances between mitochondrial *COX1* gene sequences of three *Varroa* species with 2000 bootstrap replications. The numbers on each branch indicate the genetic distances.

Table 2.

Pairwise differences and genetic divergence among samples of *V. underwoodi*, *V. destructor*, and *V. jacobsoni* calculated based on the *COX1* gene sequences polymorphism

Samples	1. MH205176, *V. underwoodi*	2. LC532104, *V. underwoodi*	3. MH205175, *V. underwoodi*	4. MH205174, *V. underwoodi*	5. MH205173, *V. underwoodi*	6. MH205177, *V. underwoodi*	7. KJ403744, *V.destructor*	8. KJ403742, *V. destructor*	9. KJ507740, *V. destructor*	10. KJ403739, *V. destructor*	11. MF462134, *V. jacobsoni*	12 AF010479, *V. jacobsoni*
	Number of nucleotide differences/number of amino acid differences											
1. MH205176, *V. underwoodi*, China, Jilin		0 /0	1 /1	1 /1	2 /2	10 /8	44 /36	46 /36	46 /36	44 /35	44 /34	44 /34
2. LC532104, *V. underwoodi*, Russia, Primorsky	0.000 /0%		1 /1	1 /1	2 /2	10 /8	44 /36	46 /36	46 /36	44 /35	44 /34	44 /34
3. MH205175, *V. underwoodi*, China, Nanchang	0.002 /0%	0.002 /0%		0 /0	1 /1	10 /8	45 /37	47 /37	47 /37	45 /36	45 /35	45 /35
4. MH205174, *V. underwoodi*, China, Jinhua	0.002 /0%	0.002 /0%	0.000 /0%		1 /1	10 /8	45 /37	47 /37	47 /37	45 /36	45 /35	45 /35
5. MH205173, *V. underwoodi*, China, Hangzhou	0.004 /0%	0.004 /0%	0.002 /0%	0.002 /0%		9 /7	45 /37	47 /37	47 /37	45 /36	45 /35	45 /35
6. MH205177, *V. underwoodi*, China, Maoming	0.022 /2%	0.022 /2%	0.022 /2%	0.022 /2%	0.020 /2%		42 /36	44 /36	44 /36	42 /35	44 /36	44 /36
7. KJ403744, *V. destructor*, Saudi Arabia, Riyadh	0.096 /10%	0.096 /10%	0.098 /10%	0.098 /10%	0.098 /10%	0.092 /9%		4 /2	4 /2	2 /1	32 /27	32 /27
8. KJ403742, *V. destructor*, Saudi Arabia, Riyadh	0.100 /10%	0.100 /10%	0.103 /10%	0.103 /10%	0.103 /10%	0.096 /10%	0.009 /1%		0 /0	4 /1	34 /27	34 /27
9. KJ507740, *V. destructor*, Saudi Arabia, Riyadh	0.100 /10%	0.100 /10%	0.103 /10%	0.103 /10%	0.103 /10%	0.096 /10%	0.009 /1%	0.000 /0%		4 /1	34 /27	34 /27
10. KJ403739, *V. destructor*, Saudi Arabia, Riyadh	0.096 /10%	0.096 /10%	0.098 /10%	0.098 /10%	0.098 /10%	0.092 /9%	0.004 /1%	0.009 /1%	0.009 /1%		32 /26	32 /26
11. MF462134, *V. jacobsoni*, Papua New Guinea	0.096 10%	0.096 /10%	0.098 /10%	0.098 /10%	0.098 /10%	0.096 /10%	0.070 /7%	0.074 /7%	0.074/ 7%	0.070 /7%		0 /0
12 AF010479, *V. jacobsoni*, Australia, Canberra	0.096 /10%	0.096 /10%	0.098 /10%	0.098 /10%	0.098 /10%	0.096 /10%	0.070 /7%	0.074 /7%	0.074 /7%	0.070 /7%	0.000 /0%	

p-distance / genetic divergence

Russia. In the *V. destructor* samples, the genetic divergence varied from 0% to 1%, p-distance varied from 0.000 to 0.022, the number of nucleotide differences from 0 to 4, and the number of amino acid differences from 0 to 1. The samples KJ403744 and KJ403739 from Saudi Arabia, Riyadh are the least different. No differences were found between KJ403742 and KJ403740 from Riyadh, Saudi Arabia. In the *V. jacobsoni* samples, no differences were found between MF462134 from Moresby, Papua New Guinea, and AF010479 from Canberra, Australia.

A NJ phylogenetic tree was constructed based on pairwise p-distances of the mitochondrial *COX1* gene sequences among three species of mites *V. underwoodi*, *V. destructor*, *V. jacobsoni*. The *COX1* gene sequence LC532104 from Primorsky Territory, Russia (haplotype *China 1 MH205176*) was clustered with all *V. underwoodi* sequences MH205173, MH205174, MH205176, MH205177 (China), which was distinct from *V. destructor* sequences KJ403739, KJ507740, KJ403742, KJ403744 (Riyadh, Saudi Arabia) and *V. jacobsoni* sequences MF462134 (Moresby, Papua New Guinea), AF010479 (Canberra, Australia) (Fig. 2). On the phylogenetic tree, the sample of *V. underwoodi* LC532104 (Primorsky Territory, Russia) was located the closest to the northernmost sample MH205176 (Jilin, China) (distance of 450 kilometers) and the farthest from the southernmost sample MH205177 (Maoming, China) (distance of 3000 kilometers). On the tree, *V. underwoodi* was genetically closer to *V. destructor* than to *V. jacobsoni*. Compared to all *V. underwoodi* samples, only the southernmost sample MH205177 (Maoming, China) was closest to the *V. destructor* samples.

DISCUSSION

The *V. underwoodi* mites in the Primorsky Territory have probably always parasitized in wild *A. cerana* colonies living in tree hollows. Conditions are more favourable for the reproduction of *V. underwoodi* in hives than in tree hollows. Kuznetsov (2005) stated that in 2002 the *V. underwoodi* mites were found only once in the *A. cerana* drone brood in the apiary, but in 2004, a massive reproduction of *V. underwoodi* was observed. In 2004, the infestation rate of *A. cerana* colonies with the *V. underwoodi* mites was 2.8% in June, 35% in July and 58% in August in the drone brood cells, and was 1% in worker brood cells in the apiary. Individual *A. cerana* drone cells contained up to five to six adults and two to three *V. underwoodi* nymphs. *V. underwoodi* imagos and nymphs were found on the same pupae of *A. cerana* drones and were rarely observed in the brood of worker bees. Female *V. underwoodi* were found mostly on young drones and very rarely on young worker bees of *A. cerana*. In summer 2004, up to 16% of young drones were infested with *V. underwoodi* mites, but in autumn, no mites were found on adult bees. In 2004, the high rate of infestation by *V. underwoodi* mites in the brood contributed to the frequent cleansing swarming of *A. cerana* colonies (Kuznetsov, 2005).

The *Varroa* mites sampled in the Primorsky Territory, Russia were assumed as subspecies *V. underwoodi* based on their morphometrics that was in the range of previously described populations (Delfinado-Baker & Aggarwal 1987; Anderson et al., 1997; Huang 2004; Wang et al., 2019) and mitochondrial gene *COX1* matched with the sample available from the province Jilin of China MH205176 (Wang et al., 2019). The body length and width of adult female *V. underwoodi* from Russia were a little bigger in comparison with other populations, which can be explained by the northernmost distribution. Since the *COX1* gene sequence of *V. underwoodi* from Primorsky Territory, Russia LC532104 was identical to that of Jilin, China MH205176, we defined both of them as a haplotype *China 1 MH205176* (Traynor et al., 2020).

The level of genetic divergence based on mitochondrial DNA sequences between insect species within the genera varied from 8% to 17%, and the genetic p-distance based on mitochondrial DNA sequences varied from 0.100 to 0.200 (Tan et al., 2011; Han et al., 2016; Eimanifar et al., 2017; Ilyasov et al., 2018, 2019). In our current study, the genetic divergence of mitochondrial *COX1* gene sequence ranges from 7% to 10%, and genetic p-distances ranges from 0.072 to 0.099 between three species of mites *V. underwoodi*, *V. destructor,* and *V. jacobsoni,* which is almost matched with the range of the mtDNA-based intraspecific level of genetic differences in insects (8-17%) (Tan et al., 2011; Han et al., 2016; Eimanifar et al., 2017; Ilyasov et al., 2018, 2019). The levels of divergence described here between *V. destructor* and *V. jacobsoni* 7% is a bit higher than 5.9%, which was described, in a recently published paper (Techer et al., 2019). This difference can be explained by averaging a different number of samples of mites used

for comparisons; we used only four samples of *V. destructor* from Saudi Arabia and only two samples of *V. jacobsoni* from Papua New Guinea and Australia, whereas in the recently published paper we used seven samples of *V. destructor* from South Korea, France, Vietnam, China, Japan, Nepal, Sri Lanka and eleven samples of *V. jacobsoni* from Indonesia, Malaysia, Laos, Borneo, Papua New Guinea (Techer et al., 2019). Previous studies based on the morphology and mitochondrial *COX1* gene had reported the occurrence of *V. underwoodi* in *A. cerana* colonies in Nepal (Delfinado-Baker & Aggarwal 1987), South Korea (Woo, 1992; Kuznetsov, 2005; Chantawannakul et al., 2016), Indonesia (Anderson et al., 1997; Chantawannakul et al., 2016), Papua New Guinea (Lee, 1995; Anderson et al., 1997; Chantawannakul et al., 2016), Vietnam (de Guzman & Rinderer, 1999; Chantawannakul et al., 2016) and China (Huang 2004; Wang et al., 2019). In the previous studies, Russia had not been included in the distribution area of *V. underwoodi* (Chantawannakul et al., 2016; Wang et al., 2019). To date, this is the first evidence of *V. underwoodi* distribution in far east Russia in native *A. c. ussuriensis* colonies using both morphometry and mitochondrial *COX1* gene sequencing methods. The distribution of *V. underwoodi* in Khasansky district, Primorsky Territory, Russia (43.5N, 131.3E) is at a distance of 450 kilometers from the distribution of *V. underwoodi* in Jilin province, North China (43.1N, 127.1E).

The presence of *V. underwoodi* in capped worker brood cells in one *A. mellifera* colony in Papua New Guinea (Roberts et al., 2015) shows that interspecies host switch can occur. To date, *V. underwoodi* has not been found yet in *A. mellifera* colonies in far east Russia. This suggests that interspecies transmission to *A. mellifera* is rare, which considerably limits chances for host switch. The population structure of *V. underwoodi* mites studied previously suggested that genetic diversity might lead to evolving lineages to reproduce in *A. mellifera* colonies. As has been the case for *V. destructor* and *V. jacobsoni*, rare events can lead to interspecies host switches with devastating effects (Rosenkranz et al., 2010; Roberts et al., 2015). Because the *V. underwoodi* samples are genetically closest to *V. destructor* samples that are a common parasite of both *A. mellifera* and *A. cerana*, *V. underwoodi* also has a high probability to host switch and parasitizing on *A. mellifera* (Roberts et al., 2020).

The infestation rate by *V. underwoodi* was significantly higher in China's northern provinces than in its southern ones (Wang et al., 2019). The higher infestation rate in the cold region is unsuspected because mass parasite reproduction is not possible when colonies desist brood rearing during winter. The higher infestation rate in northern Asia is affected by drone brood production time that is shorter in a cold climate (Wang et al., 2019). The identity of the *COX1* gene sequences of *V. underwoodi* from Primorsky Territory, Russia LC532104 and from Jilin, China MH205176 located 450 kilometers away can presumably be explained by the fact that both mite populations are predominantly adapted to parasitizing on *A. cerana* in the cold climate of northern Asia. The identity of the *COX1* sequence of the *V. underwoodi* samples from far east Russia and northern China suggests that this region is inhabited by one single population of *V. underwoodi*, parasitizing a common host, *A. cerana*. This indicates that migration between Chinese and Russian *A. cerana* populations is associated with the exchange of genes and parasites. Thus, haplotype *China 1 MH205176* of *V. underwoodi* can be the first candidate for a cross-species host switch from north Asian *A. c. ussuriensis* and north European *A. m. mellifera,* both of which inhabit a cold continental climate. The *COX1* gene sequence LC532104 from Primorsky Territory, Russia clustered with all *V. underwoodi* sequences from China and formed a common subgroup with the northernmost sample MH205176 from Jilin, China, and belonged to one haplotype *China 1 MH205176*. This suggests that the samples of *V. underwoodi* from the far east of Russia and North China are representatives of one common big North Asian population containing haplotype *China 1 MH205176*. This parasite *V. underwoodi* seems to originate from south Asian areas and later

spreads to north Asia together with *A. cerana* migration. The low level of genetic differences of *V. underwoodi* samples collected between far east Russia and North China after isolation by distance and geography can be explained by a low rate of molecular evolution due to a low level of natural selection through life in a stable environment inside *A. cerana* colonies. Similarly, no differences were found between *V. destructor* samples KJ403742 and KJ403740 from distant regions of Saudi Arabia, between *V. jacobsoni* samples MF462134 and AF010479 from distant countries Papua New Guinea and Australia. Thus, the rate of molecular evolution in all *Varroa* mite species is very low, and their genomes are more conservative than the genomes of their host species of genus *Apis*.

Until recently, the distribution area of *V. underwoodi* covered almost all countries where *cerana* is found, with the exception of Russia. On the basis of *COX1* gene sequences and morphometry analysis, we expanded the range of *V. underwoodi* by 450 km to the east to the Primorsky Territory, Russia. The identity of the *COX1* gene sequences of the *V. underwoodi* from northern China and far east Russia indicates the absence of state borders between countries for *A. cerana*, which freely migrate and spread parasites. The northernmost boundaries of the *V. underwoodi* range remain unexplored. Last year, we collected northernmost *A. cerana* samples near the Terney village of the Primorsky Territory (45.06N 136.61E) – the northern border of the species range. We assumed that the northern border of the *V. underwoodi* range coincides with *A. cerana*. Furthermore, we are going to characterize *V. underwoodi* using additional *COX3-ATP6* and *CYTB* markers. It is possible we can find differences between Russian and Chinese *V. underwoodi* samples and can demonstrate that its biogeography is more complex than previously assumed.

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