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PREVALENCE OF *NOSEMA CERANAE* (MICROSPORIDIA) IN THE *APIS MELLIFERA MELLIFERA* BEE COLONIES FROM LONG TIME ISOLATED APIARIES OF SIBERIA

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Summary. This study was conducted to determine the prevalence of the microsporidian species, *Nosema apis* and/or *N. ceranae*, in *Apis mellifera mellifera* honeybee colonies from a long time isolated Yenisei population of Krasnoyarsk krai (Siberia). *N. ceranae* was found in all investigated apiaries, including the apiary in very cold climates (near Turukhansk, 65°47′35″N, 87°57′44″E). We showed that 64.7% of bee colonies (22 out of 34 studied) in most apiaries were infected with both *Nosema* species (mixed infection). Only in the Yaksha apiary, either only *N. ceranae* infected or uninfected bee colonies were found. Infection with *N. apis* alone was not detected in any bee colonies. We also examined the proportions of *Nosema* infected individuals in 22 bee colonies with mixed infection. Colonies generally had the highest percentage of co-infected bees. However, if only *N. ceranae* infection was found in bees from all colonies, then most of the *N. apis* infections is a co-infection with *N. ceranae*. This is the first report for molecular detection of *N. ceranae* in very cold climates. We discuss the causes of the prevalence of *N. ceranae*, including host genetic variability, in the long time isolated apiaries and in very cold climates.

Key words: *Nosema ceranae*, parasitic disease, prevalence, honeybee, *Apis mellifera*, Yenisei population, Russia.

Н. В. Островерхова. Широкое распространение *Nosema ceranae* (Microsporidia) в семьях темной лесной пчелы *Apis mellifera mellifera* на длительно изолированных пасеках Сибири // Дальневосточный энтомолог. 2020. N 407. С. 8-20.

Резюме. Изучено распространение двух видов микроспоридий рода *Nosema* (*N. apis*, *N. ceranae*) в семьях темной лесной пчелы *Apis mellifera mellifera*, обитающих на длительно изолированных пасеках Красноярского края (Енисейская популяция, Сибирь). Вид *N. ceranae* был обнаружен на всех исследованных пасеках, в том числе в очень холодном климате (пасека около п. Туруханск, 65°47′35″с.ш., 87°57′44″в.д.). Показано, что 64,7% пчелиных семей (22 из 34 изученных) на большинстве пасек были заражены обоими видами *Nosema* (смешанная инвазия). Только на пасеке п. Якша были выявлены семьи, либо незараженные, либо зараженные только видом *N. ceranae*. Пчелиные семьи, зараженные только одним видом *N. apis*, не были обнаружены. Исследование доли пчел, зараженных разными видами *Nosema*, в 22 семьях со смешанной инвазией

показало, что большинство особей в семье заражено обоими видами микроспоридий. Вместе с тем, если особи, зараженные только одним видом *N. ceranae*, выявлены во всех исследованных семьях, то пчелы, зараженные только *N. apis* были единичны (паразит *N. apis* чаще регистрировался совместно с *N. ceranae*). Настоящая статья – это первое сообщение о выявлении паразита *N. ceranae* в очень холодном климате. Обсуждаются причины распространения возбудителя *N. ceranae*, такие как генетическое разнообразие хозяина, длительная изолированность пасек и очень холодный климат.

INTRODUCTION

Nosemosis is a parasitic disease of honeybees caused by two microsporidian species, *Nosema apis* (Zander, 1909) and *Nosema ceranae* Fries, Feng, da Silva, Slemenda et Pieniazek, 1996 (Fries *et al*., 1996). For a long time, it was thought that the *N. apis*, a relatively benign pathogen, was only one microsporidian parasite of the European bee *Apis mellifera* (Bailey, 1955; Fries, 1993). Since 2006, a new microsporidian parasite, *N. ceranae*, specific for the Asian honeybee, *Apis cerana*, has been identified in the European honeybee, *A. mellifera*, worldwide (Fries *et al*., 2006; Higes *et al*., 2006; Huang *et al*., 2007; Klee *et al*., 2007; Paxton *et al*., 2007; Chen *et al*., 2008; Williams *et al*., 2008; Giersch *et al*., 2009; Invernizzi *et al*., 2009), as well as in other species of Hymenoptera including bumblebees, stingless bees, and social wasp (Graystock *et al*., 2013; Plischuk, Lange, 2016; Porrini *et al*., 2017; Sinpoo *et al*., 2019).

In many regions of the world, *N. ceranae* has become the dominant species in the honeybee populations (Klee *et al*., 2007; Chen *et al*., 2008; Stevanovic *et al*., 2011; Martin-Hernandez *et al*., 2012; Tunca *et al*., 2016; Shumkova *et al*., 2018). As the sole causative agent of nosemosis, *N. ceranae* was registered in Western Asia (Nabian *et al*., 2011; Ansari *et al*., 2017; Khezri *et al*., 2018) and Southern Europe (Tlak Gajger *et al*., 2010; Papini *et al*., 2017). It is supposed that *N. ceranae* is a more aggressive parasite compared with *N. apis* and appears to be replacing *N. apis* in some honeybee populations (Klee *et al*., 2007; Fries, 2010). In addition, parasite *N. ceranae* is associated with colony declines in Mediterranean countries like Spain, Greece, Israel, and Turkey (Higes *et al*., 2008, 2009, 2010a; Bacandritsos *et al*., 2010; Soroker *et al*., 2011; Oguz *et al*., 2017).

The worldwide prevalence and different consequences of *N. ceranae* infection for honeybees are thought to be determined mainly by the climatic influence on virulence of parasite (Fries, 2010; Gisder *et al*., 2010, 2017; Papini *et al*., 2017). It is assumed that in warmer climates, *N. ceranae* is more competitive than *N. apis*, and *N. ceranae* spores are capable of surviving high temperatures (60°C) and desiccation. In contrast, in cold climates, *N. ceranae* spores appear to be much more vulnerable than the *N. apis* spores. *N. ceranae* spores are intolerant of cold and freezing, and the decrease in the viability of the *N. ceranae* spores was observed under experimental conditions even after a short exposure to low temperatures (4°C) (Martín-Hernández *et al*., 2009; Fenoy *et al*., 2009; Gisder *et al*., 2010; Higes *et al*., 2010b; Sánchez Collado *et al*., 2014).

The different sensitivity to temperatures in the two *Nosema* species may be a potential explanation for the wider *N. ceranae* prevalence in sub-tropical climate (southern countries) compared to *N. apis*, which is more prevalent in temperate climate (northern countries) (Fries, 2010). However, there was a difference in the prevalence of *N. ceranae* in similar climatic conditions, for example in the Scandinavian countries (Klee *et al*., 2007; Paxton *et al*., 2007). It is possible that the different honeybee subspecies, as well as the bee colonies and individual bees within lineage, vary in their ability to counter infection (Fontbonne *et al*., 2013; Mendoza *et al*., 2014; Huang *et al*., 2015). Unfortunately, in most studies on the *Nosema* prevalence, the honeybee subspecies and lineages and their genetic diversity have not been described (Martín-Hernández *et al*., 2018).

We have previously identified the *N. ceranae* in Siberia (Russia), in the Tomsk region (Fig. 1), characterized by severe climatic conditions with long cold winters (Ostroverkhova *et al*., 2016, 2019a). To confirm the widespread and non-random distribution of the *N. ceranae* parasite in a cold climate, we studied other areas of Siberia. In addition, we first investigated the prevalence of *Nosema* species in the dark-colored forest bee populations. The objective of this work was to study the *Nosema ceranae* infestation of the *Apis mellifera mellifera* bee colonies from the long time isolated Yenisei population (the Krasnoyarsk Krai, Siberia).

MATERIAL AND METHODS

Region. The study was conducted throughout the Krasnoyarsk Krai (the Yenisei population) in the summer of 2015–2016 (Table 1). The Krasnoyarsk Krai is a large territory in the eastern part of Siberia (Eastern Siberia) and stretched from the Yenisei River to the Baikal ridge. The climate of the Krasnoyarsk Krai is sharply continental with considerable daily and annual temperature amplitudes, relatively hot and short summers and long cold winters (5–6 months). In winter, the territory is covered with snow. The duration of the frost-free period is 103 days; the first frosts are observed already in early September and the last frosts occur in May. The average annual temperatures of studied territory (the Yenisei region) is –1.5°C. The warmest month is July; the average July temperature is about 16°C. The winter is cold; the average January temperature is about –26°C. Precipitation is, on average, 350–460 mm; mean relative humidity is 75%. The Krasnoyarsk Krai is a predominantly taiga region; forests cover about 70% of the territory (for details, see Ostroverkhova *et al*., 2019b).

In the Krasnoyarsk Krai, the honeybee *A. mellifera* was introduced about 230 years ago. We investigated a unique Yenisei population located in the deep taiga kept by the Old Believers. The Yenisei population is a long time isolated *Apis mellifera mellifera* population that existed for more than 60 years without the importation of new honeybees (Ostroverkhova *et al*., 2018). Five different geographical localities of the Yenisei population were investigated: Yartsevo (60°14′42″N, 90°13′19″E); Ostyatskoe (59°11′12″N, 91°19′24″E); Kolmogorovo (59°16′06″N, 91°19′02″E), Ozernoe (58°46′56″N, 92°08′05″E), and Yaksha (59°03′00″N, 89°19′00″E) (Fig. 1). For comparison, one bee colony received in September 2017 from a remote apiary near Turukhansk (65°47′35″N, 87°57′44″E) was also included in this survey (Fig. 1). In the Turukhansky district, located in the north of the Krasnoyarsk Krai, beekeeping is absent due to the very cold climate, but there are single apiaries. For comparison, the northernmost settlement of the Tomsk region, where the *Nosema ceranae* was registered, is indicated by a point A (for details, see Ostroverkhova *et al*., 2016, 2019a).

Biological samples. Honeybee samples were collected from six apiaries of Krasnoyarsk Krai (Fig. 1). From 1 (s. Yartsevo, s. Turuchansk) to 11 (s. Yaksha) bee colonies in each apiary were inspected; a total of 34 bee colonies were investigated. From 70 to 100 worker bees were randomly selected from each bee colony and were examined for the presence of *Nosema*. Bee samples were anesthetized on dry ice and stored in 70% ethanol until use.

Each bee colony was analyzed using the variability in the *COI-COII* mtDNA locus and morphometric parameters of the wing, such as the cubital index, the hantel index, and the discoidal shift, to determine its conformance to the *A. m. mellifera* breed standard (Ostroverkhova *et al*., 2018).

Algorithm of study included two stages. In the first stage of the study, from 60 to 70 workers from each bee colony were pooled and used for DNA isolation. The presence of nosemosis in the bee colony was examined using a polymerase chain reaction (PCR). In the second stage of the study, in the *Nosema* positive bee colonies with mixed infection (the presence of both *N. apis* and *N. ceranae*), the proportion of individuals infected with different *Nosema* infection was determined. For this, an additional 14 to 30 individuals from

each *Nosema* infected colony were investigated. The analysis was performed separately for each worker bee, for which the midgut of the bee was isolated and used for DNA extraction. A total of 412 individuals from 22 bee colonies were analyzed.

Fig. 1. The map of localization of the Yenisei population and studied apiaries (dots 1–6) of the Krasnoyarsk Krai (Eastern Siberia): 1 – Yartsevo; 2 – Kolmogorovo; 3 – Ostyatskoe; 4 – Ozernoe; 5 – Yaksha; 6 – Turuchansk. For comparison, the northernmost settlement of the Tomsk region, where the *Nosema ceranae* was registered, is indicated by a point A (for details, see Ostroverkhova *et al*., 2016, 2019a).

DNA extraction and PCR amplification. Abdomens were excised from honeybees, and DNA was obtained by a direct DNA extraction method using a DNA purification kit, PureLink™ Mini (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. DNA was first extracted from homogenized pools of 60-70 honeybees from each colony, then from separate individuals from bee colonies infected with both *Nosema* species. After DNA extraction, bee samples were submitted to duplex-PCR including the co-amplification of the 16S rRNA genes of *N. apis* and *N. ceranae* (Martin-Hernández *et al*., 2007; Hamiduzzaman *et al*., 2010). The primer sequences utilized to amplify the 218 bp fragment corresponding to the 16S ribosomal gene of *N. ceranae* were 218MITOC-FOR 5′-CGGCGACGATGTGATATGAAAATATTAA-3′ and 218MITOC-REV 5′-CCCGGTCATTCTCAAACAAAAAACCG-3′. The primer sequences used to amplify the 321 bp fragment corresponding to the 16S ribosomal gene of *N. apis* were 321APIS-FOR 5′-GGGGGCATGTCTTTGACGTACTATGTA-3′ and 321APIS-REV 5′-GGGGGGCGTTTAAAATGTGAAACAACTATG-3′ (Martin-Hernández *et al*., 2007). PCR products were electrophoresed in 2% agarose gel parallel to standard size electrophoresis and visualized using a Gel Doc XR+ system (BioRad, Foster City, CA, USA). All analyses were carried out in duplicate with use positive and negative controls, and identical results were obtained.

In addition to the use of specific primers and fragment size to identify the species present, a selection of fragments (both *N. ceranae* and *N. apis)* was verified by DNA sequencing. Sequencing was done in both directions using forward or reverse primer (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, Foster City, CA, USA). DNA sequencing was performed using ABI Genetic Analyzer 3730 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol.

RESULTS

Infestation of bee colonies with *Nosema*

We studied the infestation of honeybees with the microsporidian *Nosema* spp. in the *A. m. mellifera* bee colonies from apiaries of the Krasnoyarsk Krai (Siberia). Two species of microsporidia, *N. apis* and *N. ceranae*, were registered using molecular genetic methods (Fig. 2). To confirm the PCR findings, the DNA fragments were sequenced. Sequence analysis revealed a complete sequence identity for *N. apis* (GenBank Accession No U97150) and *N. ceranae* (GenBank Accession No DQ486027).

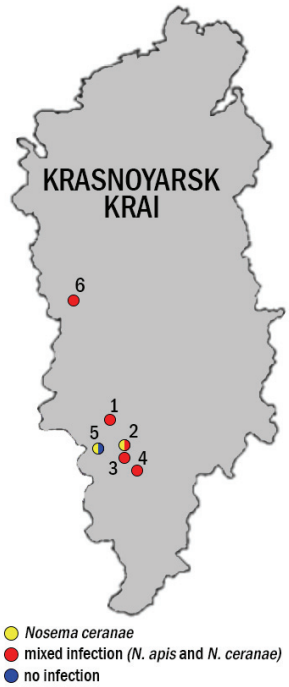

Fig. 2. Distribution of *Nosema* species in apiaries throughout the Krasnoyarsk Krai (dots 1–6 see Fig. 1). Bee colonies not infected by *Nosema* are indicated in blue. Bee colonies infected by mixed *Nosema* infection are indicated in red, bee colonies corresponding to infection by *N. ceranae* are indicated in yellow. Sectors in circles indicate representation cases (existence/absence) of an infection without frequency.

Overall, all apiaries and most bee colonies were found to be infected with *Nosema*. Detection by PCR using *N. apis* and *N. ceranae* specific primers found that all apiaries were positive for *N. ceranae*: in most apiaries (5 out of 6 apiaries, 83.33%), *N. ceranae* was detected in combination with *N. apis* (mixed infection), in one apiary – only *N. ceranae* (Yaksha) (Fig. 2). In 34 bee colonies analyzed, *Nosema* spp. DNA was detected in 88.24% of the samples. All uninfected bee colonies (4 out of 34 colonies examined) were found only in an apiary near Yaksha. Of the *Nosema* positive bee colonies, 73.33% of the samples (22 colonies out of 30 examined) contained both *Nosema* species (mixed infection) and 26.67% of the samples *N. ceranae* only (8 colonies out of 30 examined). But no bee colonies were found to be positive only for *N. apis*.

The proportion of infected individuals in the bee colony with *Nosema*

In order to determine the proportion of infected individuals and infection levels of two *Nosema* species, individual bees were analyzed from 22 colonies with mixed infection; a total of 412 individual (Table 1).

Table 1. Proportion of *Nosema* infected individuals in each colony with mixed infection

Colony	Sample collection period	Total number of analyzed indivi-duals	*Nosema* positive individuals (infection categories)							
			Total		*N. apis* single		*N. ceranae* single		Co-infection (infection with both *N. ceranae* and *N. apis*)	
			n	%	n	%	n	%	n	%
Ost-1	June 2015	16	16	100.0	2	12.50	3	18.75	11	68.75
Ost-2		16	10	62.50	0	0	6	37.50	4	25.00
Ost-3		18	15	83.33	0	0	9	50.00	6	33.33
Ost-4		16	15	93.75	0	0	11	68.75	4	25.00
Ost-5		14	11	78.57	0	0	4	28.57	7	50.00
Ost-6		24	23	95.83	0	0	2	8.33	21	87.50
Ost-7		27	26	96.30	0	0	11	40.74	15	55.56
Kol-1		16	9	56.25	0	0	7	43.75	2	12.50
Kol-2		16	15	93.75	0	0	5	31.25	10	62.50
Kol-4		14	8	57.14	1	7.14	4	28.57	3	21.43
Kol-5		14	14	100.0	0	0	5	35.71	9	64.29
Kol-6		21	20	95.24	0	0	13	61.90	7	33.33
Kol-7		19	19	100.0	0	0	7	36.84	12	63.16
Yar-1	July 2015	15	14	93.33	0	0	6	40.00	8	53.33
Oz-1	July 2016	22	20	90.91	1	4.55	2	9.09	17	77.27
Oz-2		21	17	80.95	0	0	5	23.81	12	57.14
Oz-3		18	17	94.44	1	5.56	1	5.56	15	83.33
Oz-4		21	17	80.95	0	0	3	14.29	14	66.67
Oz-5		17	13	76.47	0	0	5	29.41	8	47.06
Oz-6		19	18	94.74	0	0	9	47.37	9	47.37
Oz-7		18	10	55.56	0	0	1	5.56	9	50.00
Tur-1	September 2017	30	28	93.33	0	0	8	26.67	20	66.67

Given are the total number of analyzed individuals, the numbers (n) and proportions (%) of individuals within each infection category. Ost – Ostyatskoe, Kol – Kolmogorovo, Yar – Yartsevo, Oz – Ozernoe, Tur – Turuchansk.

In the studied colonies, the majority of the analyzed bees (more than 85%) were infected with *Nosema*. The infection variants were as follows: all individuals of colony were infected (colonies Ost-1, Kol-5, and Kol-7); a large number of individuals of colony (≥75%) were infected (colonies 3, 4, 5, 6 and 7 from Ostyatskoe, colonies 2 and 6 from Kolmogorovo, colonies 1, 2, 3, 4, 5 and 6 from Ozernoe, Yak-1, and Tur-1); about 40% of the bees of colony were not infected (Ost-2, Kol-1, Kol-4, Oz-7).

In most of the studied colonies, bees with a co-infection prevailed (both *Nosema* species were detected). In seven colonies (half of the studied colonies in apiaries in the Kolmogorovo and Ostyatskoe villages – Ost-2, Ost-3, Ost-4, Kol-1, Kol-3, Kol-4, and Kol-6), bees infected only with *N. ceranae* were predominant, and in one of these colonies (colony Kol-3), only *N. ceranae* infection was identified. *N. apis* was detected always as a low-level co-infection with *N. ceranae*; only 5/412 bees (1.21%) were infected only with *N. apis* (from colonies Ost-1, Kol-4, Oz-1, Oz-3).

The maximum number of bees with mixed infection was registered in the colony 6 from Ostyatskoe (87.50%), whereas the minimum number of bees – in the colony 1 from Kolmogorovo (12.50%). The maximum number of bees with only *N. ceranae* infection was found in the colony 4 from Ostyatskoe (68.75%), whereas the minimum number of bees – in the colonies 3 and 7 from Ozernoe (5.56%).

Thus, in most studied apiaries and bee colonies, co-infection of two *Nosema* species predominates. If only *N. ceranae* infection was found in bees from all colonies, then most of the *N. apis* infections is a co-infection with *N. ceranae*. The question arises of replacement the traditional species *N. apis* by *N. ceranae*.

DISCUSSION

This study allowed us for the first time to investigate the infestation of honeybees from the Krasnoyarsk Krai with microsporidia of the genus *Nosema* spp. and to identify the parasite *N. ceranae* in the *A. m. mellifera* bee colonies from the long time isolated apiaries in the deep taiga, where new bees have not been imported for more than 60 years. Earlier, we identified *N. ceranae* in honeybees from the northern apiaries of the Tomsk region (Ostroverkhova *et al*., 2019a). According to beekeepers, the importation of new honeybees into these apiaries has also not been carried out for a long time.

These findings were unexpected for us. First, how did the parasite spread to isolated apiaries if the *N. ceranae* infection by the importation of new bees to these apiaries is excluded? Second, the parasite *N. ceranae* was found in very cold climates (Turukhansk village).

We suggest several reasons for the presence of the *N. ceranae* parasite in the northern apiaries of Siberia: (i) the spread of the *N. ceranae* infection with beekeeping equipment and/or plants; (ii) the spread of the parasite due to the unfavorable ecological situation caused by the extreme flood in the north of the Tomsk region in 2015 (for the Tomsk region only); (iii) the natural spread of *N. ceranae* in the wild. It is unlikely that the primary *N. ceranae* infestation of honeybees in apiaries of the Krasnoyarsk Krai was due to human transportation of bee packages. However, infection could be transmitted among bees via ingestion of environmentally resistant mature spores from contaminated wax, combs, other hive interior surfaces, beekeeping material, and water (Higes *et al*., 2010a). In support of the latter assumption (iii), the findings of the causative agent *N. ceranae* in some Hymenoptera species may also indicate (Graystock *et al*., 2013; Plischuk & Lange, 2016; Porrini *et al*., 2017; Sinpoo *et al*., 2019).

It was originally thought that *N. ceranae* is a microsporidian parasite that until the 1990s infected only the Asian honeybee, *A. cerana* (Fries *et al*., 1996). Only in 2006, it was found in the European honeybee, *A. mellifera*, in Spain (Higes *et al*., 2006). Interestingly, the *N. ceranae* was later found in archived bee samples in the US dating back to 1975 (Traver & Fell, 2015) and 1995 (Chen *et al*., 2008), in bee samples collected in Mexico in 1995–1996 (Guerrero-Molina *et al*., 2016) and in Uruguay pre-1990 (Invernizzi *et al*., 2009), and also in samples of Africanized drones collected in Brazil in 1979 (Teixeira *et al*., 2013). These findings, as well as the finding in the Krasnoyarsk Krai, described in the present study, suggest

the existence of longer host-parasite relationship "*Apis mellifera* - microsporidia *Nosema ceranae*" than previously recognized. It is possible that early reports, in which only light microscopy, without ultrastructural analysis was used to detect *Nosema* infections, wrongly identified *N. ceranae* as *N. apis*. The use of highly sensitive molecular genetic methods has played a key role for detection of *N. ceranae* in *A. mellifera* (Teixeira *et al*., 2013; Guerrero-Molina *et al*., 2016).

At the same time, according to a comparative analysis of the genetic diversity, demography, and evolution of the two *Nosema* species, *N. apis* is an evolutionarily old parasite of the *A. mellifera* honeybee, and the host-parasite relationship is well balanced, while the spread of *N. ceranae* throughout the *A. mellifera* worldwide population is a relatively recent event (Maside *et al*., 2015). Based on the patterns of nucleotide polymorphism at three single copy genes (*PTP2*, *PTP3*, and *RPB1*) studied in a collection of *N. apis* and *N. ceranae* isolates obtained from *A. mellifera* colonies from all over the world, significant variability in both *Nosema* species within honeybee colonies (62-90% of the total genetic variance) was shown. However, there is a differentiation of the genetic variants (20-34% of the total number of variants) in *N. apis* isolated from honeybees of different evolutionary lineages, specifically between isolates from African (A) and European honeybee lineages (C and M). On the contrary, in *N. ceranae*, the lack of differentiation (<4% of the genetic variance) observed among host lineages (Maside *et al*., 2015).

Evidently, *N. ceranae* spread rapidly through *A. mellifera* populations worldwide and was found in various climatic regions, with different consequences of infection for honeybees in Northern and Southern temperate areas (Klee *et al*., 2007; Paxton *et al*., 2007). If *N. ceranae* might cause colony collapse in warmer climates (Higes *et a*l., 2008, 2009; Martin-Hernandez *et al*., 2007; Cepero *et al*., 2014), in colder climates, colony losses could not be associated with *N. ceranae* (Invernizzi *et al*., 2009; Genersch *et al*., 2010; Gisder *et al*., 2010). In addition, in the northern regions, for example in northeastern Germany, the spread of *N. ceranae* not necessarily result in the replacement of *N. apis* (Gisder *et al*., 2017).

We diagnosed the *N. ceranae* infection in most of the studied bee colonies from the Krasnoyarsk Krai, which were relatively healthy and lived in very cold climates for a long time. In contrast to the northeastern German honeybee populations, which also live in cold climates (Gisder *et al*., 2017), co-infection (both *Nosema* species) prevailed in the *A. m. mellifera* colonies from the Krasnoyarsk Krai (Fig. 2). As recently demonstrated, mixed infections negatively affected honeybee survival more than single *Nosema* infections (Milbrath *et al*., 2015), but this is not consistent with our findings.

Most of the individual bees in studied bee colonies from the Krasnoyarsk populations were infected with two microsporidian species or only *N. ceranae*. Individual infection patterns were similar in the studied bee colonies (Table 1), which may be due to the same time of sampling. All the studied bee colonies were collected in June-July (although in different years), except for colony from Turukhansk (September). Despite the predominance of *N. ceranae* (single or co-infection with *N. apis*) in bees studied, it is premature to suggest the replacement of the traditional *N. apis* by *N. ceranae*. As demonstrated by Sokół and Michalczyk, the microsporidian species in worker bees during the flow season is determined by sampling time (month) (Sokół & Michalczyk, 2012). Considerable intra-colony variation in infection intensity among individual workers was also detected during the spring-summer-fall seasons (Mulholland *et al*., 2012). Finally, in Northeast Germany, a general advantage of *N. ceranae* over *N. apis* and an overall replacement of *N. apis* by *N. ceranae* in the studied honeybee population are not shown. At the same time, a significant predominance of *N. ceranae* infections was revealed in summer and autumn, but not in spring (Gisder *et al*., 2017).

Thus, the *N. ceranae* parasite is widespread in various climatic areas, including in very cold climates. There is probably a climatic influence on *N. ceranae* virulence (Gisder *et al*., 2010). Despite the *N. ceranae* prevalence in Siberia, there were only two cases of mass bee colony losses probably caused by mixed *Nosema* infection (but with a predominance of the *N. apis*) in the spring 2015 in the northern apiaries of the Tomsk region (Ostroverkhova *et al*., 2019a). In addition to geographic (climatic) differences, other factors, for example the genetic variability of the host, may affect the co-adaptation of the host and *Nosema* parasite (Fontbonne *et al*., 2013; Huang *et al*., 2015; Natsopoulou *et al*., 2015). It is assumed that the variation between bee colonies in susceptibility to infection by *N. ceranae* are linked to genetic variability in workers from resistance to tolerance (Fontbonne *et al*., 2013; Huang *et al*., 2015; Martín-Hernández *et al*., 2018). However, as reported by Martín-Hernández *et al*. (2018), in most studies of artificial infections and honeybee populations, no bee subspecies or lineage were identified. It is assumed that in most regions, C-lineage honeybees (*A. m. carnica*, *A. m. ligustica*, and even backfast) were analyzed (Martín-Hernández *et al*. (2018). For the first time, we examined the infestation of the *A. m. mellifera* bee colonies (evolutionary lineage M) with *Nosema* parasites. Interestingly, in an apiary near Yaksha (Fig. 2), from 11 studied bee colonies, only 7 colonies were infected with *N. ceranae*. No *Nosema* infection was detected in 4 bee colonies. A possible explanation for this finding could be genetic differences in *N. ceranae* susceptibility between bee colonies. This uneven distribution pattern of *N. ceranae* and infection level may be caused by a combination of factors such as certain environmental conditions, the genetic background of honeybees that influences *Nosema* distribution, as well as methods beekeeper management practices. Therefore, long-term studies of the *Nosema* spp. prevalence in honeybee populations of various climatic regions, the host-parasite dynamic in specific environmental conditions, as well as the genetic characteristics of bee colonies (and the parasites) are necessary for assessing the contribution of various factors to the bee colony survival, as well as a better understanding of the consequences for beekeeping management.

CONCLUSION

Overall, our results provide evidence that *N. ceranae* infection occurs in bee colonies living in cold climates (the Krasnoyarsk Krai, Siberia), and this parasite is not associated with colony depopulation or honeybee collapse. Further research focusing on genetic diversity of the *Apis mellifera mellifera* bee colonies (as well as other subspecies and lineages) with different susceptibility to *Nosema* infection is required.

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