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Parasites of the genus *Nosema*, *Crithidia* and *Lotmaria* in the honeybee and bumblebee populations: a case study in India

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The populations of honeybees and bumblebees have been decreasing around the world in the recent decades. A variety of pathogens and parasites, including bacteria, fungi, protozoa, nematodes, mites and insects play significant role in honeybee and bumblebee colonies loss. Parasites of the genus *Nosema* (Microsporidia: Nosematidae) and the genera *Crithidia* and *Lotmaria* (Kinetoplastida: Trypanosomatidae) have a significant negative impact on honeybee and bumblebee colonies. Recent studies of nuclear DNA markers of these parasites allowed to describe new species and genetic variants. The aim of this study was to investigate the Microsporidia (*Nosema* spp.) and Trypanosomatidae (*Crithidia* spp. and *Lotmaria passim*) prevalence and genetic diversity in honeybee and bumblebee populations of Indian territories that haven't been studied before. In total 119 specimens of 4 honeybee and 5 bumblebee species were analyzed in this study. The prevalence of parasites in honeybee and bumblebee populations of the two Indian states (Jammu and Kashmir, Karnataka) were identified using PCR with primers specific for the ribosomal RNA genes cluster of *Nosema*, *Crithidia* and *Lotmaria* species. Co-infection by microsporidian and trypanosomatid parasites was detected in several honeybee and bumblebee specimens from Jammu and Kashmir state. Comparative analysis of ribosomal RNA genes sequences showed that honeybee samples from India studied were infected by *N. bombi*, *N. ceranae* and *L. passim*. Bumblebee populations were infected by *Nosema D*, *Crithidia bombi* and *Crithidia expoeki*. No honeybee's specimen with trypanosomatid infection was found in Karnataka state. For the first time *N. bombi* infection was detected in the honeybee population. The studies of distribution of microsporidia and trypanosomatid parasites among the honeybee and bumblebee populations all over the World were summarized and supplemented.

Key words: honeybees; bumblebees; infection; ribosomal RNA genes; *Nosema* spp.; *Crithidia* spp.; *Lotmaria passim*.

Паразитические организмы родов *Nosema*, *Crithidia* и *Lotmaria* в популяциях пчел и шмелей: исследование в Индии

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В последние десятилетия наблюдается резкое снижение численности популяций медоносных пчел и шмелей на территории большинства стран мира. Вклад в снижение численности данных опылителей вносят различные паразитические организмы (бактерии, грибы, простейшие, нематоды, клещи и насекомые). Паразиты рода *Nosema* (Microsporidia: Nosematidae) и родов *Crithidia* и *Lotmaria* (Kinetoplastida: Trypanosomatidae) оказывают значительное негативное влияние на численность медоносных пчел и шмелей. В недавних исследованиях, проведенных с использованием ядерных ДНК-маркеров, были описаны новые виды и генетические варианты данных паразитов. Целью настоящей работы являлось установление уровня зараженности медоносных пчел и шмелей микроспоридиями (*Nosema* spp.) и трипаносоматидами (*Crithidia* spp. и *Lotmaria passim*), а также изучение генетической вариабельности этих паразитов на ранее не исследованной территории Индии.
В работе проанализировано 119 образцов из четырех видов медоносных пчел и пяти видов шмелей. Уровни зараженности популяций пчел и шмелей паразитическими организмами на территории двух штатов (Джамму и Кашмир, Карнатака) были определены с помощью полимеразной цепной реакции с праймерами, специфичными к кластеру генов рибосомной РНК *Nosema*, *Crithidia* и *Lotmaria*. Совместное заражение популяций медоносных пчел и шмелей микроспоридиями и трипаносоматидами было зафиксировано на территории штата Джамму и Кашмир. В результате сравнительного анализа нуклеотидных последовательностей кластера генов рибосомной РНК установлено, что в популяциях медоносных пчел на территории Индии были представлены *N. bombi*, *N. ceranae* и *L. passim*. Популяции шмелей были поражены микроспоридией *Nosema D* и трипаносоматидами *Crithidia bombi* и *Crithidia*

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expoeki. В образцах медоносных пчел, собранных на территории штата Карнатака, паразиты родов *Crithidia* и *Lotmaria* не выявлены. В популяции медоносных пчел впервые выявлена микроспоридия *N. bombi*. Данные о распространении микроспоридий и трипаносоматид в популяциях медоносных пчел и шмелей по всему миру были обобщены и дополнены.

Ключевые слова: медоносные пчелы; шмели; заражение; гены рибосомной РНК; *Nosema* spp.; *Crithidia* spp.; *Lotmaria passim*.

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Microsporidia of the genus *Nosema* (Microsporidia: Nosematidae) and trypanosomatid parasites of the genera *Crithidia* and *Lotmaria* (Kinetoplastida: Trypanosomatidae) have a negative impact on the honeybees and bumblebees colonies fitness (Schmid-Hempel, 2001; Brown et al., 2003; Higes et al., 2008; Hornitzky, 2008; Yourth et al., 2008; Ravoet et al., 2013).

Nosema and *Tubulinosema* species represent the obligate intracellular spore forming organisms that are related to the Fungi (Han, Weiss, 2017). Two microsporidium species, *Nosema ceranae* (Fries et al., 1996) and *Nosema apis* (Zander, 1909), are known to infect honeybees. *Nosema bombi* is another parasite belonging to the phylum Microsporidia which is widespread in the bumblebee populations (Fantham, Porter, 1914). Analysis of standard nuclear DNA markers of *N. bombi* in bumblebee colonies from USA, Russia, China and several European countries revealed new genetic variants of the parasite (Fries et al., 2001; Tay et al., 2005; Szentgyörgyi et al., 2011; Cordes et al., 2012; Li et al., 2012; Vavilova et al., 2015). Three new *Nosema* variants (A, B, and C) isolated from bumblebees in China were suggested to be genetic variants of *N. ceranae* (Li et al., 2012; Vavilova et al., 2015). All detected genetic variants of *Nosema* species did not receive the status of separate species.

Another microsporidium species *Tubulinosema pampeana* (Microsporidia: Tubulinosematidae) was described for the first time in *Bombus araratus* individuals from Argentine (Plischuk et al., 2015). Currently, there are no cases of *T. pampeana* infections in other regions.

Crithidia and *Lotmaria* species are the protozoan flagellated trypanosomatid parasites of honeybees and bumblebees. For a long time *Crithidia mellificae* (Langridge, McGhee, 1967) was the only Trypanosomatidae species described for the *Apis mellifera* and it was poorly investigated. Resent identification of several DNA markers of the American honeybees parasites revealed their high genetic diversity (Cox-Foster et al., 2007; vanEngelsdorp et al., 2009; Runckel et al., 2011; Cornman et al., 2012). After detailed analysis of trypanosome stains the *C. mellificae* SF was redesignated as *Lotmaria passim.* Therefore at the moment two stains of *C. mellificae* (designated as ATCC 30254 and ATCC 30862) and two stains of *Lotmaria passim* (designated as BRL and SF) for honeybee populations are described (Schwarz et al., 2015). Trypanosomatid *Crithidia bombi* (Lipa, Triggiani, 1988) infecting bumblebees is highly researched (Schmid-Hempel, Reber Funk, 2004; Meeus et al., 2010; Schmid-Hempel, Tognazzo, 2010). Microsatellite data showed that several *C. bombi* genotypes circulates in bumblebee populations from Switzerland, Argentina and Chile (Schmid-Hempel, Reber Funk, 2004; Schmid-Hempel et al., 2011, 2014). Recently two new *Crithidia* species, *Crithidia expoeki* and *Crithidia mexicana*, have been identified in bumblebees from North America and Mexica, respectively (Schmid-Hempel, Tognazzo, 2010; Gallot-Lavallée et al., 2016).

Microsporidian and trypanosomatid parasites described above have a negative impact on the honeybees and bumblebees fitness. The parasites cause the rapid honeybees and bumblebees loss at both individual and colony levels (Schmid-Hempel, 2001; Brown et al., 2003; Higes et al., 2008; Hornitzky, 2008; Yourth et al., 2008; Ravoet et al., 2013).

Thus, investigation of these parasites in host populations from new geographical regions allows to characterize new genetic variants and describe it's specific distribution. Despite the high importance of honeybees and bumblebees for the economy of India no studies of their parasites have been performed so far. In this study the diversity of *Nosema*, *Crithidia*, and *Lotmaria* parasites from honeybees and bumblebees in an unexplored regions of India, states Jammu and Kashmir and Karnataka were analyzed.

Materials and methods

Sample collection, DNA extraction, PCR amplification, and sequencing. 80 samples of honeybee species (*A. cerana*, *A. dorsata*, *A. florea* and *A. mellifera*) were collected in two Indian states (Jammu and Kashmir, Karnataka) in May and June, 2007, respectively (Fig. 1, Table). 39 bumblebee specimens of *B. asiaticus*, *B. lucorum*, *B. rufofasciatus*, *B. simillimus* and *B. trifasciatus* species were collected in Jammu and Kashmir state in May, 2007 (see Fig. 1, Table).

All samples were obtained by entomological sweep nets, identified to the species level in the field and preserved in 70 % ethanol. Total DNA were extracted from abdomens of the specimens fixed in ethanol using DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's protocol.

The standard sets of primers and PCR conditions were used for PCR amplification of small subunit ribosomal RNA (SSU rRNA), internal transcribed spacer (ITS) and large subunit ribosomal RNA (LSU rRNA) genes of *Nosema* spp. and *Tubulinosema* spp. (Tay et al., 2005; Szentgyörgyi et al., 2011) and 18S rRNA genes of *Crithidia* spp. and *L. passim* (Meeus et al., 2010; Arismendi et al., 2016). Polymerase chain reactions (PCR) were performed in 20 µl volume containing 0.1 µg of genomic DNA, 10 mM Tris-HCl (pH 8.9), 1 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 µM of each of four dNTPs, 0.5 µM primers, and 2.5 units of Taq DNA polymerase. The PCR products were analyzed in 1.2 % agarose gel electrophoresis and extracted from gel with a QIAquick Gel Extraction Kit (QIAGEN). From 40 ng to 200 ng of each PCR product

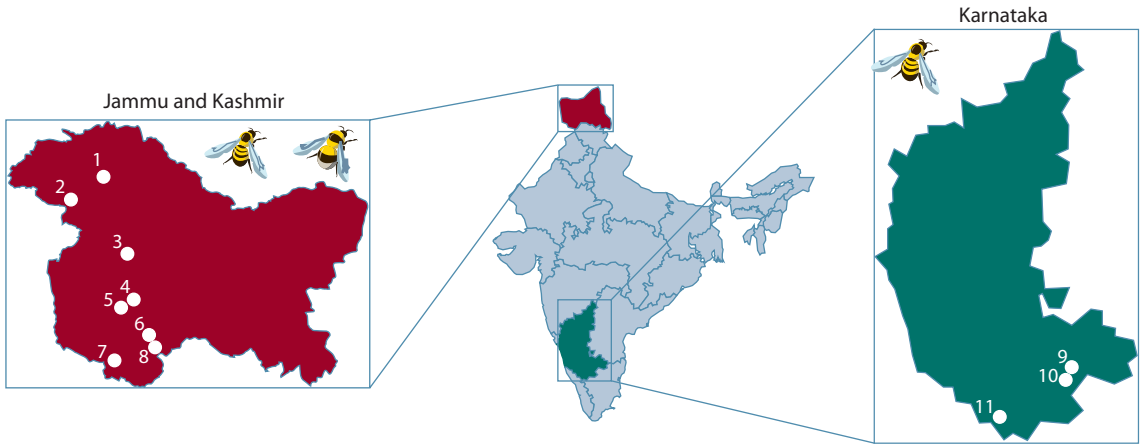

Fig. 1. Map of Indian states with collection sites designation. Collection sites are correlated with the Table.

Collection sites and list of honeybee and bumblebee species used in this study. The site areas are designated according to the Fig. 1

Indian state	Site	Coordinates of sites	Species of genus *Apis* and *Bombus*	Specimen number	Number of infected specimens (positive results of PCR)	
					Nosema spp./ *Tubulinosema* spp.	*Crithidia* spp./ *Lotmaria passim*
Jammu and Kashmir	1	34°08′50.2″ N, 74°53′01.2″ E	*Apis cerana* Fabricius, 1793	4	2/0	0/2
			Apis mellifera Linnaeus, 1758	13	7/0	0/5
	2	34°01′57.2″ N, 74°21′50.5″ E	*A. cerana*	2	0/0	0/0
	3	33°32′09.2″ N, 75°14′57.8″ E	*A. cerana*	1	0/0	0/0
			A. mellifera	5	0/0	0/1
	4	33°07′38.1″ N, 75°22′19.2″ E	*A. mellifera*	9	3/0	0/1
	6	32°59′38.0″ N, 75°42′10.9″ E	*A. cerana*	7	0/0	0/0
	7	32°43′07.4″ N, 74°51′59.1″ E	*A. cerana*	2	0/0	0/0
			Apis dorsata Fabricius, 1793	19	4/0	0/1
			Apis florea Fabricius, 1787	1	0/0	0/0
Karnataka	9	13°01′09.0″ N, 77°34′07.0″ E	*A. cerana*	6	1/0	0/0
			A. dorsata	2	1/0	0/0
			A. florea	2	1/0	0/0
	10	12°53′05.8″ N, 77°28′21.5″ E	*A. mellifera*	5	0/0	0/0
	11	12°01′07.4″ N, 76°06′06.6″ E	*A. dorsata*	2	1/0	0/0
Total specimen number				80	20/0	0/10
Jammu and Kashmir	1	34°08′50.2″ N, 74°53′01.2″ E	*Bombus simillimus* Smith, 1852	8	0/0	2/0
	2	34°01′57.2″ N, 74°21′50.5″ E	*Bombus asiaticus* Morawitz, 1875	2	0/0	1/0
			Bombus lucorum Linnaeus, 1761	1	0/0	0/0
			Bombus rufofasciatus Smith, 1852	1	0/0	0/0
	4	33°07′38.4″ N, 75°22′19.5″ E	*Bombus trifasciatus* Smith, 1852	3	2/0	0/0
	5	33°05′09.9″ N, 75°19′49.2″ E	*B. trifasciatus*	5	2/0	2/0
	8	32°57′11.1″ N, 75°43′31.5″ E	*B. asiaticus*	16	0/0	7/0
			B. trifasciatus	3	0/0	0/0
Total specimen number				39	4/0	12/0

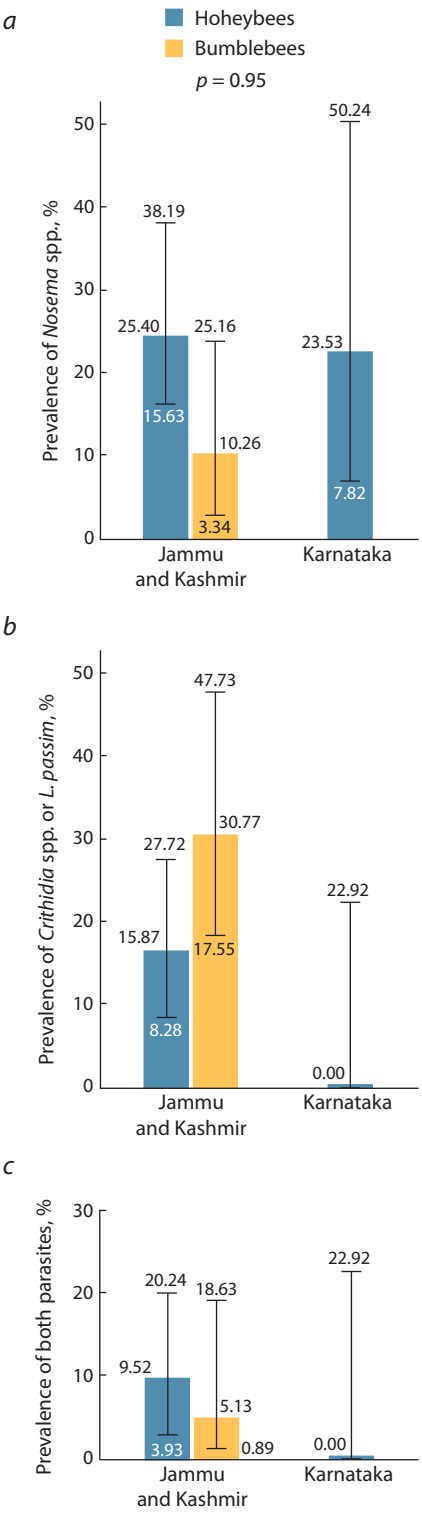

Fig. 2. Prevalence of *Nosema* spp. (*a*), *Crithidia* spp. or *L. passim* (*b*) infection and co-infection of all parasites (*c*) in honeybee and bumblebee populations from Jammu and Kashmir and Karnataka states.

Bars represent confidence intervals defined by chi-square test ($p = 0.95$) in STATISTICA. The studied parasites were characterized by low host-specificity; thus, their prevalence was calculated as percentage of the infected specimens to the total number of honeybee or bumblebee specimens in each sampling location.

was used in a 10-µl cycle sequencing reaction with the BigDye® Terminator Kit (Applied Biosystems) and analyzed on an ABI 3130XL Genetic Analyser (Applied Biosystems) at the SB RAS Genomics Core Facility (Novosibirsk, Russia, http://sequest.niboch.nsc.ru). The partial SSU rRNA sequences of *N. bombi* and *N. ceranae* were deposited in the European Nucleotide Archive (ENA) under accession numbers HG321408–HG321411 and LT548980–LT548999. The obtained SSU rRNA, ITS2, and LSU rRNA gene sequences of *Nosema* spp. were deposited to GenBank under accession numbers KF188752– KF188755. The partial sequences of 18S rRNA gene from *Crithidia* spp. and *L. passim* were placed in GenBank under accession numbers KX151671–KX151702.

Parasite prevalence. The prevalence of microsporidia and trypanosomatid parasites was calculated as percentage of the infected specimens to the total number of honeybee or bumblebee specimens in each sampling sites. Confidence intervals for parasite prevalence were defined using STATISTICA by chi-square test.

Comparative and phylogenetic analyses. Logical search was performed to identify rRNA genes sequences from insects parasites of the phylum Microsporidia and family Trypanosomatidae available from the United States National Center for Biotechnology Information. Nucleotide sequences alignments were performed by ClustalW (Larkin et al., 2007) and improved by MUSCLE algorithm (Edgar, 2004) in Unipro UGENE software (Okonechnikov et al., 2012) (http://ugene.unipro.ru) for each parasite group. Phylogenetic analyses were performed using the Neighbor-Joining (NJ) method in MEGA 6.0 and Maximum Likelihood (ML) method in PhyML 3.0 (Guindon, Gascuel, 2003; Tamura et al., 2013). Statistical support for the NJ and ML trees was evaluated by bootstrapping, 100 replications for the ML method and 1,000 replications for the NJ method (Felsenstein, 1985).

Results and discussion

Prevalence of parasite infection in honeybee and bumblebee populations

We studied 80 honeybee and 39 bumblebee specimens collected in two Indian states. *Nosema* spp. were detected by PCR amplification with primers specific to SSU rRNA sequence. *Nosema* spp. were discovered in 20 honeybee specimens from all investigated honeybee species and 4 bumblebee specimens of *B. trifasciatus*. The prevalence of *Nosema* spp. in honeybee populations was 25 and 24 % in Jammu and Kashmir, and Karnataka states, respectively (Fig. 2, *a*). In Jammu and Kashmir state the prevalence of *Nosema* spp. in bumblebee population was 10 %.

The trypanosomatid parasites were identified by PCR amplification with primers specific to 18S rRNA sequence in 10 honeybee and 12 bumblebee specimens. *A. cerana*, *A. dorsata*, *A. mellifera*, *B. asiaticus*, *B. simillimus* and *B. trifasciatus* species were infected. In Jammu and Kashmir state the prevalence of trypanosomatid parasites was 16 and 31 % in honeybee and bumblebee populations, respectively (Fig. 2, *b*). No infected honeybee specimens were found in Karnataka state.

Co-infection by *Nosema* spp. and one of the trypanosomatid parasites (*Crithidia* spp. or *L. passim*) was detected in 6 honeybee (*A. cerana* and *A. mellifera*) and 2 bumblebee (*B. trifasciatus*) specimens in Jammu and Kashmir state (Fig. 2, *c*).

Genetic diversity of *Nosema* spp. in honeybee and bumblebee populations

Comparative analyses of the SSU rRNA sequences of *Nosema* spp. Totally, we obtained 24 nucleotide sequences of *Nosema* spp. SSU rRNA gene from honeybees and bumblebees (see Table). The results of the comparative analysis showed that 13 out of 20 sequences from honeybee specimens were identical to *N. bombi* SSU rRNA sequences (KF002566, HG321391, KF188769, JN872234, and JN872233). *N. bombi* was described for bumblebees only. The remaining 7 sequences were identical to SSU rRNA of *N. ceranae* (KF640602, JX205150).

Previously *Nosema* species were considered to be the host specific parasites. *N. apis* infected only the European honeybee *A. mellifera*, while *N. ceranae* was a specific parasite for the Asian honeybee *A. cerana* (Smith, 2012). In the recent years *N. ceranae* was identified in *A. mellifera* and some bumblebee species in the different parts of the world. Moreover, some stains of *N. ceranae* are predicted to replace *N. apis* in populations of *A. mellifera* honeybees (Chen, Huang, 2010;

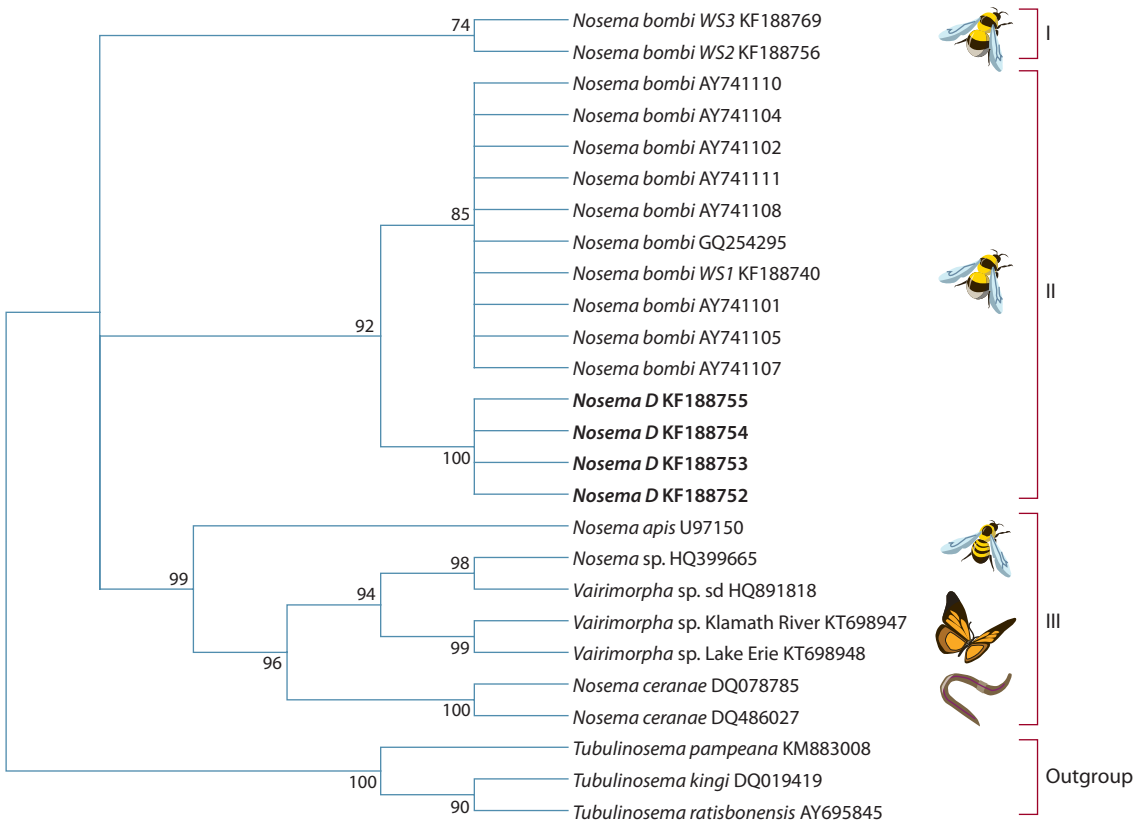

Fig. 3. Neighbor-joining (NJ) phylogenetic tree was based on SSU rRNA, ITS2, and the partial LSU rRNA nucleotide sequences of several *N. bombi* genetic variants, *Nosema* spp. and *Vairimorpha* spp. from various insects and annelids available from GenBank. *Tubulinosema* spp. were used as outgroup.

Statistical support was evaluated by bootstrapping (1000 replications). The nodes with bootstrap values over 50 % are indicated.

Martín-Hernández et al., 2012). In this study a bumblebee parasite *N. bombi* was identified in honeybees from Jammu and Kashmir and Karnataka states, thus additionally refuting the assumption of the host specificity of *Nosema* species.

All *Nosema* spp. SSU rRNA sequences from infected bumblebee specimens were almost identical to *Nosema D* sequences (JN872219–JN872229) (Li et al., 2012). A distinguish is only a single nucleotide substitution. No other *Nosema* as well as *Tubulinosema* species were found in bumblebees in this study (see Table).

Comparative and phylogenetic analyses of SSU rRNA, ITS2 and partial LSU rRNA sequences from *Nosema* spp. To expand information about *Nosema D* we obtained SSU rRNA, ITS2, and partial LSU rRNA sequences for four *B. trifasciatus* specimens. Sequences of *Vairimorpha* spp., *N. bombi*, *N. ceranae*, *N. apis*, and *Nosema* sp. from *Pieris rapae* from GenBank, as well as the obtained sequences were used for phylogenetic analysis. Sequences of several *Tubulinosema* species were taken as an outgroup. Phylogenetic tree built by the NJ method is presented in Fig. 3.

The phylogenetic tree was divided on outgroup and three clusters (see Fig. 3). The outgroup was presented by sequences of *T. pampeana*, which were described as parasite of *B. araratus* from South America and two parasites of *Drosophila* spp. (*T. ratisbonensis* and *T. kingi*). The first cluster (I) consists of sequences of *N. bombi WS2* and *N. bombi WS3* that were previously described in populations of bumblebee from West Siberia (Vavilova et al., 2015). The second cluster (II) includes two clades. The sequences of *N. bombi* previously identified from the Europe, USA and West Siberia formed the first clade (Tay et al., 2005; Sokolova et al., 2010; Szentgyörgyi et al., 2011). The second clade consists of the newly identified *Nosema D* sequences. The third cluster (III) is also split into two clades. Sequence of *N. apis*, obtained from *A. mellifera* apiary specimens in New Zealand (Gatehouse, Malone, 1998), is in the first clade of the third cluster. The second clade of this cluster consists of sequences of several *Vairimorpha* spp. from *Bombyx mori* and *Manayunkia speciosa* (Liu et al., 2012; Malakauskas et al., 2015); sequences of *N. ceranae* from Taiwan honeybees (Huang at al., 2007); and sequence of unspecified *Nosema* from *Pieris rapae* (Chen et al., 2012). Thus, the analysis of complete SSU rRNA, ITS2 and partial LSU rRNA gene sequences confirmed that *Nosema D* is a genetic variant of *N. bombi* and distributed in the bumblebee populations at least in China and India.

Genetic diversity of *Crithidia* spp. and *Lotmaria passim* in honeybee and bumblebee populations

Comparative analyses of the 18S rRNA sequences of *Crithidia* spp. and *Lotmaria passim*. We obtained 10 and 12 nucleotide sequences of 18S rRNA gene of trypanosomatid parasites in honeybees and bumblebees, respectively

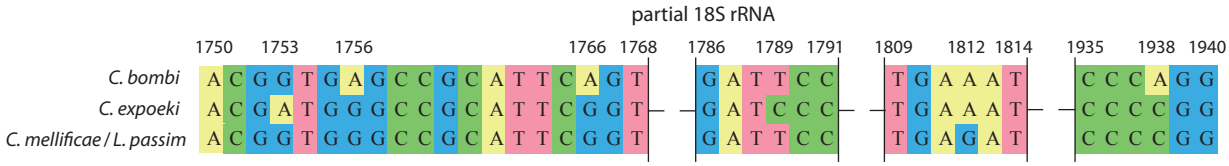

Fig. 4. Alignment of partial 18S rRNA gene sequences of *C. bombi*, *C. expoeki* and *C. mellificae*/*L. passim* specified from honeybee and bumblebee specimens in this study.

Nucleotide positions are indicated according to *Crithidia fasciculata* sequence (Y00055) of full-length rRNA gene cluster.

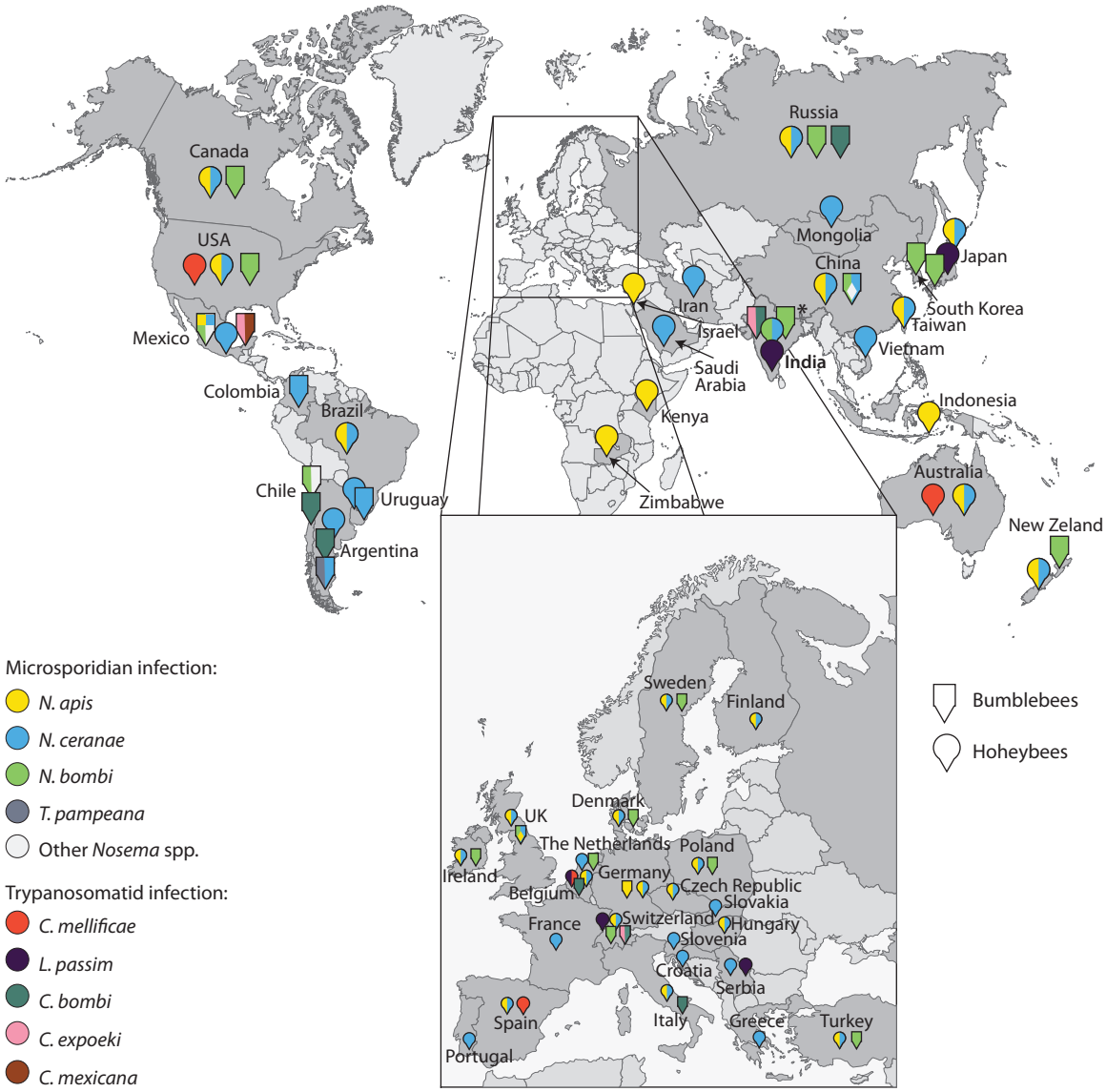

Fig. 5. World map of microsporidian and trypanosomatid distributions across the honeybee and bumblebee populations.

* *Nosema D*, which was determined in bumblebee populations from China (Li et al., 2012) and from India (the present study).

(see Table). The results of comparative analysis showed that nine of infected bumblebee specimens refer to *C. expoeki* (KM980187) and three others refer to *C. bombi* (FN546181, KM980184, KM980185). The distinguish between *C. bombi* and *C. expoeki* sequences amounted five nucleotide substitutions (Fig. 4).

All ten 18S rRNA gene sequences were identical and they could belong to either *C. mellificae* or *L. passim* parasites. Sequences of *C. mellificae*/*L. passim* differ in 4 and 3 nucleotide substitutions from *C. bombi* and *C. expoeki*, respectively (see Fig. 4). Using the primers for 18S rRNA specific to *L. passim* and to *C. mellificae* (Arismendi et al., 2016) on the next

step, we proved that all the obtained sequences belonged to *L. passim* (KJ713378, KM980188, KT252553, KX953206).

Summarizing the data about microsporidian and trypanosomatid parasites in honeybee and bumblebee population from India, we identified that two *A. cerana* and one *A. mellifera* specimens were co-infected by *N. ceranae* and *L. passim*; three specimens of *A. mellifera* were infected by both *N. bombi* and *L. passim*. Co-infection by *Nosema D* and *C. expoeki* in bumblebee populations was established in two *B. trifasciatus* specimens. No cases of *Nosema D* and *C. bombi* co-infection were found in this study.

Co-infection by *N. ceranae* and *L. passim* was also previously established in honeybee samples from Switzerland (Tritschler et al., 2017). Infection of both *N. ceranae* and *C. mellificae* parasites was described for honeybees from Belgian apiaries (Ravoet et al., 2013) (see Fig. 4). Nevertheless, sequences of *C. mellificae* 18S rRNA, identified by Ravoet et al. (2013), were identical for *C. mellificae* and *L. passim*. Thus, these data should be clarified. Gallot-Lavallée et al. (2016) investigated co-infection by *Nosema* spp. and *Crithidia* spp. in the bumblebee populations from Mexico and established the cases of shared parasite infection (Fig. 5). However, species of *Nosema* and *Crithidia* genera found in infected bumblebee samples were not specified. Our data about co-infection of honeybee and bumblebee specimens by microsporidia and trypanosomatid parasites coincide with previously described studies. For the first time *N. bombi*/*L. passim* and *N. bombi*/*C. expoeki* co-infection were detected.

Geographic distribution of *Nosema* spp., *Crithidia* spp. and *L. passim* in honeybee and bumblebee populations

The results of this study supplement the knowledge of the distribution of microsporidia parasites among the honeybee and bumblebee populations all over the world (see Fig. 5).

Apian parasites of genus *Nosema* (*N. apis* and *N. ceranae*) are wildly distributed in honeybee populations. Joint presence of these parasites was described in numerous studies (Table S1 in the Supplementary material)[1]. Nevertheless, there are cases in the several countries such as Indonesia, Israel, Kenya and Zimbabwe of honeybee infections by *N. apis* only (see Table S1). Presence of *N. ceranae* only was established in the honeybee's population from countries of Latin America (except Brazil), several European countries, Iran, Mongolia, Saudi Arabia and Vietnam (see Table S1). In this study, we discovered that honeybee populations were infected by *N. ceranae*. There were no cases of *N. apis* presence. Presence of *N. bombi* parasite in honeybee specimens was detected for the first time (see Fig. 5).

N. bombi is widespread in the natural and commercial bumblebee populations of North and South America, Eurasia and New Zealand (Gallot-Lavallée et al., 2016; Brown, 2017). Several *N. bombi* genetic variants (*WS1*, *WS2* and *WS3*) were described in Siberian bumblebee populations (Vavilova et al., 2015). Four new *Nosema* variants (A, B, C and D) were isolated from bumblebees in China (Li et al., 2012). The microsporidian parasite, *T. pampeana*, was described in bumblebee populations from Argentina (Plischuk et al., 2015). In the recent decades the cases of bumblebees infection by apian parasites *N. ceranae* (Argentina, China, Colombia, Mexico, UK and Uruguay), *N. apis* (Mexico and UK) and other *Nosema* species (Chile, China and Mexico) have been described (Gallot-Lavallée et al., 2016; Brown, 2017). We established the presence of *Nosema D* in bumblebee population from Jammu and Kashmir state (India). *Nosema D* was previously described by (Li et al., 2012) (see Fig. 5).

Presence of two trypanosomatid parasites, *C. mellificae* and *L. passim*, was indicated in honeybee populations globally. *C. mellificae* was found in honeybee specimens from Australia, Belgium, USA and Spain (Table S2). *L. passim* infections were described for honeybees from Belgium, Japan, Serbia and Switzerland (see Table S2). In this study distribution of *L. passim* in Indian honeybee populations were established (see Fig. 5). There were no cases of honeybee infection by *C. mellificae*.

The cases of trypanosomatid infections were determined in commercial and native populations of bumblebees on the territories of North and South America and Eurasia. *C. bombi* is the most common trypanosomatid parasite that infects bumblebees from Argentina, Belgium, Chile, Germany, Italy, Russia, Switzerland and UK (Table S3). The second species *C. expoeki* is presented in Mexican and Swiss bumblebee populations (Schmid-Hempel, Tognazzo, 2010; Gallot-Lavallée et al., 2016). Bumblebee infection by *C. mexicana* was indicated in Mexico (Gallot-Lavallée et al., 2016). Both *C. bombi* and *C. expoeki* are distributed among bumblebee populations from India (see Fig. 5).

Thus, in this study the prevalence of *Nosema*, *Crithidia* and *Lotmaria* parasites in honeybee and bumblebee populations of Jammu and Kashmir and Karnataka states were identified. In addition, co-infection by Microsporidia and Trypanosomatidae parasites was identified in several honeybee and bumblebee specimens from Jammu and Kashmir state. Honeybee and bumblebee specimens from India studied were infected by several microsporidian parasites (*N. bombi*, *N. ceranae* and *Nosema D*). Trypanosomatid parasites of *C. bombi*, *C. expoeki* and *L. passim* species were detected in honeybee and bumblebee populations. Moreover, for the first time *N. bombi* infection was detected in the honeybee population. Thus, further investigations are required to determine distribution of microsporidia and trypanosomatid parasites among the honeybee and bumblebee populations all over the World.

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Conflict of interest

The authors declare no conflict of interest.

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[1] Supplementary materials are available in the online version of the paper: http://www.bionet.nsc.ru/vogis/download/pict-2017-21/appx15.pdf

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