J. Entomol. Res. Soc., 23(3): 305-319, 2021 *Research Article*
Doi: 10.51963/jers.v23i3.2021 *Online ISSN:2651-3579*

Molecular Characterization of the *Vairimorpha (Nosema) ceranae* Infection from *Bombus terrestris* (Linnaeus, 1758) (Hymenoptera: Apidae) in Turkey

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ABSTRACT

The current study aimed to determine the natural Microsporidium pathogen of the *Bombus terrestris* L. (Hymenoptera: Apidae) in Turkey (Mersin, Antalya, Muğla, İzmir, Aydın). During 2019 and 2020, the commercial and wild populations of *B. terrestris* were investigated in this survey. In the studies, natural microsporidiosis was detected in commercial *B. terrestris* populations. Fresh oval spores were measured as 4.91 ± 0.48 (6.12 - 3.73) µm in length and 2.54 ± 0.31 (3.27 - 1.88) µm in width (n=60). Both SSU rRNA and RPB1 gene sequences of the current microsporidium were top hits with the *Vairimorpha (Nosema) ceranae* isolates. While the SSU rRNA gene sequence matched with the *Vairimorpha ceranae* clone NCS44 (LC510228) isolated from the *Apis cerana japonica* at 99.24% identity (100% coverage), the RPB1 gene sequence was matched with the *Vairimorpha ceranae* isolate 1994 (KJ473287) at 99.02% identity (100% coverage). Based on the light microscopy and molecular phylogeny the current microsporidium was a new isolate of the *V. ceranae* and named here in as *Vairimorpha ceranae* Tr-07.

Key words: *Bombus terrestris*, microsporidiosis, RPB1, SSU rRNA, *Vairimorpha ceranae*.

Tosun, O. & Bekircan, Ç. (2021). Molecular characterization of the *Vairimorpha* (*Nosema*) *ceranae* infection from *Bombus terrestris* (Linnaeus, 1758) (Hymenoptera: Apidae) in Turkey. *Journal of the Entomological Research Society*, 32(3), 305-319.

INTRODUCTION

Bees are infected by lots of pathogens and parasites that cause abnormalities in their metabolism, immune system, behavior and perception (Antúnez et al, 2009; Gómez-Moracho, Heeb, & Lihoreau, 2017; Li, Chen & Cook, 2018). As a result of these infections, bee individuals and colonies lose their fitness. Undoubtedly, *Vairimorpha* (*Nosema) ceranae* is the most common pathogen in bee species. The infection caused by *V. ceranae*, show different symptoms on their hosts at the physiological levels as changing gene expression in the brain, inhibiting the apoptosis of epithelial cells and deregulating immune responses (Holt, Aronstein, & Grozinger, 2013; Martín-Hernández et al, 2017; 2018) and behavioral levels as starting foraging earlier in life, exhibiting more frequent but shorter foraging flights, reducing homing abilities and lowering olfactory learning performances (Wolf et al, 2014; Dosselli, Grassl, Carson, Simmons, & Baer, 2016; Perry, Søvik, Myerscough, & Barron, 2016; Gage et al, 2018).

In recent years*, V. ceranae* has been frequently identified in wild bee species, especially bumblebees (*Bombus* spp.) (Plischuk et al, 2009; Li et al, 2012; Graystock, Yates, Darvill, Goulson, & Hughes, 2013). The *V. ceranae* infection in bumblebees causes reduced foraging performance of all colonies and damages their cognitive skills (Piiroinen & Goulson, 2016).

Numerical declines and local extinctions in bumblebee species have been reported in recent years. Studies have shown that four species have begun to disappear in Europe, and two species have been completely extinct in the British Isles (Goulson, Lye, & Darvill, 2008). Also, significant decreases were found in the populations of different bumblebees in North America, England and Ireland (Fitzpatrick et al, 2007; Grixti, Wong, Cameron, & Favret, 2009; Williams & Osborne, 2009). it has been stated that one of the important causes of these losses is parasites and pathogens (Cox-Foster et al, 2007; Cameron et al, 2011).

Bumblebees, which are important in pollination was determined about a hundred years ago, have been mass-produced for the past 25 years and are widely used as pollinators in greenhouse cultivation (Güler, Aytekin, & Dikmen, 2011; Argun Karslı & Gürel, 2015). More than one million bumblebees are commercially produced annually in the world, and more than 90% of this is *Bombus terrestris* L. (Hymenoptera: Apidae) (Velthius & Doorn, 2006). Parasites and pathogens in bumblebees must be accurately and rapidly identified to prevent damage to native species and to safely carry out commercial bumblebee colony breeding. For this aim, the present study tries to determine the natural pathogen and parasites of the *Bombus terrestris* L. in Turkey.

MATERIALS AND METHODS

Specimen collection and Light microscopical observation

In this study, commercially produced and wild-type members of the *B. terrestris* were collected and were examined for parasites and pathogens. While the commercially produced members were collected from greenhouses in five different provinces

(Mersin, Antalya, Muğla, İzmir, Aydın), the wild type adult members of the *B. terrestris* were collected from the north-east part of Turkey (Artvin, Trabzon, Rize, Giresun, Ordu) in 2019-2020. During the field study, due to the low population densities of the wild type, the sample numbers were less than expected. Samples were caught with sweep nets and live samples were transported immediately to the laboratory for further examinations. On the other hand, the samples which were collected commercially produced members from greenhouses lands or hives, had been found dead when collected. Members of *B. terrestris* morphologically identified according to Mauss (1994) and for wet mount preparation samples were dissected with Ringer's solution and examined under the light microscope (Tosun, 2020; Yıldırım & Bekircan, 2020). Infection positive slides were photographed using Zeiss AXIO microscope equipped with an Axicam ERc5s digital camera. The necessary measurements and analysis were made using ZEN 2.3 Blue Edition imaging software. While some infection-positive remaining tissues were preserved in 95% ethanol for molecular studies, others were preserved in 2.5% glutaraldehyde in PBS for transmission electron microscopy.

DNA extraction, Amplification and Molecular analysis

Ethanol-fixed infected tissues were washed with distilled water 3 times (15 min) to remove ethanol. The genomic DNA was extracted using the QIAGEN DNA Isolation Kit, No: 69504 according to the manufacturer's instructions. To amplify the SSU rRNA gene, the QIAGEN Multiplex PCR Kit (No. 206143) and 18F/1537R primer set was used (Baki & Bekircan, 2018). Amplification processes were performed according to the kit's protocol in a 50 µl reaction system. Amplification conditions were as follows: an initial denaturation step at 95 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 60°C for 90 s, elongation at 72 °C for 2 min and a final extension at 72 °C for 10 min. Also, in the present study, the largest subunit of RNA polymerase II (RPB1) gene alignments were amplified as in the SSU rRNA gene. To amplify, the QIAGEN Multiplex PCR Kit (No. 206143) and primer set were used (Tosun, 2020; Yıldırım, 2021). The base sequences of the SSU rRNA and RPB1 gene were determined in the Macrogen Inc. Company, The Netherlands.

The sequences fragments were assembled using BioEdit and obtained consensus sequences (Hall, 1999)"mendeley":{"formattedCitation":"(Hall, 1999. Sequences with high similarity were determined according to the BLAST search and those of our interest were retrieved from the NCBI GenBank database and the literature (Table 1). The new combinations were used as re-assigned in 2020 by Tokarev et al, in the phylogenetic analysis (Tokarev et al, 2020). In the analysis, all sequences were aligned with CLUSTAL_W. Pairwise genetic distances were determined using the Kimura-2 parameter. Phylogenetic analyses were conducted using the maximum likelihood (ML) method in MEGA 10. Bootstrap confidence values were calculated with 1000 repetitions and the optimal evolutionary model was determined as GTR +I + G.

Table 1. Small subunit (SSU) ribosomal RNA and RNA polymerase II largest subunit (RPB1) gene sequences used for phylogenetic analyses.

	Accession No	Organism name	Host	Order	Family
SSU rRNA	**MW396669**	***Vairimorpha* (*Nosema*) *cerenae*Tr-07**	***Bombus terrestris***	**Hymenoptera**	**Apidae**
	LC510251	*Vairimorpha ceranae* (Japan)	*Apis cerana*	Hymenoptera	Apidae
	LC510228	*Vairimorpha ceranae* (Japan)	*Apis cerana*	Hymenoptera	Apidae
	DQ673615	*Vairimorpha ceranae* (Switzerland)	*Apis mellifera*	Hymenoptera	Apidae
	DQ329034	*Vairimorpha ceranae* (Spain)	*Apis mellifera*	Hymenoptera	Apidae
	KU937104	*Vairimorpha ceranae* (India)	*Apis mellifera*	Hymenoptera	Apidae
	KC680654	*Vairimorpha ceranae* (Thailand)	*Apis mellifera*	Hymenoptera	Apidae
	KC680650	*Vairimorpha ceranae* (Thailand)	*Bombus sp.*	Hymenoptera	Apidae
	JN872261	*Vairimorpha ceranae* (China)	*Bombus sp.*	Hymenoptera	Apidae
	DQ235446	*Vairimorpha apis* (Spain)	*Apis mellifera*	Hymenoptera	Apidae
	FJ789796	*Vairimorpha apis* (Australia)	*Apis mellifera*	Hymenoptera	Apidae
	U11047	*Vairimorpha vespula*	*Vespula vulgaris*	Hymenoptera	Vespidae
	Y00266	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
	HM370543	*Nosema bombi* (Russia)	*Bombus lucorum*	Hymenoptera	Apidae
	KF002566	*Nosema bombi* (Mexico)	*Bombus ephippiatus*	Hymenoptera	Apidae
	JN872231	*Nosema bombi* (China)	*Bombus sp.*	Hymenoptera	Apidae
	MF776532	*Nosema bombi* (Thailand)	*Bombus sp.*	Hymenoptera	Apidae
	AY741105	*Nosema bombi* (Ireland)	*Bombus pascuorum*	Hymenoptera	Apidae
	KF916504	*Nosema bombi* (Turkey)	*Bombus sp.*	Hymenoptera	Apidae
	D85503	*Nosema bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
	KT020736	*Nosema fumiferanae*	*Epiphyas postvittana*	Lepidoptera	Tortricidae
	L39109	*Endoreticulatus schubergi*	*Cholistoneura fumiferana*	Lepidoptera	Tortricidae
RPB1	**MW415412**	***Vairimorpha* (*Nosema*) *cerenae*Tr-07**	***Bombus terrestris***	**Hymenoptera**	**Apidae**
	KJ473287	*Vairimorpha ceranae* (Chile)	*Apis mellifera*	Hymenoptera	Apidae
	KM001627	*Vairimorpha ceranae* (China)	*Apis ceranae*	Hymenoptera	Apidae
	DQ996230	*Vairimorpha apis*	*Apis mellifera*	Hymenoptera	Apidae
	AF060234	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
	DQ996236	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
	JX213749	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Lymantria
	JX239748	*Vairimorpha disparis*	*Lymantria dispar*	Lepidoptera	Erebidae
	MT461295	*Nosema fumiferanae* TY61	*Apomyelois (Ectomyelois) ceratoniae*	Lepidoptera	Pyralidae

Table 1. Continued.

	Accession No	Organism name	Host	Order	Family
	HQ457435	*Nosema fumiferanae*	*Choristoneura fumiferana*	Lepidoptera	Tortricidae
	HQ457436	*Nosema sp.*	*Choristoneura occidentalis*	Lepidoptera	Tortricidae
	AJ278948	*Nosema tyriae*	*Tyria jacobaeae*	Lepidoptera	Arctiidae
	DQ996231	*Nosema bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
	DQ996234	*Nosema trichoplusiae*	*Trichoplusia ni*	Lepidoptera	Noctuidae
	HQ457438	*Nosema disstriae*	*Malacasoma disstria*	Lepidoptera	Lasiocampidae
	DQ996232	*Nosema empoascae*	*Empoasca fabae*	Homoptera	Cicadellidae
	DQ996233	*Nosema granulosis*	*Gammarus duebeni*	Amphipoda	Gammaridae
	XM 014708712	*Ordospora colligata*	*Daphnia magna*	Cladocera	Daphniidae

RESULTS

Light microscopy

In the present study, the commercially produced members of the *B. terrestris* were collected from greenhouses where tomato production was carried out in five different provinces: Mersin, Antalya, Muğla, İzmir and Aydın. In this survey, 547 samples were collected from greenhouses and examined during 2019-2020. As a result of the examinations, 51 samples were infected by the microsporidian pathogen (infection rate: 9.32%). Determined fresh oval spores were measured as 4.91 ± 0.48 (6.12 - 3.73) µm in length and 2.54 ± 0.31 (3.27 - 1.88) µm in width (n=60). Infected members gut systems fully filled with the oval mature spores (Fig. 1). In addition, during this study 171 wild members were collected from the provinces (Artvin, Rize, Trabzon, Giresun and Ordu) where those of determined before. As a result of the examinations, no microsporidiosis was found in the smears prepared from wild members (Table 2). Therefore, this group was not included in subsequent statistical analysis.

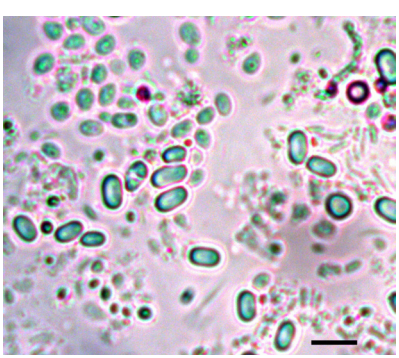

Fig. 1. The light micrograph of the *V. ceranae* Tr-07 fresh oval spores, bar: 5µm.

The infection prevalence was calculated based on the rate of bumblebees determined to be microsporidiosis positive with the microscopic examination in this study. After the analysis, infection prevalence based on provinces was determined as 14.28% in Mersin, 15.74% in Antalya, 14.28% in Muğla, respectively. No infection was detected in İzmir and Aydın. When comparing the infection rates on a year and month basis it was seen that the infection rate was 9.81% in 2019 and 8.86% in 2020 where in months, these rates were a range from 9.65% in May, 10.16% in June and 8.15% in July, respectively (Table 2).

Molecular phylogeny

The molecular phylogeny of the current microsporidium which isolated from infected *B. terrestris* tissues was based on the partial SSU rRNA and RPB1 gene. An 1177 nucleotide section of the SSU rRNA and 674 nucleotides of the RPB1 were obtained with 35.9% and 32.5% GC content after the sequencing. And these sequences of the current microsporidium were deposited in GenBank with MW396669 and MW415412 accession codes. Each sequence was subjected to BLAST analysis that matched only microsporidian records. Both SSU rRNA and RPB1 gene sequences of the current microsporidium were top hits with the *V. ceranae* isolates. While the SSU rRNA gene sequence matched with the *Vairimorpha ceranae* clone NCS44 (LC510228) isolated from the *Apis cerana japonica* at 99.24% identity (100% coverage), the RPB1 gene sequence was matched with the *Vairimorpha ceranae* isolate 1994 (KJ473287) at 99.02% identity (100% coverage).

The pairwise distance analysis that carried for the SSU rRNA gene sequence, was conducted with 22 microsporidian sequences. Pairwise phylogenetic distances between the current microsporidium and other species ranged from 0.010 to 0.505. The distance between the current microsporidium and the type species of the genus, *Vairimorpha necatrix* (Pilley, 1976) was determined as 0.068 (Table 3). Also, it was differentiating from the *Nosema bombycis* (Nägeli, 1857), the type species of *Nosema* genus, with 0.243 difference.

For RPB1 gene sequence, 18 microsporidian sequences were used in the pairwise phylogenetic distance analysis. And the distances were ranged from 0.010 to 0.388. In the analysis made on the RPB1 gene, they gave results that support the results of the analysis made with the SSU rRNA gene. And, the current microsporidium was more closely related to the *Vairimorpha* species (Table 3).

In conclusion, based on the morphological and molecular information, the current microsporidium isolated from *B. terrestris* was a new isolate of *Vairimorpha ceranae*.

Table 2. *Vairimorpha* (*Nosema*) *cerenae* infection in *B. terrestris* from the different sampling localities and months.

	Sample type	Province	Months	Dissected samples	Total	Infected samples	Infection rate (%)	Total infection rate (%)
2019	Commercial	Mersin	May	15	48	4	26.67	14.58
			June	20		3	15	
			July	13		-	-	
		Antalya	May	20	62	3	15	19.35
			June	22		5	22.73	
			July	20		4	20	
		Muğla	May	15	52	2	13.33	13.46
			June	17		3	17.64	
			July	20		2	10	
		İzmir	May	15	55	-	-	-
			June	20		-	-	
			July	20		-	-	
		Aydın	May	17	48	-	-	-
			June	13		-	-	
			July	18		-	-	
	Wild	Artvin	April	9	18	-	-	-
			May	6		-	-	
			June	3		-	-	
		Rize	April	6	15	-	-	-
			May	4		-	-	
			June	5		-	-	
		Trabzon	April	10	21	-	-	-
			May	7		-	-	
			June	4		-	-	
		Giresun	April	5	14	-	-	-
			May	3		-	-	
			June	6		-	-	
		Ordu	April	3	13	-	-	-
			May	5		-	-	
			June	5		-	-	

Table 2. Continued.

	Sample type	Province	Months	Dissected samples	Total	Infected samples	Infection rate (%)	Total infection rate (%)
2020	Commercial	Mersin	May	22	57	3	13.63	14.03
			June	20		2	10	
			July	15		3	20	
		Antalya	May	18	65	3	16.66	12.30
			June	22		2	9.09	
			July	25		3	12	
		Muğla	May	20	60	2	10	13.33
			June	18		2	11.11	
			July	22		4	18.18	
		İzmir	May	22	55	-	-	-
			June	18		-	-	
			July	15		-	-	
		Aydın	May	14	45	-	-	-
			June	13		-	-	
			July	18		-	-	
	Wild	Artvin	May	4	13	-	-	-
			June	6		-	-	
			July	3		-	-	
		Rize	May	9	19	-	-	-
			June	5		-	-	
			July	5		-	-	
		Trabzon	May	11	25	-	-	-
			June	6		-	-	
			July	8		-	-	
		Giresun	May	5	15	-	-	-
			June	6		-	-	
			July	4		-	-	
		Ordu	May	6	18	-	-	-
			June	8		-	-	
			July	4		-	-	
			GENERAL TOTAL		**718**			**7.10%**

Table 3. Comparison of current microsporidium and other related microsporidia based on the small subunit ribosomal RNA gene (SSU rRNA) and the largest subunit of RNA polymerase II (RPB1) gene by query cover, by nucleotide identity, by Pairwise distance analysis, and GC% content.

SSU rRNA	MW396669	*Vairimorpha* (*Nosema*) *cerenae*Tr-07	Query cover %	Pairwise distances	GC content (35.9%)
	LC510251	*Vairimorpha ceranae* (Japan)	99	0.01046	36.3
	LC510228	*Vairimorpha ceranae* (Japan)	99	0.01046	38.1
	DQ673615	*Vairimorpha ceranae* (Switzerland)	98	0.01752	38.8
	DQ329034	*Vairimorpha ceranae* (Spain)	98	0.01752	36.1
	KU937104	*Vairimorpha ceranae* (India)	99	0.01046	40
	KC680654	*Vairimorpha ceranae* (Thailand)	92	0.01046	36.1
	KC680650	*Vairimorpha ceranae* (Thailand)	92	0.01046	37.3
	JN872261	*Vairimorpha ceranae* (China)	42	0.01046	41.3
	DQ235446	*Vairimorpha apis* (Spain)	99	0.09585	38.7
	FJ789796	*Vairimorpha apis* (Australia)	92	0.09585	38.5
	U11047	*Vairimorpha vespula*	99	0.03184	36.8
	Y00266	*Vairimorpha necatrix*	99	0.06882	37.4
	HM370543	*Nosema bombi* (Russia)	58	0.06883	35.8
	KF002566	*Nosema bombi* (Mexico)	38	0.06883	35.8
	JN872231	*Nosema bombi* (China)	40	0.07266	35.7
	MF776532	*Nosema bombi* (Thailand)	22	0.07065	33.8
	AY741105	*Nosema bombi* (Ireland)	97	0.06550	35.9
	KF916504	*Nosema bombi* (Turkey)	25	0.07266	36.3
	D85503	*Nosema bombycis*	93	0.24367	34.1
	KT020736	*Nosema fumiferanae*	94	0.24862	32.3
	L39109	*Endoreticulatus schubergi*	71	0.50510	51
RPB1	**MW415412**	*Vairimorpha(Nosema)cerenae*Tr-07	Query cover %	Pairwise distances	GC content (32.5%)
	KJ473287	*Vairimorpha ceranae*	100	0.01077	32.4
	KM001627	*Vairimorpha ceranae*	100	0.01319	32.2
	DQ996230	*Vairimorpha apis*	98	0.22293	31.2
	AF060234	*Vairimorpha necatrix*	98	0.23869	32.5
	DQ996236	*Vairimorpha necatrix*	98	0.23869	30.9
	JX213749	*Vairimorpha lymantriae*	93	0.22134	36
	JX239748	*Vairimorpha disparis*	94	0.23456	36.4
	MT461295	*Nosema fumiferanae* TY61	96	0.27543	36.2

Table 3. Continued.

	MW396669	*Vairimorpha* (*Nosema*) *cerenae*Tr-07	Query cover %	Pairwise distances	GC content (35.9%)
RPB1	HQ457435	*Nosema fumiferanae*	94	0.27867	36.4
	HQ457436	*Nosema sp.*	94	0.26972	36.8
	AJ278948	*Nosema tyriae*	98	0.26806	36.7
	DQ996231	*Nosema bombycis*	98	0.26759	36.6
	DQ996234	*Nosema trichoplusiae*	98	0.27150	36.7
	HQ457438	*Nosema disstriae*	96	0.27711	
	DQ996232	*Nosema empoascae*	95	0.34913	43.6
	DQ996233	*Nosema granulosis*	94	0.29770	42.9
	XM 014708712	*Ordospora colligata*	89	0.38837	43.3

"-"No significant similarity found.

DISCUSSION

This survey of pathogens of the *B. terrestris* from different provinces of Turkey showed that while the microsporidiosis originated from *V. ceranae* was commonly occur at commercial bumblebee populations in Turkey, no infection was found in wild populations. If it was necessary to make a self-criticism of the study here, it can be said that the reason for the no determination of any infection in wild populations was due to the low sample count. Because recent studies showed that the wild *Bombus* species were frequently infected with the microsporidian species like a *V. ceranae* (Li et al, 2012; Plischuk & Lange, 2016; Sinpoo, Disayathanoowat, Williams, & Chantawannakul, 2019).

The current microsporidium detected from commercial members of the *B. terrestris* was determined to be the first *V. ceranae* isolate of Turkey as a result of both microscopical and molecular examinations. The microsporidial taxonomy was constructed based on light microscopy and measurements (Kudo, 1924; Weiser, 1977; Sprague, Becnel, & Hazard, 1992). In the examinations made in this context, it was determined that the current microsporidium spores had similar features to the data presented by numerous studies previously conducted for the definition and detection of *V. ceranae* (Fries, Feng, da Silva, Slemenda, & Pieniazek, 1996; Chen et al, 2009). Especially in the last quarter, the microsporidial taxonomy has been constructed with the molecular phylogeny and species identifications are made on this basis (Baker, Vossbrinck, Maddox, & Undeen, 1994; Baker, Vossbrinck, Didier, Maddox, & Shadduck, 1995; Huang, Tsai, Lo, Soichi & Wang 2004; Bekircan, 2020; Tokarev et al, 2020; Tosun, 2020) 1968 (Microsporidia: Nosematidae. Therefore, in this study partial sequences of SSU rRNA and RPB1 genes of the current microsporidium were analyzed. In the BLAST analysis conducted with partial sequences of the SSU rRNA and RPB1 genes, the current microsporidium showed high similarities with *V.*

ceranae isolates (Table 3). According to SSU rRNA pairwise distance analysis, the current microsporidium was differentiating with only 0.010 from *V. ceranae* Thailand (KC680650) and China (JN872261) isolates, which were isolated from *Bombus* species (Sinpoo et al, 2019). The phylogenetic trees, which were constructed with SSU rRNA and RPB1 gene sequences, displayed two distinct clades: *Nosema* and *Vairimorpha* (Fig. 2). In both trees, the current microsporidium was grouping with the type species (*V. necatrix*) of the *Vairimorpha* genus and branched with the *V. ceranae* isolates. In the SSU rRNA tree, the current microsporidium settled the same node with the Switzerland (DQ673615) and Spain (DQ329034) isolates of the *V. ceranae* which were isolated from the *Apis mellifera* (Higes, García-Palencia, Martín-Hernández, & Meana, 2007; Martín-Hernández et al, 2007) (Table 3). And the distances between the current microsporidium and these isolates were determined as the same (0.017). Also, in the RPB1 tree, the current microsporidium settled the same node with the *V. ceranae* isolates (KJ473287 and KM001627) as in the SSU rRNA tree (Fig. 2).

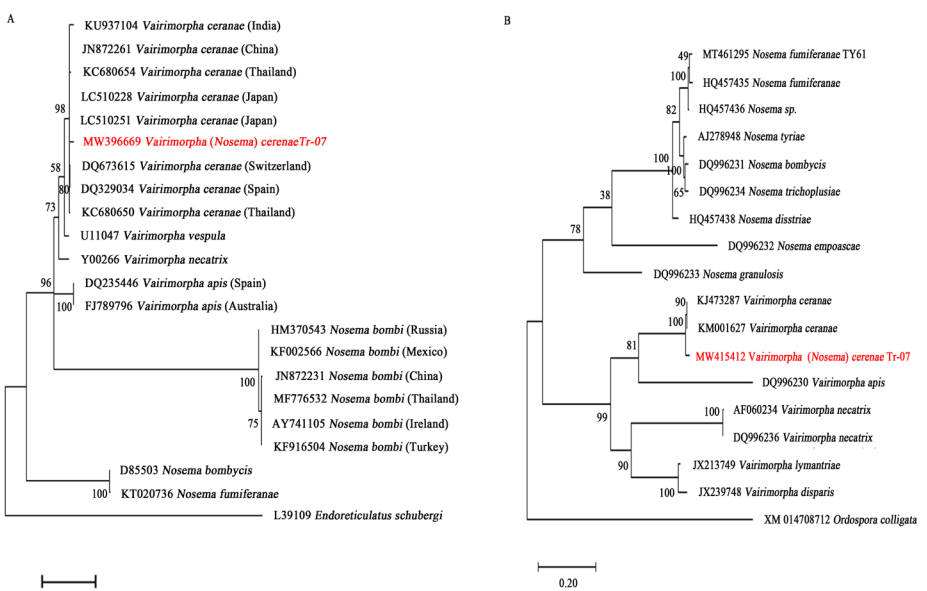

Fig. 2. Phylogenetic trees constructed by maximum likelihood (ML) revealed that the current microsporidium whose sequences were obtained in the present study was most closely related to the *V. ceranae* isolates. *Endoreticulatus schubergi* (L39109) and *Ordospora colligata* (XM014708712) were used as outgroups. The analysis was done on 1000 bootstrapped data sets. Bootstrap values were shown at each node. The scale bar represented substitutions per nucleotide site. A: 16S SSU rRNA tree B: RPB1 tree.

In conclusion, phylogenetical analysis showed that the current microsporidium from *B. terrestris* was almost identical to *V. ceranae* isolates. So, based on the light microscopy and phylogenetical status the current microsporidium was a new isolate of the *V. ceranae* and named herein as *Vairimorpha ceranae* Tr-07 (MW396669).

In addition, the prevalence of the *V. ceranae* Tr-07 infection from commercial *B. terrestris* members was evaluated in this study. Infection was detected in three of the five provinces where the samples were collected, and the province where the disease was most common was determined as Antalya (15.74%). When assessed the prevalence according to the months, June was the month that the infection was peaked (10.16%) (Table 2). Although greenhouses are areas where controlled air conditions are provided, this situation is eliminated in order to reduce costs in summer months and natural weather conditions are valid in these areas. And in the greenhouses where samples were collected, natural climatic conditions prevailed. There are many studies revealing the variability of *V. ceranae* infection according to weather conditions and months (Gisder et al, 2010; Tosun, 2012; Özgör, Güzerin, & Keskin, 2015). In 2015, Özgör et al, determined that *V. ceranae* formation in Turkey was directly affected by the temperature and humidity. Similarly, in the current study, the peak point was determined in June which the average data of the temperature and humidity were high relatively. Although the three provinces where *V. ceranae* infection was detected are geographically relatively close to each other, the infection was most frequently detected in Antalya. This situation can be explained due to the variability of the artificial diets of businesses as stated in Gómez-Moracho, Durand, Pasquaretta, Heeb, & Lihoreau in 2021.

Finally, the current study revealed the first *V. ceranae* infection at the *B. terrestris* in Turkey and its current status.

ACKNOWLEDGEMENTS

We are thankful to Beekeeper Mehmet TURKUT for making this study possible. All authors designed the project, provided samples and contributed to the preparation of the manuscript.

REFERENCES

Antúnez, K., Martín-Hernández, R., Prieto, L., Meana, A., Zunino, P., & Higes, M. (2009). Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). *Environmental Microbiology*, 11(9), 2284-2290. https://doi.org/10.1111/j.1462-2920.2009.01953.x

Argun Karslı, B. & Gürel, F. (2015). Identification of some important Bumblebee (*Bombus terrestris* L.) parasites by molecular methods. *Uludağ Arıcılık Dergisi*, 14(2), 88-98. http://dergipark.gov.tr/uluaricilik/issue/34048/376896

Baker, M.D., Vossbrinck, C.R., Maddox, J.V., & Undeen, A.H. (1994). Phylogenetic relationships among *Vairimorpha* and *Nosema* species (Microspora) based on ribosomal RNA sequence data. *Journal of Invertebrate Pathology*, 64(2), 100-106. https://doi.org/10.1006/jipa.1994.1077

Baker, M.D., Vossbrinck, C.R., Didier, E.S., Maddox, J.V., & Shadduck, J.A. (1995). Small subunit ribosomal DNA phylogeny of various microsporidia with emphasis on AIDS related forms. *The Journal of Eukaryotic Microbiology*, 42(5), 564-570. https://doi.org/10.1111/j.1550-7408.1995.tb05906.x

Baki, H. & Bekircan, Ç. (2018). A new microsporidium, *Vairimorpha subcoccinellae* n. sp. (Microsporidia: Burenellidae), isolated from *Subcoccinella vigintiquatuorpunctata* L. (Coleoptera: Coccinellidae). *Journal of Invertebrate Pathology*, 151, 182-190. https://doi.org/10.1016/j.jip.2017.12.004

Bekircan, Ç. (2020). Assignment of *Vairimorpha leptinotarsae* comb. nov. on the basis of molecular characterization of *Nosema leptinotarsae* Lipa, 1968 (Microsporidia: Nosematidae). *Parasitology*, 147(9), 1019-1025. https://doi.org/10.1017/S0031182020000669

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., & Griswold, T.L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the United States of America*, 108(2), 662-667. https://doi.org/10.1073/pnas.1014743108

Chen, Y.P., Evans, J.D., Murphy, C., Gutell, R., Zuker, M., Gundensen-Rindal, D., & Pettis, J.S. (2009). Morphological, molecular, and phylogenetic characterization of *Nosema ceranae*, a microsporidian parasite isolated from the European honey bee, *Apis mellifera*. *Journal of Eukaryotic Microbiology*, 56(2), 142-147. https://doi.org/10.1111/j.1550-7408.2008.00374.x

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., VanEngelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J.F., Lipkin, W.I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science*, 318(5848), 283-287. https://doi.org/10.1126/science.1146498

Dosselli, R., Grassl, J., Carson, A., Simmons, L.W., & Baer, B. (2016). Flight behaviour of honey bee (*Apis mellifera*) workers is altered by initial infections of the fungal parasite *Nosema apis*. *Scientific Reports*, 6, 1-11. https://doi.org/10.1038/srep36649

Fitzpatrick, Ú., Murray, T.E., Paxton, R.J., Breen, J., Cotton, D., Santorum, V., & Brown, M.J.F. (2007). Rarity and decline in bumblebees - A test of causes and correlates in the Irish fauna. *Biological Conservation*, 136(2), 185-194. https://doi.org/10.1016/j.biocon.2006.11.012

Fries, I., Feng, F., da Silva, A., Slemenda, S.B., & Pieniazek, N.J. (1996). *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). *European Journal of Protistology*, 32(3), 356-365. https://doi.org/10.1016/S0932-4739(96)80059-9

Gage, S.L., Kramer, C., Calle, S., Carroll, M., Heien, M., & DeGrandi-Hoffman, G. (2018). *Nosema ceranae* parasitism impacts olfactory learning and memory and neurochemistry in honey bees (*Apis mellifera*). *Journal of Experimental Biology*, 221(4), 1-11. https://doi.org/10.1242/jeb.161489

Gisder, S., Hedtke, K., Möckel, N., Frielitz, M.C., Linde, A., & Genersch, E. (2010). Five-year cohort study of *Nosema* spp. in Germany: Does climate shape virulence and assertiveness of *Nosema ceranae*? *Applied and Environmental Microbiology*, 76(9), 3032-3038. https://doi.org/10.1128/AEM.03097-09

Gómez-Moracho, T., Heeb, P., & Lihoreau, M. (2017). Effects of parasites and pathogens on bee cognition. *Ecological Entomology*, 42, 51-64. https://doi.org/10.1111/een.12434

Gómez-Moracho, T., Durand, T., Pasquaretta, C., Heeb, P., & Lihoreau, M. (2021). Artificial diets modulate infection rates by *Nosema ceranae* in bumblebees. *Microorganisms*, 9(1), 1-18. https://doi.org/10.3390/microorganisms9010158

Goulson, D., Lye, G.C., & Darvill, B. (2008). Decline and conservation of bumble bees. *Annual Review of Entomology*, 53, 191-208. https://doi.org/10.1146/annurev.ento.53.103106.093454

Graystock, P., Yates, K., Darvill, B., Goulson, D., & Hughes, W.O.H. (2013). Emerging dangers: Deadly effects of an emergent parasite in a new pollinator host. *Journal of Invertebrate Pathology*, 114(2), 114-119. https://doi.org/10.1016/j.jip.2013.06.005

Grixti, J.C., Wong, L.T., Cameron, S.A., & Favret, C. (2009). Decline of bumble bees (*Bombus*) in the North American Midwest. *Biological Conservation*, 142(1), 75-84. https://doi.org/10.1016/j.biocon.2008.09.027

Güler, Y., Aytekin, A.M., & Dikmen F. (2011). Bombini and Halictidae (Hymenoptera: Apoidae) fauna of Afyonkarahisar province of Turkey. *Journal of the Entomological Research Society*, 13(1), 1-22. https://doi.org/10.51963/jers.v23i2

Hall, T.A. (1999). PdfBioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Higes, M., García-Palencia, P., Martín-Hernández, R., & Meana, A. (2007). Experimental infection of *Apis mellifera* honeybees with *Nosema ceranae* (Microsporidia). *Journal of Invertebrate Pathology*, 94(3), 211-217. https://doi.org/10.1016/j.jip.2006.11.001

Holt, H.L., Aronstein, K.A., & Grozinger, C.M. (2013). Chronic parasitization by *Nosema microsporidia* causes global expression changes in core nutritional, metabolic and behavioral pathways in honey bee workers (Apis mellifera). *BMC Genomics*, 14(1). https://doi.org/10.1186/1471-2164-14-799

Huang, W.F., Tsai, S.J., Lo, C.F., Soichi, Y., & Wang, C.H. (2004). The novel organization and complete sequence of the ribosomal RNA gene of *Nosema bombycis*. *Fungal Genetics and Biology*, 41(5), 473-481. https://doi.org/10.1016/j.fgb.2003.12.005

Kudo, R. (1924). A biologic and taxonomic study of the Microsporidia. *Illinois Biological Monographs*, 9, 1-268.

Li, J., Chen, W., Wu, J., Peng, W., An, J., Schmid-Hempel, P., & Schmid-Hempel, R. (2012). Diversity of nosema associated with bumblebees (*Bombus* spp.) from China. *International Journal for Parasitology*, 42(1), 49-61. https://doi.org/10.1016/j.ijpara.2011.10.005

Li, W., Chen, Y., & Cook, S.C. (2018). Chronic *Nosema ceranae* infection inflicts comprehensive and persistent immunosuppression and accelerated lipid loss in host *Apis mellifera* honey bees. *International Journal for Parasitology*, 48(6), 433-444. https://doi.org/10.1016/j.ijpara.2017.11.004

Martín-Hernández, R., Meana, A., Prieto, L., Salvador, A.M., Garrido-Bailón, E., & Higes, M. (2007). Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. *Applied and Environmental Microbiology*, 73(20), 6331-6338. https://doi.org/10.1128/AEM.00270-07

Martín-Hernández, R., Higes, M., Sagastume, S., Juarranz, Á., Dias-Almeida, J., Budge, G.E., Meana, A., & Boonham, N. (2017). Microsporidia infection impacts the host cell's cycle and reduces host cell apoptosis. *PLoS ONE*, 12(2), 1-17. https://doi.org/10.1371/journal.pone.0170183

Martín-Hernández, R., Bartolomé, C., Chejanovsky, N., Le Conte, Y., Dalmon, A., Dussaubat, C., García-Palencia, P., Meana, A., Pinto, M.A., Soroker, V., & Higes, M. (2018). *Nosema ceranae* in *Apis mellifera*: a 12 years postdetection perspective. *Environmental Microbiology*, 20(4), 1302-1329. https://doi.org/10.1111/1462-2920.14103

Mauss, V. (1994). *Bestimmungsschlüssel für Hummeln*. Deutscher Jugendbund für Naturbeobachtungen (DJN), 55 s.

Nägeli, K.W. (1857). Überdie neue Krankheit der Seidenraupe und verwandte Organismen. *Botanische Zeitung*, 15, 60-761.

Özgör, E., Güzerin, E., & Keskin, N. (2015). Determination and comparison of *Nosema apis* and *Nosema ceranae* in terms of geographic and climatic factors. *Hacettepe Journal of Biology and Chemistry*, 43(1), 9-15. https://doi.org/10.15671/HJBC.20154311200

Perry, C.J., Søvik, E., Myerscough, M.R., & Barron, A.B. (2016). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proceedings of the National Academy of Sciences of the United States of America*, 113(30), E4431. https://doi.org/10.1073/pnas.1610243113

Piiroinen, S. & Goulson, D. (2016). Chronic neonicotinoid pesticide exposure and parasite stress differentially affects learning in honeybees and bumblebees. *Proceedings of the Royal Society B: Biological Sciences*, 283(1828). https://doi.org/10.1098/rspb.2016.0246

Pilley, B.M. (1976). A new genus, *Vairimorpha* (Protozoa: Microsporida), for *Nosema necatrix* Kramer 1965: Pathogenicity and life cycle in *Spodoptera exempta* (Lepidoptera: Noctuidae). *Journal of Invertebrate Pathology*, 28(2), 177-183. https://doi.org/10.1016/0022-2011(76)90119-1

Plischuk, S., Martín-Hernández, R., Prieto, L., Lucía, M., Botías, C., Meana, A., Abrahamovich, A.H., Lange, C., & Higes, M. (2009). South American native bumblebees (Hymenoptera: Apidae) infected by *Nosema ceranae* (Microsporidia), an emerging pathogen of honeybees (*Apis mellifera*). *Environmental Microbiology Reports*, 1(2), 131-135. https://doi.org/10.1111/j.1758-2229.2009.00018.x

Plischuk, S. & Lange, C.E. (2016). *Bombus brasiliensis* Lepeletier (Hymenoptera, Apidae) infected with *Nosema ceranae* (Microsporidia). *Revista Brasileira de Entomologia*, 60(4), 347-351. https://doi.org/10.1016/j.rbe.2016.06.003

Sinpoo, C., Disayathanoowat, T., Williams, P.H., & Chantawannakul, P. (2019). Prevalence of infection by the microsporidian *Nosema* spp. in native bumblebees (*Bombus* spp.) in northern Thailand. *PLOS ONE*, 14(3), e0213171. https://doi.org/10.1371/journal.pone.0213171

Sprague, V., Becnel, J.J., & Hazard, E.I. (1992). Taxonomy of phylum microspora. *Critical Reviews in Microbiology*, 18(5-6), 285-395. https://doi.org/10.3109/10408419209113519

Tokarev, Y.S., Huang, W.F., Solter, L.F., Malysh, J.M., Becnel, J.J., & Vossbrinck, C.R. (2020). A formal redefinition of the genera *Nosema* and *Vairimorpha* (Microsporidia: Nosematidae) and reassignment of species based on molecular phylogenetics. *Journal of Invertebrate Pathology*, 169 (October 2019), 107279. https://doi.org/10.1016/j.jip.2019.107279

Tosun, O. (2012). *Bal Arılarında (Apis mellifera L., 1758) Nosemosis (Nosematosis) Hastalığının Doğu Karadeniz Bölgesinde Bulunan Arı Kolonilerindeki Varlığı, Dağılımı ve Hastalık Etkenlerinin Karakterizasyonu*. [Doctora tezi, Karadeniz Teknik Üniversitesi]. Fen Bilimleri Enstitüsü, 112 s.

Tosun, O. (2020). A new isolate of *Nosema fumiferanae* (Microsporidia: Nosematidae) from the date moth *Apomyelois (Ectomyelois) ceratoniae*, Zeller, 1839 (Lepidoptera: Pyralidae). *Parasitology*, 147(13), 1461-1468. https://doi.org/10.1017/S0031182020001481

Velthius, H.W. & Doorn, A. Van. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*, 37, 421-451.

Weiser, J. (1977). Contribution to the classification of Microsporidia. *Vestnik Ceskoslovenske Spolecnosti Zoologicke*, 41, 308-320.

Williams, P.H. & Osborne, J.L. (2009). Bumblebee vulnerability and conservation world-wide. *Apidologie*, 40(3), 367-387. https://doi.org/10.1051/apido/2009025

Wolf, S., McMahon, D.P., Lim, K.S., Pull, C.D., Clark, S.J., Paxton, R.J., & Osborne, J.L. (2014). So near and yet so far: Harmonic radar reveals reduced homing ability of nosema infected honeybees. *PLoS ONE*, 9(8). https://doi.org/10.1371/journal.pone.0103989

Yıldırım, H. (2021). The first record of *Vairimorpha hostounsky* sp. nov. Infection in the blue shieldbug, *Zicrona caerulea* Linnaeus, 1758 (Hemiptera: Pentatomidae). *Microbial Pathogenesis,* 155, 104883. https://doi.org/10.1016/j.micpath.2021.104883

Yıldırım, H. & Bekircan, Ç. (2020). Ultrastructural and molecular characterization of *Nosema alticae* sp. nov. (Microsporidia: Nosematidae), pathogen of the flea beetle, *Altica hampei* Allard, 1867 (Coleoptera: Chrysomelidae). *Journal of Invertebrate Pathology*, 170(November 2019). https://doi.org/10.1016/j.jip.2019.107302

Received: July 14, 2021 *Accepted: September 09, 2021*