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Research paper

Phylogenetic analysis based on the 16S rDNA, *gltA*, *gatB*, and *hcpA* gene sequences of *Wolbachia* from the novel host *Ceratozetes thienemanni* (Acari: Oribatida)

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ABSTRACT

We determined the occurrence of intracellular endosymbionts (*Wolbachia*, *Cardinium*, *Arsenophonus*, *Rickettsia*, *Spiroplasma*, *Hamiltonella*, flavobacteria, and microsporidia) in oribatid mites (Acari: Oribatida) with the use of PCR technique. For the first time we looked for and detected *Wolbachia* in parthenogenetic oribatid mite *Ceratozetes thienemanni* Willmann, 1943. The 16S rDNA, *gatB*, *hcpA*, and *gltA* sequences of *Wolbachia* in *C. thienemanni* showed the highest similarity (≥ 90%) to the genes of *Wolbachia* from springtails (Collembola) and oribatid mite *Gustavia microcephala*. We found the unique sequence 5'-GGGGTAATGGCC-3′ in 16S rDNA of *Wolbachia* from *C. thienemanni* and collembolan representing group E. The phylogeny of *Wolbachia* based on the analysis of single genes as well as concatenated alignments of four bacterial loci showed that the bacteria from *C. thienemanni* belonged to *Wolbachia* group E, like the endosymbionts from springtail hosts and *G. microcephala*. Considering coexisting of representatives of Oribatida and Collembola in the same soil habitat and similar food, it is possible that the source of *Wolbachia* infection was the same. Residues of dead invertebrates could be in organic matter of their soil food, so the scenario of infection transferred by eating of remains of soil cohabitates is also possible. It could explain the similarity and relationship of the *Wolbachia* in these two arthropod groups. Oribatid mite *C. thienemanni* is a parthenogenetic mite which is a unique feature in the genus *Ceratozetes*. Moreover, this species, within the entire genus *Ceratozetes*, is characterized by the most northerly distribution. It is difficult to determine either it is parthenogenesis or the presence of endosymbionts that are in some way responsible for this kind of evolutionary success. Maybe we are dealing here with a kind of synergy of both factors?

1. Introduction

The understanding of the biology of eukaryotic organisms requires knowledge on their microbiome and an inside into the roles of these symbiotic associates. The most known heritable bacteria in Arthropoda are *Wolbachia.* It was estimated that 52% of terrestrial arthropod species are infected (Weinert et al., 2015). However, there are groups of invertebrates poorly researched regarding the occurrence of maternally inherited microbes. In our opinion, oribatid mites (Acari: Oribatida) have been omitted in studies on endosymbionts distribution, which is difficult to understand as in the quantitative aspect, they are the dominant group of the arthropods in the organic layer of soil in temperate zone forests in the number as high as over several hundreds of thousand individuals per square meter. Together with springtails (Collembola), they make the most abundant and important component of the soil mesofauna and contribute to fragmentation and decay of organic matter (Schatz and Behan-Pelletier, 2008). Despite the fact that Oribatida are ubiquitous invertebrates, little is known about their endosymbionts. So far *Oppiella nova* has been reported to be infected by *Wolbachia* (Weeks et al., 2003; Konecka and Olszanowski, 2015) and *Cardinium* (Weeks et al., 2003). *Wolbachia* has been also found in *Gustavia microcephala*, and *Cardinium* in *Microzetorchestes emeryi* (Konecka and Olszanowski, unpublished results) and *Achipteria coleoptrata* (Konecka and Olszanowski, 2019). Additionally, an unidentified endosymbiont has been detected in the *Hermannia gibba* ovaries (Liana and Witaliński, 2010). Still, the information of maternally inherited endosymbiont infections in Oribatida is scarce.

The explanation of the riddle concerning the origin and the meaning of endosymbionts is extremely important in the understanding of the evolution process of their hosts. The functions performed by the

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intracellular microbes of Arthropoda have been broadest characterized in relation to *Wolbachia.* These bacteria have substantial ecological and evolutionary impacts, affecting their hosts' reproduction (Ma and Schwander, 2017; Duplouy and Hornett, 2018), defense against pathogens (Becerra et al., 2015), fitness host (Zug and Hammerstein, 2015), and even genome evolution (Charlat et al., 2003). In the long term, the microorganism can increase extinction risk by reducing genetic diversity. In parthenogenetic populations, the lack of sex leads to the accumulation of mutations, and lower evolvability (Charlat et al., 2003). Moreover, the *Wolbachia* genome contains a high number of mobile elements (Siguier et al., 2014; Sutton et al., 2014), simple repetitive DNA sequences (Moran et al., 2008; Sanchez-Contraras and Vlisidou, 2008; Werren et al., 2008; Chrostek and Teixeira, 2015), and shows general plasticity (Ramírez-Puebla et al., 2016) as well as frequent rearrangement between strains (Comandatore et al., 2015; Newton et al., 2016), and also between the bacteria and host genomes (Kondo et al., 2002; Harris et al., 2010; Brelsfoard et al., 2014; Morrow et al., 2015; Kajtoch and Kotásková, 2018). Gene transfer from the endosymbiont into invertebrate genomes is considered to be common (Hou et al., 2014) and some invertebrate genomes have gene insertions which range from several hundred bp to nearly the entire *Wolbachia* genome of size >1 Mb (Dunning Hotopp et al., 2007). Novel gene functions may be the result of such insertions (Werren et al., 2008). The bacteria can also cause host speciation (Heddi et al., 1999; Frati et al., 2004).

Phylogenetic analysis using bacterial 16S rDNA and housekeeping genes allowed to distinguish 16 supergroups of *Wolbachia*, designated from A to Q (Vandekerckhove et al., 1999; Lo et al., 2002; Czarnetzki and Tebbe, 2004; Hise et al., 2004; Casiraghi et al., 2005; Lo et al., 2007; Haegeman et al., 2009; Merçot and Poinsot, 2009; Ros et al., 2009; Harris et al., 2010; Augustinos et al., 2011; Bing et al., 2014; Glowska et al., 2015). The examples of genes useful in differentiating bacteria and determining their relationship are: *groEL* coding for 60 kDa heat-shock protein (Ros et al., 2009), *hcpA* coding for conserved hypothetical protein (Baldo et al., 2006), *aspC* coding for aspartate aminotransferase (Paraskevopoulos et al., 2006), and *aspS* coding for aspartyl-tRNA synthetase (Crainey et al., 2010). The analysis of the sequences of 16S rDNA and housekeeping genes allows characterizing *Wolbachia* strains in a diverse range of hosts. These data is also an important source of sequence data for extensive comparative genetics for investigating molecular evolutionary processes in these bacteria (Baldo et al., 2006). However, according to some authors the whole-genome information should be used for *Wolbachia* typing (Bleidorn and Gerth, 2017).

The aim of our study was to determine the distribution of endosymbiotic microorganisms in oribatid mites (Acari: Oribatida). Apart from *Wolbachia*, we were identifying also other microbes: *Cardinium*, *Arsenophonus*, *Rickettsia*, *Spiroplasma*, *Hamiltonella*, flavobacteria, and microsporidia. The intracellular symbionts cannot be cultivated in vitro and traditional methods are ineffective for their detecting and characterizing, so we used PCR technique for detection of endosymbionts. The phylogenetic analysis of *Wolbachia* was based on bacterial 16S rDNA and three housekeeping genes: *gatB* coding for glutamyltRNA(Gln) amidotransferase, *gltA* coding for citrate synthase, and *hcpA* coding for a conserved hypothetical protein.

2. Materials and methods

2.1. Oribatid mites

The sample of moss was collected in the mixed forest near Ostrowiec Świętokrzyski, Świętokrzyskie voivodeship in Poland (50°59′N 21°20′E; May 3, 2018). Mites were extracted by using high-gradient Tullgren funnels, segregated intravitally and immediately placed directly in 96% ethanol for genetic analysis. Some comparative specimens were conserved in 70% ethanol and then determined to the species or genus level by using the key of Weigmann (2006).

2.2. Identification of endosymbionts

Detection of endosymbionts *Wolbachia*, *Cardinium*, *Arsenophonus*, *Rickettsia*, *Spiroplasma*, and microsporidia was carried out according to procedure described previously (Konecka and Olszanowski, 2015). Identification of *Hamiltonella* and flavobacteria was conducted as presented by Moran et al. (2005), and Majerus and Majerus (2010), respectively.

Overall DNA of each mite specimen was extracted with Genomic Mini kit (A&A Biotechnology) for universal genomic DNA isolation according to the manufacturer's recommendation. The genes of endosymbionts were detected by PCR amplification with primers which sequences and annealing temperatures have been presented by Webb et al. (1990), Weisburg et al. (1991), Baker et al. (1995), Fukatsu and Nikoh (2000), Brown et al. (2001), Thao and Baumann (2004), Zchori-Fein and Perlman (2004), Moran et al. (2005), and Majerus and Majerus (2010). PCR reactions were carried out in mixtures containing: 10–30 ng of DNA, 1 μl 10× PCR buffer (Bioron), 0.4 μl 5 mM dNTP (Novazym), 0.6 μM each primer (Oligo.pl), 1 U HighTaq DNA polymerase (Bioron) and sterile bidistilled water to a volume of 10 μl. In each reaction a sample without template DNA was included as a negative control. Additional reaction with universal primers for invertebrate 28S rRNA gene (Tiawsirisup et al., 2008) was conducted as a positive control of DNA isolation and amplification. PCR products were electrophoresed in agarose gel (NOVA Mini, Novazym) and sequenced with BigDye Terminator v3.1 in an ABI Prism 3130XL Analyzer (Applied Biosystems). BLASTn algorithm was used to compare the sequences of amplicons to the data deposited in the GenBank (International Nucleotide Sequence Database Collaboration).

2.3. Molecular analysis of Wolbachia genes

Molecular characterization of *Wolbachia* was performed based on the analysis of sequences of 16S rDNA and housekeeping genes: *gatB*, *coxA*, *hcpA*, *fbpA* (Baldo et al., 2006), *gltA* (Casiraghi et al., 2005), *groEL* (Ros et al., 2009), and *ftsZ* (Holden et al., 1993; Werren et al., 1995; Baldo et al., 2006). To obtain a sequence of almost entire *Wolbachia* 16S rDNA gene, two PCR reactions were performed: (1) with the specific primer EHR16SD (Brown et al., 2001) and universal eubacterial primer 1513R (Weisburg et al., 1991), and (2) with the specific primer EHR16SR (Brown et al., 2001) and universal eubacterial primer 63F (Fredriksson et al., 2013). The primer sequences and annealing temperatures are presented in Table 1.

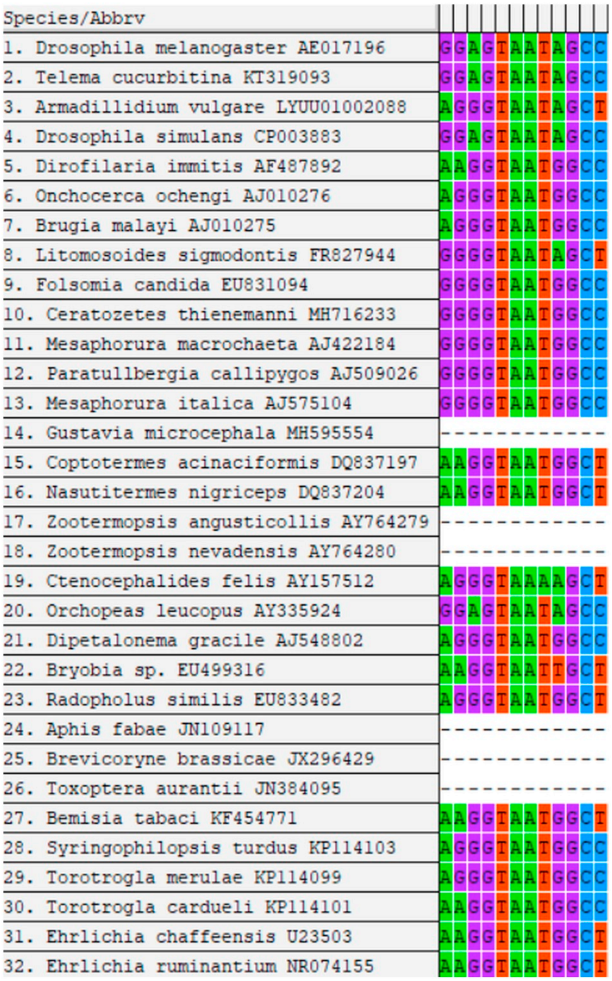
Amplifications were performed in a 10 μl mixtures containing: 10–30 ng of DNA, 1 μl 10× PCR DreamTaq Buffer (Thermo Scientific), 0.4 μl 5 mM dNTP (Novazym), 0.6 μM each primer (Oligo.pl), 0.8 U DreamTaq Hot Start DNA Polymerase (Thermo Scientific), and sterile bidistilled water to a total volume of 10 μl. Negative controls without template DNA were included in each reaction. Positive controls were not included. The PCR cycling profile was as follows: 95 °C for 5 min; 40 cycles of 95 °C at 30 s, annealing (Table 1) for 30 s, and 72 °C for 45 s; 72 °C for 5 min. Amplicons were electrophoresed, sequenced and analyzed with BLASTn as described above. The 16S rDNA, *hcpA*, *gatB*, and *gltA* sequences of *Wolbachia* in *Ceratozetes thienemanni* were deposited in GenBank. Additionally, the 16S rDNA sequence of *Wolbachia* in *C. thienemanni* was inspected for the nucleotides varied between different bacterial supergroups that are located in the regions corresponding to the particular positions of *Escherichia coli* 16S rDNA as proposed by Czarnetzki and Tebbe (2004). The tested nucleotide positions were: 76, 92, 94, 156, 165, 422, 562, 841, 845, 989, 1216, 1252, 1254, 1283, and 1305.

Table 1
Primer sets used for characterization of *Wolbachia* in oribatid mites.

Primer designation	Primer sequence (5′ → 3′)	Gene	Product	Annealing temperature (°C)	Reference
EHR16SD	GGTACCYACAGAAGAAGTCC	16S rDNA	16S rRNA	55	Brown et al. (2001)
1513R	ACGGYTACCTTGTTACGACTT				Weisburg et al. (1991)
EHR16SR	TAGCACTCATCGTTTACAGC				Brown et al. (2001)
63F	CAGGCCTAACACATGCAAGTC				Fredriksson et al. (2013)
gatB_F1	GAKTTAAAYCGYGCAGGBGTT	*gatB*	glutamyltRNA(Gln) amidotransferase	54	Baldo et al. (2006)
gatB_R1	TGGYAAYTCRGGYAAAGATGA				
hcpA_F1	GAAATARCAGTTGCTGCAAA	*hcpA*	conserved hypothetical protein	54	Baldo et al. (2006)
hcpA_R1	GAAAGTYRAGCAAGYTCTG				
fbpA_F1	GCTGCTCCRCTTGGYWTGAT	*fbpA*	fructose-bisphosphate aldolase	59	Baldo et al. (2006)
fbpA_R1	CCRCCAGARAAAAYYACTATTC				
coxA_F1	TTGGRGCRATYAACTTTATAG	*coxA*	cytochrome *c* oxidase	54	Baldo et al. (2006)
coxA_R1	CTAAAGACTTTKACRCCAGT				
WgltAF1	TACGATCCAGGGTTTGTTTCTAC	*gltA*	citrate synthase	55	Casiraghi et al. (2005)
WgltARev1	CTCATTAGCTCCACCGTGTG				
groEL-F	CAACRGTRGSRRYAACTGCDGG	*groEL*	60 kDa heat-shock protein	50	Ros et al. (2009)
groEL-R	GATADCCRCGRTCAAAYTGC				
ftsZ_F1	ATYATGGARCATATAAARGATAG	*ftsZ*	prokaryotic cell division protein	54	Baldo et al. (2006)
ftsZ_R1	TCRAGYAATGGATTRGATAT				
ftsZ1	CCGTATGCCGATTGCAGAGCTTG			60	Holden et al. (1993)
ftsZ2	GCCATGAGTATTCACTTGGCT				
ftsZf1	GTTGTCGCAAATACCGATGC			50	Werren et al. (1995)
ftsZr1	CTTAAGTAAGCTGGTATATC				

Table 2
GenBank accession nos. of sequence data used in this study to align *Wolbachia* in phylogenetic analysis.

Wolbachia host	*Wolbachia* supergroup	GenBank accession no.			
		16S rRNA	*gatB*	*gltA*	*hcpA*
Drosophila melanogaster	A	AE017196	AE017196		DQ842415
Telema cucurbitina	A	KT319093			KU057809
Wasmannia auropunctata	A		JX499049		JX499045
Armadillidium vulgare	B	LYUU01002088	DQ842420	FJ390331	DQ842383
Drosophila simulans	B	CP003883	CP003883	NC_021084	
Dirofilaria immitis	C	AF487892	KU255376	AJ609641	
Onchocerca ochengi	C	AJ010276	KU255392	HE660029	
Brugia malayi	D	AJ010275	KU255368	AJ609643	DQ842384
Litomosoides sigmodontis	D	FR827944		AJ609645	FJ390171
Folsomia candida	E	EU831094	KT799605	AJ609649	KT799610
Gustavia microcephala	E	MH595554		MH605024	
Mesaphorura italica	E	AJ575104			
Ceratozetes thienemanni	E	MH716233	MH716234	MH716235	MH716236
Coptotermes acinaciformis	F	DQ837197			FJ390173
Nasutitermes nigriceps	F	DQ837204		FJ390333	
Zootermopsis angusticollis	H	AY764279		AY764281	FJ390174
Zootermopsis nevadensis	H	AY764280		AY764282	FJ390175
Ctenocephalides felis	I	AY157512		AJ609650	
Orchopeas leucopus	I	AY335924			
Dipetalonema gracile	J	AJ548802	KU255375	FJ390335	FJ390176
Bryobia sp.	K	EU499316		EU499326	
Radopholus similis	L	EU833482			
Aphis fabae	M	JN109117		JN316248	
Brevicoryne brassicae	M	JX296429		JN316250	
Toxoptera aurantii	N	JN384095			
Bemisia tabaci	O	KF454771	KF452588	KF587270	KF454726
Syringophilopsis turdus	P	KP114103			
Torotrogla merulae	P	KP114099			
Torotrogla cardueli	Q	KP114101		KP114115	
Outgroup strains					
Ehrlichia chaffeensis		U23503	CP000236	AF304142	
Ehrlichia ruminantium		NR074155	NR074155	DQ513390	



Species/Abbrv	
1. Drosophila melanogaster AE017196	GGAGTAATAGCC
2. Telema cucurbitina KT319093	GGAGTAATAGCC
3. Armadillidium vulgare LYUU01002088	AGGGTAATAGCT
4. Drosophila simulans CP003883	GGAGTAATAGCC
5. Dirofilaria immitis AF487892	AAGGTAATGGCC
6. Onchocerca ochengi AJ010276	AGGGTAATGGCC
7. Brugia malayi AJ010275	AGGGTAATGGCC
8. Litomosoides sigmodontis FR827944	GGGGTAATAGCT
9. Folsomia candida EU831094	GGGGTAATGGCC
10. Ceratozetes thienemanni MH716233	GGGGTAATGGCC
11. Mesaphorura macrochaeta AJ422184	GGGGTAATGGCC
12. Paratullbergia callipygos AJ509026	GGGGTAATGGCC
13. Mesaphorura italica AJ575104	GGGGTAATGGCC
14. Gustavia microcephala MH595554	------------
15. Coptotermes acinaciformis DQ837197	AAGGTAATGGCT
16. Nasutitermes nigriceps DQ837204	AAGGTAATGGCT
17. Zootermopsis angusticollis AY764279	------------
18. Zootermopsis nevadensis AY764280	------------
19. Ctenocephalides felis AY157512	AGGGTAAAAGCT
20. Orchopeas leucopus AY335924	GGAGTAATAGCC
21. Dipetalonema gracile AJ548802	AGGGTAATGGCC
22. Bryobia sp. EU499316	AAGGTAATTGCT
23. Radopholus similis EU833482	AGGGTAATGGCT
24. Aphis fabae JN109117	------------
25. Brevicoryne brassicae JX296429	------------
26. Toxoptera aurantii JN384095	------------
27. Bemisia tabaci KF454771	AAGGTAATGGCT
28. Syringophilopsis turdus KP114103	AGGGTAATGGCC
29. Torotrogla merulae KP114099	AGGGTAATGGCC
30. Torotrogla cardueli KP114101	AAGGTAATGGCC
31. Ehrlichia chaffeensis U23503	AAGGTAATGGCT
32. Ehrlichia ruminantium NR074155	AAGGTAATGGCT

Fig. 1. An alignment showing the unique sequence 5’-GGGGTAATGGCC-3’of 16S rDNA of *Wolbachia* supergroup E from *Ceratozetes thienemanni*, *Folsomia candida*, *Mesaphorura macrochaeta*, *Paratullbergia callipygos*, and *Mesaphorura italica*.

2.4. Phylogeny of *Wolbachia*

The sequences of 16S rDNA, *gatB*, *hcpA*, and *gltA* genes of *Wolbachia* from *C. thienemanni* were aligned to the *Wolbachia* loci representing different phylogenetic supergroups (A – Q) by applying MEGA 6.0 software (Tamura et al., 2013). NCBI accession numbers for sequences used for phylogenetic analysis are given in Table 2. The alignments of the obtained sequences were constructed with the use of CLUSTAL W (Thompson et al., 1994). The sequences of single genes were aligned and then a multiple gene alignment was created by using the data of 29 *Wolbachia* strains from different hosts together with *C. thienemanni*. An outgroup of *Ehrlichia* spp. sequences was added to the analysis. jModelTest 2 software (Posada and Crandall, 1998; Guindon and Gascuel, 2003; Darriba et al., 2012) was used for choosing the appropriate model of sequence evolution. The maximum likelihood bootstrap support was determined by using 500 bootstrap replicates. The selected model was General Time Reversible with gamma distributed among site rate variation (GTR + G) for *gltA* sequence and for concatenated sequence data of the four genes (16S rDNA, *gatB*, *gltA*, and *hcpA*). The Hasegawa-Kishino-Yano model with gamma distributed among site rate variation (HKY + G) was chosen for 16S rDNA and *gatB* sequence data. For the *hcpA* sequence data, the Tamura-Neil model with gamma distributed among site rate variation (TrN + G) was used. Recombination in genes between strains was detected by a φ test using SplitsTree4 software (Huson and Bryant, 2006).

3. Results

We undertook the issue of the occurrence of heritable endosymbionts in mites (Acari: Oribatida). For the first time we identified *Wolbachia* in parthenogenetic species *Ceratozetes thienemanni* collected from moss sample gathered in mixed forest. *Cardinium*, *Arsenophonus*, *Rickettsia*, *Spiroplasma*, *Hamiltonella*, flavobacteria, and microsporidia were not found.

The 1395-bp region of 16S rDNA was obtained for *Wolbachia* in *C. thienemanni*. The amplicon sequence analysis by using BLASTn showed the similarity of 98% to *Wolbachia* 16S rDNA from springtails *Folsomia candida* (Collembola: Isotomidae) deposited in GenBank under accession nos. EU330430 and AF179630. We amplified three genes (*gatB*, *hcpA*, and *gltA*) of *Wolbachia* in *C. thienemanni* (Table 2). The PCR products of *fbpA*, *coxA*, *groEL*, and *ftsZ* were not observed. NCBI accession numbers for the obtained *Wolbachia* sequences are as follow: MH716233 (16S rDNA), MH716234 (*gatB*), MH716235 (*gltA*), and MH716236 (*hcpA*). The amplicons of *gatB*, *gltA*, and *hcpA* of 385 bp, 630 bp, and 442 bp, respectively, also revealed the highest similarity (90–92%) to the bacterial sequences of *F. candida* (accession nos. CP015510 and KT799605). The 16S rDNA and *gltA* sequences showed also high similarity of 98% and 90%, respectively, to the *Wolbachia* genes from *G. microcephala* (accession nos. MH595554 and MH605023). The *hcpA* and *gatB* gene sequences of *Wolbachia* in *G. microcephala* were not available in GenBank. The sequence of *hcpA* gene of other oribatid *Oppiella nova* was also deposited in GenBank database (accession no. KF511576) and revealed high similarity of 87% to the sequence of *Wolbachia* in *C. thienemanni*.

The phylogeny of *Wolbachia* in *C. thienemanni* was based on the analysis of sequences of four bacterial genes: 16S rDNA, *gatB*, *gltA*, and *hcpA* (2852 bp in total). Both single genes as well as four-gene phylogenetic characterization showed the membership of the bacteria from the oribatid mite in *Wolbachia* supergroup E together with endosymbionts found in collembolan hosts (*F. candida* and *Mesaphorura italica*) and in other Oribatida mite *Gustavia microcephala*. We excluded the possibility that *Wolbachia* in *C. thienemanni* could be a recombinant between strains of other supergroup by the φ test which did not reveal statistically significant evidence for recombination ($P = 1$). Phylogenetic trees reconstructing the relationships of bacteria based on the analyses of individual genes are presented in Figs. S1–S4 (available in the online Supplementary Material). Phylogeny based on the concatenated set of 16S rDNA, *gatB*, *gltA*, and *hcpA* gene sequences is presented in Fig. S5. Moreover, in the 16S rRNA gene region of *Wolbachia* in *C. thienemanni* that corresponds to the positions 76–1305 of *Escherichia coli* 16S rDNA we noted the following nucleotides: (1) G in 76, 165, 1216, and 1254 positions, (2) A in 92, 94, 845, and 1305 positions, (3) T in 156, 841, and 1252 positions, and (4) C in 422, 562, 989, and 1283 positions. Additionally, we found the sequence 5′-GGG GTAATGGCC-3′ in 16S rDNA gene of *Wolbachia* supergroup E discovered in *C. thienemanni*, *F. candida* (accession no. EU831094), *M. italica* (accession no. AJ575104), *P. callipygos* (accession no. AJ509026), and *M. macrochaeta* (accession no. AJ422184). The sequence was not found in 16S rDNA of other *Wolbachia* strains used in this study as a comparative material (Table 2). The location of the above nucleotide sequence was determined in 243–254 positions in reference to 16S rDNA of *Wolbachia* from *F. candida* (accession no. CP015510). An alignment showing the unique sequence of 16S rDNA of *Wolbachia* was presented in Fig. 1.

4. Discussion

We discovered the *Wolbachia* endosymbiont in *Ceratozetes thienemanni* (Acari: Oribatida). It is the first report looking for the bacteria in this oribatid mite species. Apart from this mite, the microorganism was also detected in two others Oribatida species: *Oppiella nova* (Weeks et al., 2003; Konecka and Olszanowski, 2015) and *Gustavia microcephala*

(Konecka and Olszanowski, unpublished). Four *Wolbachia* genes were amplified: 16S rDNA, *gatB*, *gltA*, and *hcpA*. The gene sequences showed the highest similarity to those of *Wolbachia* from springtails *Folsomia candida* (Collembola) and *G. microcephala*. The PCR products of *fbpA*, *coxA*, *groEL*, and *ftsZ* were not obtained, which could be evidence of differences in sequences complementary to the primers used and confirms the diversity of *Wolbachia* strains. However, it is difficult to draw unambiguous conclusions because positive controls were not included in the PCR reactions. These negative results may also derive from reasons different than the absence or sequence diversity, such as lack of sensitivity and/or errors in the primers synthesis.

The analysis of the 16S rDNA sequence of *Wolbachia* in *C. thienemanni* revealed the nucleotide profile G, A, A, T, G, T, A, C, G, T, G, and C in the corresponding positions of 16S rDNA of *Escherichia coli*: 76, 92, 94, 156, 165, 841, 845, 989, 1216, 1252, 1254, and 1283. The set of the nucleotides was mixed of the nucleotide patterns found in the endosymbiont representing phylogenetic supergroup E found in Collembola. The nucleotides in positions 76, 92, 94, 156, 989, and 1216 were the same as reported for *Wolbachia* from springtails *Mesaphorura macrochaeta*, *M. italica*, and *Paratullbergia callipygos*. The nucleotides in positions 165, 841, 845, 1252, and 1254 were identical as those reported for *Wolbachia* in collembolan *F. candida* (Czarnetzki and Tebbe, 2004). Additionally, nucleotides in 841 and 845 positions of 16S rDNA *Wolbachia* in *C. thienemanni* were the same as those of the bacteria in *G. microcephala*. In position 989 of *Wolbachia* in *C. thienemanni* there was C (identical as for the endosymbiont in *M. macrochaeta*, *M. italica*, and *P. callipygos*) and in *G. microcephala* it was T (as in *F. candida*) (Konecka and Olszanowski, unpublished results). Determination of nucleotide variety between *Wolbachia* in *C. thienemanni* and *G. microcephala* in other mentioned above positions was impossible as the 16S rDNA sequence of the microorganism in *G. microcephala* available in GenBank was shorter and it did not include these positions.

The phylogeny of *Wolbachia* strains based on 16S rDNA, *gatB*, *gltA*, and *hcpA* genes showed that the bacteria of *C. thienemanni* clustered with *Wolbachia* of supergroup E infecting springtail hosts (Collembola) and oribatid mite *G. microcephala*. Czarnetzki and Tebbe (2004) have reported a unique nucleotide profile C, C, A in 422, 562, and 1305 positions of 16S rDNA for *Wolbachia* supergroup E of collembolans. We also noticed this pattern in *Wolbachia* in *C. thienemanni*, which additionally confirmed the *Wolbachia* similarity and relationship to the bacteria from springtails. It is extremely interesting why the bacterial strains of Oribatida and Collembola are closely related. Acarine and collembolan hosts branch off quite early in the evolution, well before other Arthropoda (de la Filia et al., 2015). May be the phylogenetic relations among endosymbionts of oribatid mites and springtails reflect evolution relationship among their hosts? On the other hand, food can be the source of *Wolbachia* infection and horizontal transmission of *Wolbachia* between hosts may result from sharing the same food substrates (Haine et al., 2005; Sintupachee et al., 2006; Li et al., 2016) or even ingesting dead invertebrates that were infected (Brown and Lloyd, 2015). Considering coexistence of these two arthropod groups in the same soil habitat (Kováč et al., 2001; Huhta et al., 2010) and their similar food sources (Moore et al., 1988), it could not be excluded that bacterial infection in Oribatida and Collembola had the same origin. Opportunistic predation on nematodes which are common hosts of *Wolbachia* is also underestimated (Schuster, 1956; Luxton, 1972). Additionally, residues from dead invertebrates could be present in organic matter of their soil food, so the scenario of infection transferring by eating the remains of soil cohabitates is also possible (Schneider et al., 2004). It could explain the similarity and relationship of the *Wolbachia* in these two arthropod groups if they were infected by eating each other after death.

The *Wolbachia* from the E supergroup in Collembola may play various roles. It is suspected to induce parthenogenesis (Czarnetzki and Tebbe, 2004; Pike and Kingcombe, 2009) and, additionally, the endosymbiont was found to be necessary for survival of offspring in pathenogenetic springtails (Timmermans and Ellers, 2009). Other research suggests that *Wolbachia* of supergroup E does not affect reproduction in collembolan hosts but initiates or accelerates speciation (Frati et al., 2004). Oribatid mite *C. thienemanni* is a parthenogenetic mite which is a unique feature in the genus *Ceratozetes* (Fischer et al., 2014) and any of the *Wolbachia* functions mentioned above could not be excluded in reference to this mite. This species, within the entire genus *Ceratozetes*, is characterized by the most northerly distribution, being found in subalpine heath in Finland, subarctic conditions in Canada and Alaska and in the arctic coastal plain in Russia (Behan-Pelletier, 1984). It is difficult to determine either it is parthenogenesis or the presence of endosymbionts that are in some way responsible for this kind of evolutionary success. Maybe we are dealing here with a kind of synergy of both factors?

In conclusion, we report for the first time the occurrence of *Wolbachia* in parthenogenetically reproducing oribatid mite *C. thienemanni*. The amplicon sequences of 16S rDNA and housekeeping genes of the bacteria showed the highest similartity to *Wolbachia* found in Collembola and other Oribatida host *G. microcephala*. Phylogenetic analysis revealed that the endosymbionts of these invertebrates clustered together with *Wolbachia* from supergroup E.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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