First report of Wolbachia in Damaeus onustus (Acari: Oribatida)

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Abstract

Purpose: Little is known about the distribution and phylogeny of bacterial endosymbionts in oribatid mites (Acari: Oribatida). Thus, we undertook the issue of occurrence of these microbial symbionts in this arthropod group.

Methods: We used PCR technique for detection of Wolbachia in Damaeus onustus. Phylogenetic analysis of the bacterium was conducted based on the 16S rDNA sequence.

Results: To the best of our knowledge, we present a novel finding of Wolbachia infection in the sexually reproducing oribatid mite, D. onustus. The presence of uninfected individuals (ca. 93%) suggests that the bacteria do not function as primary symbionts. A comparison of the bacterial 710-bp 16S rDNA sequence detected in the oribatid mite with the sequences deposited in GenBank revealed its 92–93% similarity to the 16S rDNA sequences of Wolbachia identified in some springtails (Collembola) and Bryobia sp. mite. Bacteria from D. onustus showed phylogenetic relationships with Wolbachia from springtails, Megalothorax minimus and Neelus murinus, which were included by other authors into a separate Wolbachia clade.

Conclusion: Our finding suggests that the strains of Wolbachia from D. onustus may form a new Wolbachia supergroup.

Keywords: Wolbachia, 16S rDNA, Oribatida, Phylogenetic analysis

Findings

Wolbachia is one of the most frequent intracellular symbiont of invertebrates: arthropods and nematodes. It is estimated that 52% of arthropod species are infected with Wolbachia (Weinert et al. 2015; Huang et al. 2019). The bacterium is responsible mainly for manipulating its host reproduction (Ali et al. 2016; Mariño et al. 2017) and causing sex-ratio distortion in the infected population (Salunkhe et al. 2014; Duplouy and Hornett 2018). However, the range of its impact is much broader and includes host fitness (Zug and Hammerstein 2015; Liu et al. 2018), viral infection inhibition (Geoghegan et al. 2017; Tan et al. 2017), and defense against pathogens through the involvement in the production of host anti-predator and alarm pheromones (Becerra et al. 2015).

Wolbachia is transmitted vertically through the egg cytoplasm, from mother to offspring within the host population (Zhao et al. 2013; Guo et al. 2018). Horizontal transmission of the endosymbiont between hosts can also occur (Kremer and Huigens 2011; Brown and Lloyd 2015; Ahmed et al. 2016; Pietri et al. 2016) and is usually inferred from the presence of similar or identical bacterial strains in two unrelated host species. Food may be a medium for Wolbachia transmission among similarly feeding invertebrates, and sharing the same diet may promote horizontal transmission of these bacteria (Haine et al. 2005; Sintupachee et al. 2006; Li et al. 2016; Chrostek et al. 2017). The ingestion of infected carcasses or eggs could be a possible source of Wolbachia introduction, and eating dead invertebrates with bacterial cells inside their tissues may facilitate horizontal transmission of Wolbachia (Brown and Lloyd 2015).
Outside the host tissue, *Wolbachia* cannot be cultured in laboratory conditions using conventional bacteriological techniques. Identification and distribution of the endosymbiont in different hosts rely on molecular PCR-based screening methods. Sequence analysis of 16S rDNA and housekeeping genes of *Wolbachia* provides information useful in typing, evolutionary research, and phylogeny of these bacteria (Baldo et al. 2006; Werren et al. 2008). Different sets of genes are applied in the symbiont characterization. Phylogenetic analysis is based on 16S rDNA and housekeeping genes, for example, *atpD* (ATP synthase beta chain), *dnaA* (chromosomal replication initiator protein), and *topI* (DNA topoisomerase I) (Crainey et al., 2010). The *wsp* gene coding for the *Wolbachia* surface protein is also a reliable tool in the bacteria phylogeny (Baldo et al. 2006). Currently, strains of genus *Wolbachia* are divided into supergroups A-Q (Glowska et al. 2015).

Although a few studies on endosymbionts in oribatid mites (Acari: Oribatida) have been conducted (Pierrot-Minnot and Norton 1997; Weeks et al. 2003; Liana and Witaliński 2010; Konecka and Olszanowski 2015, Konecka and Olszanowski 2019a, Konecka and Olszanowski 2019b, Konecka and Olszanowski 2019c, Konecka et al. 2019), still little is known about the distribution and phylogeny of microorganisms in this arthropod group. We identified *Wolbachia* in *Damaeus onustus*. Phylogenetic analysis of the bacterium was conducted based on the 16S rDNA sequence.

Fifteen individuals of the oribatid mite, *D. onustus* (Acari: Oribatida) were isolated from a sample of soil and litter collected in a deciduous forest in the Wkrzańska Forest, West Pomeranian Voivodeship in Poland (53° 58′ N, 14° 43′ E).

DNA was extracted using the Genomic Mini kit (A&A Biotechnology). Amplifications of the 781-bp product of *Wolbachia* 16S rDNA were performed in a standard PCR mixture with 553F_W (5′-CTTCATRYACTCGAGT TGCWGAGT-3′) and 1334R_W (5′-GAKTTAAAYCGYGCAGGGTTT-3′) primers, as presented by Simões et al. (2011). A negative control without DNA template was included in the reaction. The PCR program was as follows: 94 °C for 2 min; 35 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 45 s; and 72 °C for 10 min (Simões et al. 2011). Amplicons were electrophoresed, sequenced with BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems), and analyzed with BLASTn. The 710-bp 16S rDNA sequence was deposited in GenBank under accession no. MH921824.

The 16S rDNA sequence of *Wolbachia* from *D. onustus* was aligned with the loci identified in other invertebrate hosts. The alignment of 32 *Wolbachia* sequences was constructed with the use of CLUSTAL W (Thompson et al. 1994). An outgroup of *Ehrlichia* spp. sequences was added. The jModelTest 2 software (Darriba et al. 2012) was used to select the optimal model of sequence evolution. The General Time Reversible model with gamma distribution among site rate variation (GTR +G) was selected. Phylogenetic analysis was conducted using MEGA.

### Table 1  *Wolbachia* strains used in phylogenetic analysis

| Designation of Wolbachia supergroup | Host of Wolbachia |
|-------------------------------------|-------------------|
| A                                   | *Drosophila melanogaster*, *Telemeca cucurbitina* |
| B                                   | *Drosophila simulans*, *Armadillidium vulgare* |
| C                                   | *Dirofilaria immitis*, *Onchocerca ochengi* |
| D                                   | *Litomosoides sigmodontis* |
| E                                   | *Ceratozetes thienemanni*, *Mesaphorura italica*, *Gustavia microcephala*, *Folsomia candida*, *Megalothorax incertus* |
| F                                   | *Coptotermes acinaciiformis*, *Nasutitermes nipriceps* |
| H                                   | *Zoospermopsis angusticalis*, *Zoospermopsis nevadensis* |
| I                                   | *Ctenocephalides felis*, *Orchopeas leucopus* |
| J                                   | *Dipetalonema gracile* |
| K                                   | *Bryobia* sp. |
| L                                   | *Radopholus similis* |
| M                                   | *Brevicoryne brassicae*, *Aphis fabae* |
| N                                   | *Tauxoptera aurantii* |
| O                                   | *Bemisia tabaci* |
| P                                   | *Syringophilopsis turdus*, *Torotrogla merulae* |
| Q                                   | *Torotrogla cardueli* |
| ?                                   | *Damaeus anustus*, *Megalothorax minimus*, *Neelus murinus* |
version 6.0 (Tamura et al. 2013). The maximum likelihood bootstrap support was determined by using 1000 bootstrap replicates. Recombination in genes between strains was detected by the φ test using the SplitsTree4 software (Huson and Bryant 2006).

To the best of our knowledge based on an extensive literature search, this is the first report of Wolbachia infection in the sexually reproducing oribatid mite D. onustus. We examined 15 specimens of D. onustus and only one of them was infected with Wolbachia. The low occurrence of infected individuals in this small sample (ca. 7%) suggests that the bacteria do not function as primary symbionts.

The 710-bp 16S rDNA sequence of Wolbachia was deposited in GenBank under accession no. MH921824. The φ test did not find statistically significant evidence of recombination (φ = 0.4885). A comparison of the bacterial 16S rDNA sequence detected in D. onustus with the sequences deposited in GenBank revealed similarity of 92–93% to the 16S rDNA sequences of Wolbachia identified in springtails (Collembola): Megalothorax minimus (accession no. KC767945), M. incertus (accession no. KT799584), and Neelus murinus (accession no. KC767946). The Wolbachia sequence was also highly similar (92%) to mite, Bryobia sp. (accession no. EU499316). These sequences were included in phylogenetic analysis of bacteria together with Wolbachia sequences representing supergroups A-Q (Table 1).

Phylogeny based on the 16S rDNA and ftsZ gene sequences of M. minimus and N. murinus bacteria was presented by Tanganelli et al. (2014). These authors found that Wolbachia from the two species of springtails did not cluster with known Wolbachia supergroups and formed a separate

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**Fig. 1** Maximum likelihood reconstruction of Wolbachia phylogeny based on the sequences of 16S rDNA. Strains are designated by the names of their hosts, except for the outgroup. NCBI accession numbers for sequences are presented after the names of hosts. Bar, substitutions per nucleotide. Bootstrap values based on 1000 replicates are shown on the branches.
and M. mini-chia
tus from keeping genes is required to explain the membership of bacteria phylogeny based on the sequences of house-
two separate supergroups. Further analysis, including the bacteria that infected Collembola also clustered into
tantly related supergroups and confirmed the fact that (Konecka et al. 2019) and
Mesaphor-
supergroup E bacteria from other springtails, mus
nelli et al. (2014) that
are phylogenetically distinct from supergroup E bacteria from other springtails, (Konecka and Olszanowski 2019a).
Our results sug-
gested that Wolbachia from Oribatida formed two dis-
tantly related supergroups and confirmed the fact that the bacteria that infected Collembola also clustered into
itself and into two separate supergroups. Further analysis, including bacteria phylogeny based on the sequences of house-
keeping genes is required to explain the membership of Wolbachia from D. onustus to a potentially new Wolba-
ch supergroup.

In conclusion, our study presents for the first time the occurrence of Wolbachia infection in Oribatida D. onus-
the 16S rDNA sequence of Wolbachia from the mite indicated similarity and phylogenetic relationship with bacteria found in springtails, M. mini-
us and N. murinus. Our discovery suggested that the strains may form a new Wolbachia supergroup. The role
of these bacteria in D. onustus remains unknown and also needs further investigations. Nevertheless, the effect of parthenogenesis induction by Wolbachia could be excluded considering the fact that D. onustus is a sexually
producing species.

Competing interests
The authors declare no conflicts of interest.

Ethics approval and consent to participate
All work performed in studies involving invertebrate animals (mites) was
done in compliance of the ethical standards following for the environmental
t primarily used in studies involving invertebrate animals (mites) was
done in compliance of the ethical standards following for the environmental
samples. This article does not contain any studies with human participants, laboratory animals, or vertebrate animals. The informed consent was not
applicable.

Authors’ contributions
EK and ZO designed the study and planned the experiments. AJ collected
the sample. EK, ZO, and AJ carried out the experiments. EK analyzed the data
and wrote the manuscript with input from ZO. The authors read and
approved the final manuscript.

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