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Genomic and metagenomic analyses of the domestic mite *Tyrophagus putrescentiae* identify it as a widespread environmental contaminant and a host of a basal, mite-specific *Wolbachia* lineage (supergroup Q)

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ABSTRACT

Tyrophagus putrescentiae (mould mite) is a global, microscopic trophic generalist that commonly occurs in various human-created habitats, causing allergies and damaging stored food. Its ubiquity and extraordinary ability to penetrate research samples or cultures through air currents or by active walking through tights spaces (such as treads of screw caps) may lead to sample contamination and introduction of its DNA to research materials in the laboratory. This prompts a thorough investigation into potential sequence contamination in public genomic databases. The trophic success of *T. putrescentiae* is primarily attributed to the symbiotic bacteria housed in specialized internal mite structures, facilitating adaptation to varied nutritional niches. However, recent work suggests that horizontal transfer of bacterial/fungal genes related to nutritional functionality may also contribute to the mite's trophic versatility. This aspect requires independent confirmation. Additionally, *T. putrescentiae* harbors an uncharacterized and genetically divergent bacterium, *Wolbachia*, displaying blocking and microbiome-modifying effects. The phylogenomic position and supergroup assignment of this bacterium are unknown. Here, we sequenced and assembled the *T. putrescentiae* genome, analyzed its microbiome, and performed detailed phylogenomic analyses of the mite-specific *Wolbachia*. We show that *T. putrescentiae* DNA is a substantial source of contamination of research samples. Its DNA may inadvertently be co-extracted with the DNA of the target organism, eventually leading to sequence contamination in public databases. We identified a diversity of bacterial species associated with *T. putrescentiae*, including those capable of rapidly developing antibiotic resistance, such as *Escherichia coli*. Despite the presence of diverse bacterial communities in *T. putrescentiae*, we did not detect any recent horizontal gene transfers in this mite species and/or in astigmatid (domestic) mites in general. Our phylogenomic analysis of *Wolbachia* recovered a basal, mite-specific lineage (supergroup Q) represented by two *Wolbachia* spp. from the mould mite and a gall-inducing plant mite. Fluorescence in situ hybridization confirmed the presence of *Wolbachia* inside the mould mite. The discovery of an early derivative *Wolbachia* lineage (supergroup Q) in two phylogenetically unrelated and ecologically dissimilar mites suggests that this endosymbiotic bacterial lineage formed a long-term association with mites. This finding provides a unique insight into the early evolution and host associations of *Wolbachia*. Further discoveries of *Wolbachia* diversity in acariform mites are anticipated.

☆ Note: Supplementary data associated with this article.

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1. Introduction

The mould mite, *Tyrophagus putrescentiae*, is a common, microscopic, and globally distributed species of domestic mites (Hughes, 1976; Klimov and OConnor, 2009a). It is a generalist species, living in nearly every terrestrial habitat with relative humidity > 65% (Canfield and Wrenn, 2010; Erban et al., 2015). It frequently infests stored products, resulting in economic loss, and it causes anaphylaxis in sensitized individuals consuming mite-contaminated food (Sanchez-Borges and Fernandez-Caldas, 2015). After pyroglyphid house dust mites, *T. putrescentiae* is the second most medically important species responsible for indoor allergies in humans (Arlian et al., 1984; Cui et al., 2016; Hubert et al., 2019) and domesticated animals (Mueller et al., 2016). Due to its long body setae, it can disperse via air currents, attaching to human clothing and household items, or move rapidly on its own (Freitag and Kells, 2013; Hubert et al., 2018). *Tyrophagus putrescentiae* is arguably the first known animal space hitchhiker found onboard a human-inhabited spacecraft in low Earth orbit (Ott et al., 2004). The ubiquity of *T. putrescentiae* is well documented by a large body of literature (Fan and Zhang, 2007); however, its reliable identification was only possible after 2007–2009, when accurate methods based on morphology and DNA sequences were developed (Fan and Zhang, 2007; Klimov and OConnor, 2009a). Even though the mite has been previously reported to infest fungal, insect, and plant tissue cultures in laboratory and industrial settings (Walter et al., 1986; Duek et al., 2001), its role as a significant factor in DNA sequence contamination is currently underappreciated. Since most bioinformatics tools focus on bacterial and human DNA contamination, DNA of a microscopic eukaryote may evade the National Center for Biotechnology (NCBI) GenBank standard quality check procedure and be inadvertently incorporated into public sequence databases. A detailed investigation of the prevalence of contaminated sequences in GenBank is urgently needed.

Tyrophagus putrescentiae is a vector of various bacterial and fungal microorganisms in human-related habitats and agricultural settings (Hubert et al., 2003, 2018). The presence of bacteriocytes (i.e., large compartmentalized bacterial colonies in the mite parenchymal tissues) is one of the most distinctive anatomical features of *T. putrescentiae* (Erban et al., 2016a). Bacteria may be very important in the mite's nutritional ecology, cooperating with their acarine host to use nutrients from different sources. For example, the associated bacteria may provide chitinolytic enzymes digesting fungal cell walls (Smrz et al., 2016), making them ecologically important for mobilizing nitrogen from chitin (Smrz and Catska, 2010). Previous work suggests that bacterial or fungal genes related to nutritional functionality may have been incorporated into the mite genome (Xiong et al., 2022). For example, the horizontally transferred genes encoding uridine 5-diphospho-glucuronosyltransferases (UDP glucuronosyltransferases) and several fungal cell wall lytic enzymes could enable detoxification and digestive functions in their acarine hosts (Xiong et al., 2022). It is thus important to quantify the frequencies and the functional significance of horizontal gene transfer (HGT) events in *T. putrescentiae*.

Bacterial associations of *T. putrescentiae* include a Gram-negative bacterium, *Wolbachia*, which had been previously identified in several *T. putrescentiae* populations through 16S rRNA and protein sequencing methods (Hubert et al., 2019; Erban et al., 2021). *Wolbachia* is an intracellular endosymbiont associated with various arthropods and nematodes. This bacterium may not only form nutritional symbiosis with several hosts (Nikoh et al., 2014), but also has the potential to modify communities of other symbionts in the hosts, thereby serving as a beneficial disease control agent (Walker et al., 2011). A microbiome blocking/modifying effect has been recently shown for *Wolbachia* from *T. putrescentiae* (Hubert et al., 2019), suggesting that this *Wolbachia* may be potentially useful for disease/pest control applications. However, the relationship of the species with other *Wolbachia* is unknown because 16S rRNA alone is insufficient to resolve its phylogenetic relationship.

Here, we sequenced the whole genome of *T. putrescentiae* using Illumina short reads from a single female specimen from North America. Together with transcriptomics data previously generated by us for several mite populations from Europe and the USA, we answer the following questions: i) Can the mite be a significant source of DNA contamination in the laboratory? ii) Are there recent horizontal gene transfers from bacteria/fungi that can account for the mite's extended nutritional functionality? iii) What are the phylogenomic affinities of the previously uncharacterized mite-specific *Wolbachia*?

2. Materials and methods

2.1. Sample, library preparation, sequencing, and metagenomic assembly

Genomic sequencing was done from a single female reared in a culture maintained at the University of Michigan Museum of Zoology, Ann Arbor, MI, USA, at room temperature, relative humidity 75–100%, using Tetra® TetraMin Large Tropical Flakes (USA) as the food source. This culture was started from specimens with the following collection data: MEXICO: Ciudad de México, Parque Ecológico de Xochimilco, nr. Lago Acitlalin, 19.297115–99.092799, rotten reed stalk (*Typha*), 03 Jan 2017, P. Klimov (coll.), University of Michigan Museum of Zoology (UMMZ) accession BMOC 17-0108-002.

Genomic DNA was extracted from a single female specimen using a QIAamp DNA Micro kit (Qiagen, USA) under sterile conditions. An Illumina sequencing library was generated from a single mite female using the KAPA HyperPlus Kit (Roche, USA) under sterile conditions. The insert size was 322 bp. Sequencing was done on an Illumina HiSeq-4000 instrument, generating 755,504,138 (377,752,069x2) 150x2 bp paired-end reads. Read quality was evaluated in FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). Quality filtering and adaptor content removal was done in bbtools v. 38.23 (https://sourceforge.net/projects/bbmap/) as detailed in Supplementary Data S1 (section 1). Four metagenomic assemblies were run, in Megahit (Li et al., 2015) and MetaSPAdes 3.12.0 (Nurk et al., 2017), with three different sets of kmer sizes ("-k"): 21,33,55; 21,33,55,77; 21,33,55,111 (Supplementary Table S1). For assembly evaluation, the following three statistics were used: (i) basic, reference-free statistics, e.g., N50, L50 in QUAST v.5.0.0 (Gurevich et al., 2013) (Supplementary Table S2); (ii) alignment against the transcriptome using HISAT2 v.2.1.0 (Kim et al., 2019) and RNAquast v.1.5.1 (Bushmanova et al., 2016) (Supplementary Table S1), and (iii) alignment against the contaminated "*Rhagoletis zephyria*" genome in QUAST (Supplementary Table S3). These programs were run using Unix shell scripts (for detail see Supplementary Data S1 section 2). In addition, our final decontaminated assembly (see below) was evaluated by finding sets of single-copy, orthologous genes specific to Arachnida in BUSCO v.5.4.7 (Simão et al., 2015). Our assembly was submitted to GenBank on Jan 2020 and since then was used in several studies (Hou et al., 2022; Klimov et al., 2022; Zhou et al., 2023). However, these studies had different objectives and are not repeating our results. We acknowledge that there are two other *T. putrescentiae* assemblies, both published in GenBank in 2022. Of those, we compared our data with assembly GCA_021730765.1 (Hong Kong) (Xiong et al., 2022), and discussed it in the text. The other

chromosome-level assembly GCA_024499935.1 (China) (Zhou et al., 2023) was published after our manuscript was completed. Our transcriptome assembly (GenBank Transcriptome Shotgun Assembly (TSA) accession number GIFQ00000000.1) was described earlier (Hubert et al., 2019). For the transcriptomic sequencing, we used 30–40 mg of mites by fresh weight sourced from pure cultures maintained at the Crop Research Institute in Prague, Czech Republic. Our transcriptome assembly, with the GenBank TSA accession number GIFQ00000000.1, has been previously documented (Hubert et al., 2019).

2.2. Assembly annotation

Gene prediction and annotation was done in the maker genome annotation pipeline v2.31.10 (Cantarel et al., 2008) in three steps: (i) we directly used our transcriptome and non-redundant GenBank proteins from Ecdysozoa as the annotation evidence (est = transcriptome.fas; protein = Ecdysozoa_prot.fa; est2genome = 1; protein2genome = 1 in the configuration file maker_opts.ctl); these imperfect gene models (ii) were then used to train the gene prediction program SNAP (Korf, 2004) bundled with maker (snaphmm = snap1.hmm; est2genome = 0; protein2genome = 0); a new set of gene annotations generated in this step (iii) was then used to train the gene predictor yet again (snaphmm = snap2.hmm). For conserved protein domains, gene ontologies were determined in InterProScan v.5.38–76.0 (Jones et al., 2014). These ontologies and standardized gene names were assigned to maker annotations using maker accessory scripts. Top gene ontologies were summarized in WEGO v2.0 (Ye et al., 2018) using the InterProScan output.

Dermatophagoides. farinae microRNAs (miRNAs) annotated by miRDeep2 (An et al., 2013) software and curation of miRNAs based on standard miRNA features. *T. putrescentiae* miRNAs were found using nucleotide (nt) BLAST to identify regions homologous to *D. farinae* miRNAs as well as those deposited for chelicerate arthropods in miRbase (https://www.mirbase.org). Ago/Piwi homologs were similarly found with nt BLAST. Each putative Ago/Piwi protein was verified to encode a PAZ and PIWI domain.

Mitochondrial genome annotation was done using multiple lines of evidence: sequence similarity with two related species, *Sancassania berlesei* (KF499016) and *Aleuroglyphus ovatus* (NC_023778.1) (Sun et al., 2014a, 2014b); *T. putrescentiae* expressed sequence tags (EST) data (GenBank accession number: SAMN00174981 ID: 174981); ARWEN (Laslett and Canback, 2008) with minimal tRNA search methodology as described previously (Klimov and OConnor, 2009b); and the Mitos Web Server for automatic prediction of all mitochondrial genes (Bernt et al., 2013).

2.3. Mite phylogenomics

We analyzed 48 genomes of acariform mites (n = 34), including our *T. putrescentiae* assembly, parasitiform mites (outgroups, n = 13) and *Limulus polyphemus* (distant outgroup, n = 1). BUSCO v5.3.0 (Simão et al., 2015; Seppey et al., 2019) analyses were run to identify phylogenetically conserved, single-copy genes using the arachnida_odb10 database. Alignments were done in mafft v7.490 (Katoh and Standley, 2013): mafft --thread $proc --inputorder --bl 62 $i > $i.mafft. Alignment quality trimming was done in trimAl v.1.4.1 (Capella-Gutierrez et al., 2009): trimal -in $i -out $i.trimal -automated1 -resoverlap 0.75 -seqoverlap 80. A subset of 415 protein alignments (occupancy 0.8 and length 200) were used for a partitioned analyses in IQ-TREE v.2.2.0 with automatic protein model selection and partition merging (Nguyen et al., 2015): iqtree2 -s $ipf -p $ipf --seqtype AA -T AUTO --merge -rclusterf 10 -m MFP -alrt 1000 -bb 1000 -safe --prefix $ipr, where the variable $ipf represents the directory of alignment (-s) and partition (-p) files, and the variable $ipr sets the analysis name prefix.

2.4. Metagenomic decontamination

A detailed decontamination procedure is described in Supplementary Data S1 (section 3). Briefly, to classify the 176,943 initial metagenomic scaffolds, we ran a local nt BLAST v.2.7.1 (Altschul et al., 1990). Hits on contaminated GenBank assemblies, Illumina technical sequences (PhiX), and *Homo sapiens* contamination were removed; high-scoring hits on Bacteria and other non-eukaryotic organisms were filtered out; then high-scoring hits on "*Rhagoletis zephyria*" and *Tyrophagus* (bitscore >=300 or identity >=95%) were filtered and their coverages were noted. Entries unclassified by nt BLAST were classified as mite sequences based on their coverages > 600; all sequences shorter than 300 bp were removed. The mitochondrial DNA and rRNA scaffolds were identified, annotated manually, and trimmed. The final assembly, named here P3.F6, had a total of 19,731 scaffolds (length 95,135,691 bp). This Whole Genome Shotgun project has been deposited at GenBank under the accession number JAAALH000000000 (BioSample SAMN13712654, BioProject PRJNA598686, assembly GCA_012066115.1); the source short Illumina reads (SRA accession: PRJNA598686; SRA run: SRR11069688); assembled metatranscriptome of *T. putrescentiae* (GIFQ00000000) and the source short Illumina reads (SRR7903714-SRR7903734); assembled transcriptome of *Wolbachia* endosymbiont of *T. putrescentiae* (GIJY01000000).

2.5. Metagenomic profiling

Detailed methodology is given in Supplementary Data S1 (section 4). Briefly, raw Illumina reads were processed to remove adapter sequences, low quality data, and artefacts in bbmap 38.51. For these processed reads, we assigned taxonomic classifications in Kraken2 v2.0.8-beta (Wood and Salzberg, 2014) with the confidence parameter of 0.1, followed by abundance estimation in Bracken (Lu et al., 2017). Three analyses were run, each using a different Kraken library: (i) Basic with standard Kraken databases: archaea, bacteria, viral, human, plant, fungi, protozoa; (ii) Custom1 (basic plus the '*Rhagoletis zephyria*' genome); Custom2 (basic plus the *T. putrescentiae* P3.F6 assembly).

To classify scaffolds, we used BLAST searches with the nucleotide blastdb5 database (downloaded 16 May 2019). We also constructed several custom BLAST databases using our metagenome, metatranscriptome, *T. putrescentiae* assembly (P3.F6), and the '*Rhagoletis zephyria*' genome. The intersection between the DNA and RNA assemblies was determined using the following criteria to classify sequences as belonging to the same species / operational taxonomic unit (OTU): bitscore 500 and identity 95%. Full taxonomic lineage information was added to blast/diamond outputs using a custom script (Supplementary Data S1 section 6). For metagenomic profiling of assemblies, we used BlobTools (Laetsch and Blaxter, 2017), an analysis which uses three lines of evidence: coverage, GC content, and BLAST (nucleotides) or DIAMOND (protein) classification. For assigning a unique classification to multiple nt BLAST hits, we used the 'bestsum' algorithm in BlobTools. Because this and other BlobTools classification algorithms may return false positives, we did not use BlobTools to automatically remove contaminants.

2.6. HGT

Previously described methodology was used (Crisp et al., 2015). Using the Unix command awk, we parsed the Uniref50 proteins (downloaded 11 July 2022) into two groups: (1) no Metazoa; and (2) Metazoa minus Arthropoda. TaxIds were extracted from the

Uniref50 fasta headers. The TaxId 178,133 (plant + phytophagous eriophyoid mite) and 46 TaxIds not found in GenBank taxonomy were removed. Diamond v0.9.14.115 was used to run the mite coding sequences (see section 2.2 above) against two databases built from the two sets of proteins. Using bitscores from the two diamond searches, HGT indices were calculated for each coding sequence as described previously (Crisp et al., 2015). Our entire HGT discovery pipeline was documented as a Unix shell script in Supplementary Data S1 (section 7). Because UniRef50 protein clusters (50% sequence similarity) are labelled by common taxonomy of the cluster (rather than by the representative taxon) (Suzek et al., 2015), we considered protein clusters having a high taxonomic rank as conserved. For example, regardless of its HGT score, a cluster labelled as "cellular organisms|Eukaryota|" was deemed as conserved across Eukaryota, rather than being the result of HGTs.

2.7. Wolbachia endosymbiont: metatranscriptomic assembly and phylogenetics

We sequenced a metatranscriptome of *T. putrescentiae* from Europe (GIFQ00000000) and assembled it in CLC Genomics Workbench v11 (Qiagen). Collection detail and bioinformatics methodology for this sample were described previously (Hubert et al., 2019). *Wolbachia* contigs were identified using nt BLAST and DIAMOND v0.9.24.125 (Buchfink et al., 2015). Because the rRNA fraction was depleted in the transcriptome, the 16S rRNA gene was recovered separately by mapping adaptor-free, artefact-free, quality-trimmed and filtered reads onto the known 16S *Wolbachia* sequence (GCA_000829315.1) following assembly in rnaSPAdes v3.13.0. The final *Wolbachia* transcriptomic assembly had 280 contigs with a total length of 925,767 bp (average coverage 742.8), approaching the typical *Wolbachia* genome size range, 1.3–1.6 Mb (Wu et al., 2004). Phylogenetic inferences were done for four datasets: genomic, five standard phylogenetic loci (Glowska et al., 2015), five multilocus sequence typing (MLST) loci (Baldo et al., 2006), and 16S rRNA (see Supplementary Table S4: standard phylogenetic loci accession ids; Supplementary Data S3-5: nexus alignments). For the former analysis, we used 169 GenBank genomes downloaded from GenBank plus our assembly: 31 outgroups (*Ehrlichia*, *Anaplasma*) and 139 *Wolbachia* ingroups. BUSCO5 analyses was run to identify phylogenetically conserved, single-copy proteins at the level of Rickettsiales. A Maximum Likelihood tree was inferred in IQ-TREE v.2.2.0, using a partitioned analysis and automatic model selection (Nguyen et al., 2015). Our entire workflow is described in detail in Supplementary Data S1 (section 5).

2.8. Fluorescence in situ hybridization (FISH)

FISH was performed using universal and specific bacterial probes. *Tyrophagus putrescentiae* adults were first fixed in 4% formaldehyde. For the hybridizations, we followed the FISH protocol described in Perotti et al. (2007), then mounted those as whole specimens. Samples were incubated at 45 °C in darkness for up to 20 h, washed for 1 h in hybridization buffer followed by PBTA (phosphate buffer with Triton X-100 plus sodium azide) at room temperature. Then, mites were mounted in PBS/glycerol and photographed under the confocal microscope. A number of bacterial probes were used in different observations: EUB-338 (Amann et al., 1990) and EUB-338 II and III (Daims et al., 1999), *Rickettsia* (Perotti et al., 2006) and *Wolbachia* (Balmand et al., 2013) (equimolar mixed in the hybridization buffer (following remarks of ProBase)). No probe and competition suppression controls were performed. A Confocal Zeiss LSM510 microscope with Coherent Multiphoton laser was used. For these experiments, we used cultures originating from the Crop Research Institute, Prague (Czech Republic) (Hubert et al., 2019) and the University of Reading laboratory, UK (maintained since 2008, stock colony received from the Food and Environment Research Agency, UK).

3. Results

3.1. Genome assembly of T. putrescentiae

We conducted a series of independent metagenomic assemblies in metaSpades and Megahit (Supplementary Tables S1-S4**)**. Based on different metrics, most importantly, transcriptome mapping statistics (Supplementary Table S1**)**, we selected the metaSpades (k = 21,33,55) assembly as our preferred assembly, consisting of 176,943 scaffolds, with a total length of 151,679,586 bp and average coverage of 518.3. Of those, 9,303 scaffolds (79.5 Mb) matched the transcriptomic assembly (Supplementary Fig. S1**)**. Of the 176,943 initial scaffolds, nt BLAST taxonomically classified 125,524 scaffolds (136,606,678 bp, average coverage = 440.4) (Supplementary Fig. S2A**)**. This analysis revealed that our metagenomic assembly had a substantial portion of non-mite sequences, mostly bacterial DNA. Most of the non-mite scaffolds had low coverage, i.e. below 100x, with the notable exception of the bacterium *Alcaligenes faecalis*, which had a coverage of slightly above 100x (Supplementary Fig. S2A**)**. Many scaffolds lacking BLAST hits, therefore, still could be confidently classified as belonging to the mite based on their higher coverages, i.e. > 600 (Supplementary Fig. S2A: grey color). After filtering scaffolds using a combination of the BLAST classification result and/or coverage information (detailed in section 2.4 above), our final decontaminated mite assembly had a total of 19,731 scaffolds, with a length of 95,135,691 bp and average coverage of 1024.7 (Fig. 1A, B; Supplementary Table S1; Supplementary Fig. S2B**)**. For our assembly, the BUSCO statistic using the arachnida_odb10 database (2934 genes) was superior to the current GenBank reference assembly of *T. putrescentiae*: complete BUSCO: 87.5% (GCA_024499935.1) versus 89.4% (our assembly); additionally, our assembly was compatible with another, non-reference GenBank assembly of *T. putrescentiae* (GCA_021730765.1), which achieved 89.8% of complete BUSCOs (Supplementary Fig. S3). Our assembled mitochondrial genome has the typical gene order of Astigmata (Klimov and OConnor, 2009b; Sun et al., 2014b) (Fig. 1B). Top gene ontologies (in terms of gene percentages) summarized for three subontologies were as follows: membrane and membrane part (cellular component), catalytic activity and binding (molecular function), and metabolic and cellular processes (biological process) (Supplementary Fig. S4**)**. Our phylogenomic analysis inferred *T. putrescentiae* within the Astigmata, a major general mite lineage (Fig. 1C). Astigmata evolved within soil mites (Oribatida), which was supported with absolute Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap values (Fig. 1C), while other high-level relationships were similar to those inferred previously (Klimov et al., 2022; Xiong et al., 2022).

3.2. Extensive sequence contamination of GenBank databases by T. putrescentiae DNA

Our nt BLAST searches detected extensive contamination of public sequence databases with *T. putrescentiae* sequences (Table 1). For example, the GenBank reference genome database contained a '*Rhagoletis zephyria*' genome GCF_001687245 (Dowle et al., 2020) heavily contaminated with *T. putrescentiae* DNA, 4901 scaffolds (Table 1). We previously reported this (Hubert et al., 2019), albeit without providing in-depth details, and these sequences are now flagged as contaminants by GenBank. Another important example is the mite sequence identified as a bacterium

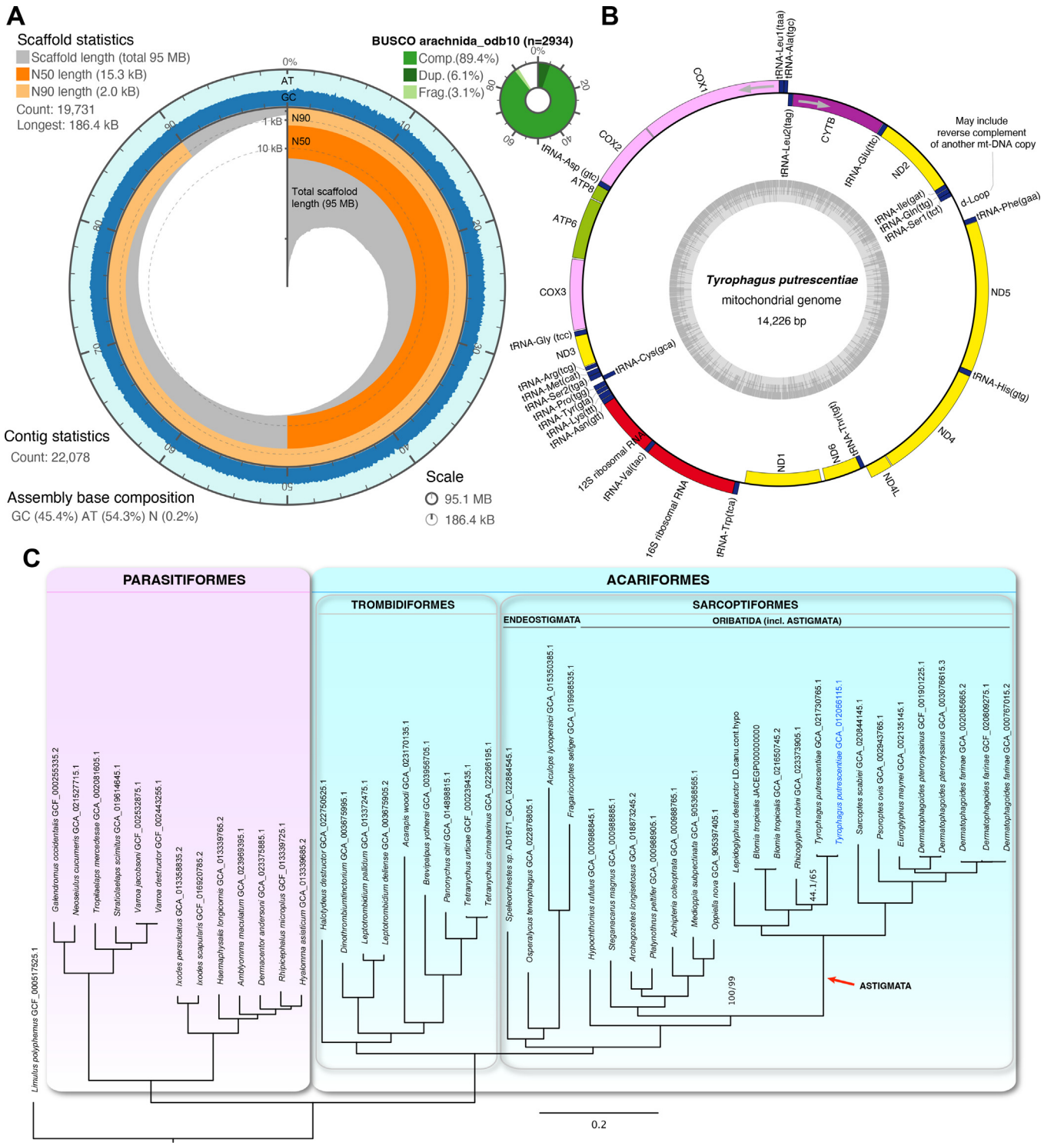

Fig. 1. Decontaminated genomic assembly of *Tyrophagus putrescentiae* from Mexico and phylogenomic tree of acariform mites. (A) Basic assembly statistics. (B) Mitochondrial genome (for each strand, arrows show direction of transcription; inner circle shows GC content). (C) Maximum likelihood phylogenomic tree of acariform mite relationships, for each branch, Shimodaira-Hasegawa approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrap support values are given (in that order) unless both measures equal 100%.

"*Shinella* sp." from healthcare settings (Brooks et al., 2017) (Table 1). Other contaminated GenBank sequences include many species of insects and fungi, as well as vertebrates, round worms, bacteria, and plants (Table 1); these sequences also have matches on the contaminated '*Rhagoletis zephyria*' genome, which offers independent confirmation of contamination. We also ran BlobTools analyses based on the GenBank nucleotide database and our raw or clean assemblies of *T. putrescentiae* as a query. The BlobTools analysis largely agrees with our results, identifying approximately 96.5 and 94.5 Mb of *T. putrescentiae* DNA in the contaminated '*R. zephyria*' genome when run on the raw and clean assemblies, respectively (Supplementary Fig. S2). In addition, the following taxa were identified by BlobTools as 'contaminated' with the mite: *Ixodes scapularis*, *Drosophila biramipes*, *Plutella xylostella*, *Cyprin-*

Table 1
Select GenBank sequences attributable to *Tyrophagus putrescentiae* contamination based on a National Center for Biotechnology (NCBI) nucleotide Basic Local Alignment Search Tool (BLAST) search.

GenBank title	GenBank ID	Identity (%)	Bitscore	TP scaffold ID	RZ	Country
Insects						
Rhagoletis zephyria genome	GCF_001687245.1	94.0[a]	909[a]	4901 scaffolds total	y	USA
Ostrinia nubilalis	AF398406.1	99.1	623	947	y	USA
Simulium damnosum sp.complex	KY631747.1	99.0	695	3270	y	Nigeria
Predatory mites						
Neoseiulus cucumeris	AY099366	98	678	692	[b]	USA
Cheyletus malaccensis	KP938898.1	98.7	689	692	[b]	China
Vertebrates						
Mus musculus	AK041295.1, AK041150.1	97.5–99.4	1118–1308	2104, 6946, 10,139	y	Japan
Ardea herodias	AF447969.1	99.02	366	4160	y	USA
Round worms						
Ancylostoma caninum	DQ841142.1, DQ841148.1	98.5–99.1	580–963	1820, 7390	y	USA
Fungi						
Colletotrichum gloeosporioides	JQ862580.1	99.0	1317	905	y	Colombia
Parastagonospora forlicesenica	KY769662.1	100	1905	3270	y	Italy
Fusarium equiseti	MG751111.1,MG751119.1,MG751114.1	99.1–100	327–545	43, 57, 3166	y (first)	USA
Fusarium graminearum	MG751125.1,MG751131.1	97.2–97.8	388–424	273, 6583	y	USA
Plants						
Intsia palembanica	FJ448223.2,FJ448461.2,FJ448035.2	97	407–749	650, 3618, 4637	y (first two)[c]	Singapore
Bacteria						
Shinella sp.	QFOR01000162	100	2353	6121	y	USA[d]

TP, a match with our decontaminated P3.F6 assembly of *T. putrescentiae* (scaffold IDs are given); RZ, a match with the GenBank "*Rhagoletis zephyria*" genome (contaminated with *T. putrescentiae* DNA); this information offers independent validation of our results.
[a] Values are averaged.
[b] The GenBank "*Rhagoletis*" genome does not have the mite mitochondrial DNA; this sequence matches GenBank *T. putrescentiae* mitochondrial genome (from China).
[c] The laboratory that produced these sequences is known to maintain *T. putrescentiae* cultures.
[d] Hospital surfaces and sink.

odon variegatus (Supplementary Fig. S2B), with low score/low identity, and probably representing false positives (not included in Table 1).

3.3. Mould mite T. putrescentiae harbors diverse bacterial communities

We profiled our metagenome (Mexico) and metatranscriptome (Europe) datasets using (i) Kraken (Wood and Salzberg, 2014) to classify raw reads and (ii) nt BLAST to assign a taxonomic classification to scaffolds (assembled reads). For the two classification strategies in metagenomic profiling, we used standard databases plus our clean genomic assembly of *T. putrescentiae* (GCA_012066115) to avoid false positive hits, i.e. incorrect classifications of *T. putrescentiae* sequences as different eukaryotic organisms. Among the bacterial metagenomic reads, Kraken identified the following bacterial species with an abundance of more than 2% (Table 2): *Alcaligenes faecalis* (58%), *Pseudomonas aeruginosa* (9%), and others (all 3–4%): *Stenotrophomonas maltophilia*, *Stenotrophomonas* sp. PAMC25021, *Advenella kashmirensis*, and *Achromobacter denitrificans* (Fig. 2A). *Stenotrophomonas maltophilia* was among the low-abundance bacteria (<10%); however, it was identified as a potential contaminant of DNA extraction kits (Glassing et al., 2016), so further confirmation of this record is needed. Kraken identified the yeast *Candida dubliniensis* as the most abundant fungal species (99% of all fungi, magnitude 245,252). However, the nt BLAST search did not find this OTU at all; instead, a different species, *Candida parapsilosis*, was detected in trace amounts (1,717 bp assembly, 42 mapped reads) (Table 2). We also detected trace amounts of low-scoring reads for the Apicomplexa (Table 2), which are known internal parasites of mites (Poinar and Poinar, 1998).

The metatranscriptomic dataset contained *Escherichia coli*, *Wolbachia* (unidentified divergent species, see below), and *Bacillus* (including *Bacillus thuringiensis*, *Bacillus cereus*) with high abundance, 15–36% of all bacterial reads (Table 2). Fungi were represented by *Fusarium* and apicomplexans were represented by an unidentified coccidian taxon (different from the Apicomplexa from the metagenomic dataset), whose nuclear rRNA was similar to that of *Adelina* (92%) (Table 2). Here, many species reported by the Kraken software in the transcriptome (European mite populations) could not be confirmed by nt BLAST searches (Table 2). This is an expected outcome, as Kraken's use of short k-mers inherently leads to lower accuracy.

Based on the intersection of the DNA and RNA samples by nt BLAST, only one species was found to occur in both samples, *Cutibacterium acnes* (Table 2). However, this bacterium is a prevalent and dominant contaminant in DNA extraction kits, constituting as much as 26–34% of all bacterial operational taxonomic units (OTUs) (Glassing et al., 2016). We consider its presence a probable artifact, but refer to Campisano et al. (2014) for alternative explanations.

FISH detected *Wolbachia* (Fig. 3A) and *Rickettsia* (Fig. 3B), in the mite's parenchymal tissue bacteriocytes; Eubacteria were mostly associated with the digestive tract and parenchymal tissue (Fig. 3E, F); *Wolbachia* was also found in the ovaries and eggs (Fig. 3A, G).

3.4. No recent horizontal gene transfers in the mould mite T. putrescentiae or Astigmata

Using the standard methodology (Crisp et al., 2015), we detected eight putative HGT events in our mite genomic assembly from the following lineages: Bacteria (five), Fungi (two), and Amoebozoa (one) (Table 3, Fig. 2B-D, Supplementary Figs. S5–S11, Supplementary Data S2). Among them was the D-Ala-D-Ala dipeptidase gene, previously suggested to be horizontally transferred to Astigmatid mites (Xiong et al., 2022). However, all these putative HGT hits had very low amino acid similarity (40.3–60.71%) to the corresponding proteins of *T. putrescentiae* (Table 3) and had significant matches to Oribatida, Endeostigmata, or Trombidiformes, which are major early-derivative acariform mite lineages compared with Astigmata (Table 3). This evidence strongly indicates that no HGT events occurred at the origin and during the evolution of Astigmata, a major lineage that includes the mould mite and other domestic mites.

Table 2
Metagenomic profiles of two geographically isolated samples of *Tyrophagus putrescentiae* (Mexico, Europe) based on raw reads (Kraken) and assembled contigs (BlobTools).

Species	Kraken % of bacterial/fungal/eukaryotic reads	Kraken magnitude	Blob reads mapped on assembly	Blob assembly size (bp)	BLAST best hit (bistcore)	Identity for BLAST best hit
		Metagenome (Mexico)				
	Bacteria					
Alcaligenes faecalis	58	1,154,214	2,450,807	4,220,682	370,900	98.881
Pseudomonas aeruginosa	9	186,466	2,129,316	4,743,839	10,405	100.000
Stenotrophomonas sp. PAMC25021	4	76,490	1,191	9,969	5,317	99.286
Achromobacter denitrificans	4	74,386	94,154	1,554,668	2,248	99.043
Advenella kashmirensis	3	67,222	331,132	3,470,439	6,248	98.477
Stenotrophomonas maltophilia	3	63,477	307,833	3,505,590	5,345	99.456
Cutibacterium acnes[h]	2	36,049	54,577	1,060,224	2,830	99.677
	Fungi (Dikarya)					
Candida dubliniensis[i]	96	221,846	42[a]	1,717 [a]	713 [a]	100 [a]
	Apicomplexa					
Low-scoring OTU(s)	0.0002	592	10[c]	101[c]	556[b]	98.418[b]
		Metatranscriptome (Europe)				
	Viruses					
Wolbachia phage	not recovered	not recovered	507	2,573	1908	81.732
	Bacteria					
Escherichia coli	36	130,213	14,451,554	119,701	9,356	99.98
Bacillus spp	18	63,648	83,438	122,804	12,412 [d]	99.911 [d]
Wolbachia	15	54,356	1,592,451	622,805	10,035	80.264
Salmonella enterica[i]	8	30,667	not recovered	not recovered	2,564	99.434
Yersinia pestis[i]	6	22,138	not recovered	not recovered	not recovered	not recovered
Cutibacterium acnes[h]	0.7	2,675	13,221	1,361	2,071	94.635
	Fungi (Dikarya)					
Zymoseptoria tritici[i]	40	11,594	not recovered	not recovered	187	78.84
Fusarium	8	2,423	18,016,619[f]	58,270[f]	11,753 [e]	99.597 [e]
Botrytis cinerea[i]	8	2,224	not recovered	not recovered	not recovered	not recovered
Colletotrichum higginsianum[i]	7	1,980	n/a	n/a	87.9	85.714
Sporisorium graminicola[i]	7	1,941	not recovered	not recovered	not recovered	not recovered
	Apicomplexa					
Low-scoring OTU(s)	0.05	104,121	9,053,673	434,697	2857, 1890[g]	82.403, 92.528[g]

OTU, operational taxonomic unit.
[a] Not found by nucleotide (nt) BLAST search; values given for *Candida parapsilosis*.
[b] For *Babesia bigemina*.
[c] For *Babesia ovata*.
[d] *Bacillus thuringiensis* and *Bacillus cereus*.
[e] For *Fusarium proliferatum*.
[f] High abundance in the transcriptome due to the presence of highly expressed mitochondrial genes.
[g] For *Klossiella equi* (mitochondrion) and *Adelina bambarooniae* (18S rRNA), respectively.
[h] Found in both samples.
[i] Spurious Kraken result, not confirmed by nt BLAST.

3.5. An early derivative, mite-specific supergroup of Wolbachia

Our phylogenomic analysis recovered two species of *Wolbachia* associated with mites (the mould mite *T. putrescentiae* and the gall mite *Fragariocoptes setiger*) forming a monophyletic group in a basal portion of the tree (Fig. 4). As genomic-scale data are not available for many *Wolbachia*, we attempted to identify this lineage through the use of 16S or multi-locus analyses (many taxa, few loci). These analyses can demonstrate the affinity of an unknown sequence, but generally they cannot resolve phylogenetic relationships among the *Wolbachia* supergroups. In the five-gene analyses, *Wolbachia* from *T. putrescentiae* (wTp) was grouped with *Wolbachia* from the quill mite *Torotrogla cardueli,* classified previously in supergroup Q (Glowska et al., 2015) (Supplementary Fig. S12). 16S also placed wTp within a general grouping that includes *Wolbachia* associated with pratylenchid nematodes, *T. cardueli* and *Bryobia* (a plant-feeding mite) (Supplementary Fig. S13). We therefore identified genomic sequences of wTp as part of supergroup Q, which was proposed previously based on five-locus sequence data and a different set of mite hosts (Glowska et al., 2015).

Supergroup Q was sister to supergroup M (*Wolbachia* from the banana aphid *Pentalonia nigronervosa*). The lineage representing supergroups Q + M was recovered as sistergroup to supergroup L from the lesion pratylenchid nematode *Pratylenchus penetrans* (plus an environmental soil sample). The entire lineage L(Q + M) was recovered as a sister group to the remaining diversity of *Wolbachia.* In addition, our genomic-scale analysis showed that previously proposed *Wolbachia* supergroup O (Bing et al., 2014; Glowska et al., 2015) is nested within supergroup B (Fig. 4).

4. Discussion

The common domestic mould mite *T. putrescentiae* is a ubiquitous generalist species associated with human-created habitats such as houses, retail stores, storehouses, food-processing facilities,

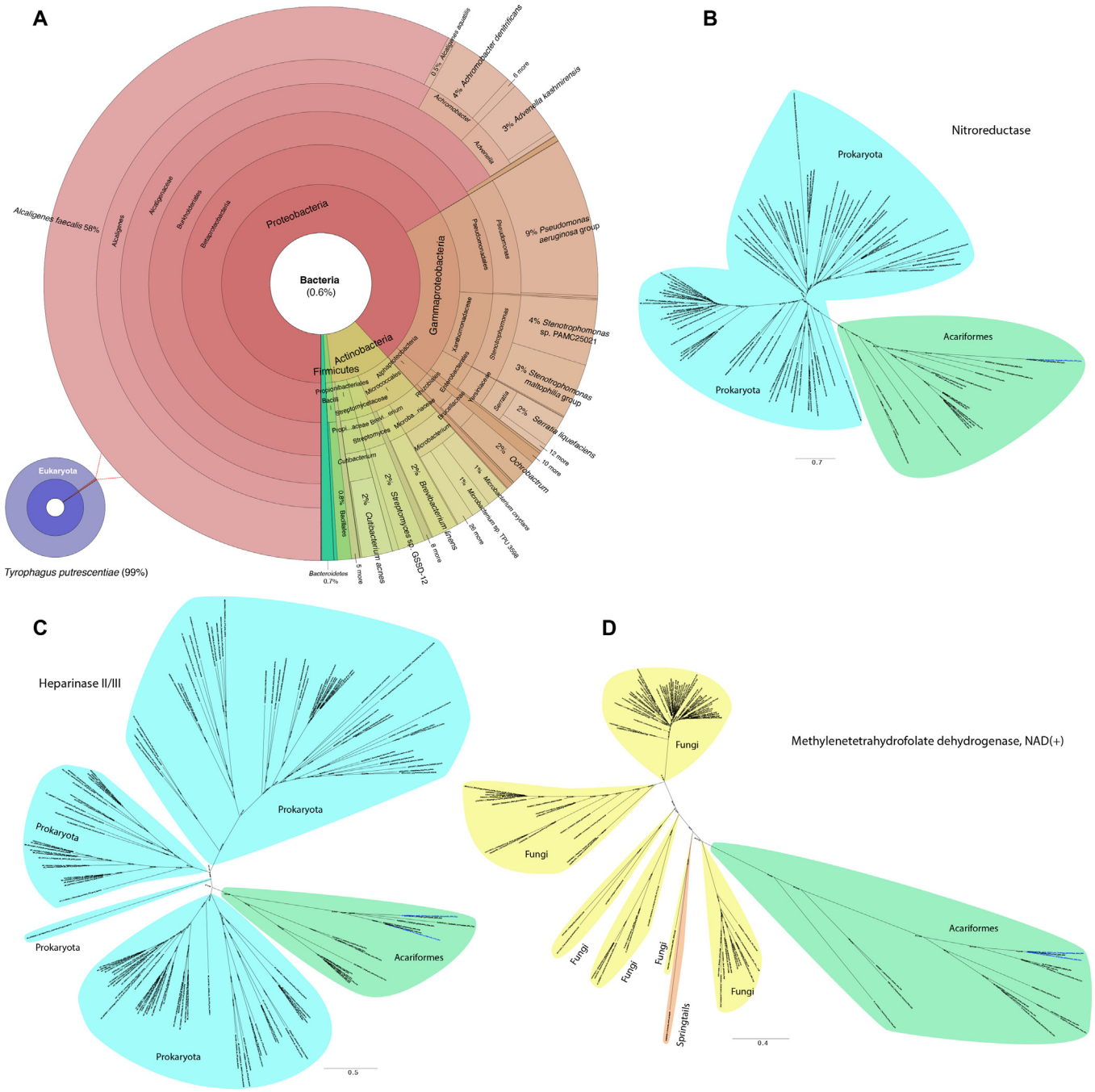

Fig. 2. Bacterial metagenomic profile of *Tyrophagus putrescentiae* from Mexico (A) and putative ancient horizontal transfer events (B-C). (A) Bacterial metagenomic profile of the Mexican sample; bacterial abundance was estimated by Kraken2/Bracken analyses based on Illumina short reads. (B-D) Putative ancient horizontal transfer events from bacteria (B, C) or fungi (D) to mites: (B) Nitroreductase, (C) Heparinase II/III-like protein, (D) NAD-dependent 5,10-methylenetetrahydrofolate dehydrogenase. Horizontal transfer events were inferred from phylogenetic gene trees, showing the target lineage incorporated within an unrelated source lineage; for details, see Table 3 and Supplementary Data S2.

and research laboratories. Here we discuss several questions related to whether it can be a source of significant DNA contamination in public databases, and whether its microbial trophic symbiosis and/or horizontal gene transfers can contribute to its remarkable ability of being a broad dietary generalist. Furthermore, we elucidate the phylogenetic relationship of a novel *Wolbachia* bacterium associated with this mite.

Bacteria and microscopic fungi are expected to introduce sequence contamination into whole eukaryotic genome sequences because they can be symbionts and/or originate from the environment, laboratory equipment, DNA extraction kits or reagents (Goodrich et al., 2014; Salter et al., 2014). Non-fungal eukaryotic DNA usually is not considered a significant source of contamination, except for human DNA (Breitwieser et al., 2019). Here, we show that the mould mite *T. putrescentiae* is an important environmental contaminant that can make its way inside various DNA samples (Table 1), and its contaminating DNA can evade detection by metagenomic methods as commonly used in practice (Goodrich et al., 2014; Breitwieser et al., 2019). The most conspicuous example of contamination was the GenBank reference genome of a fly

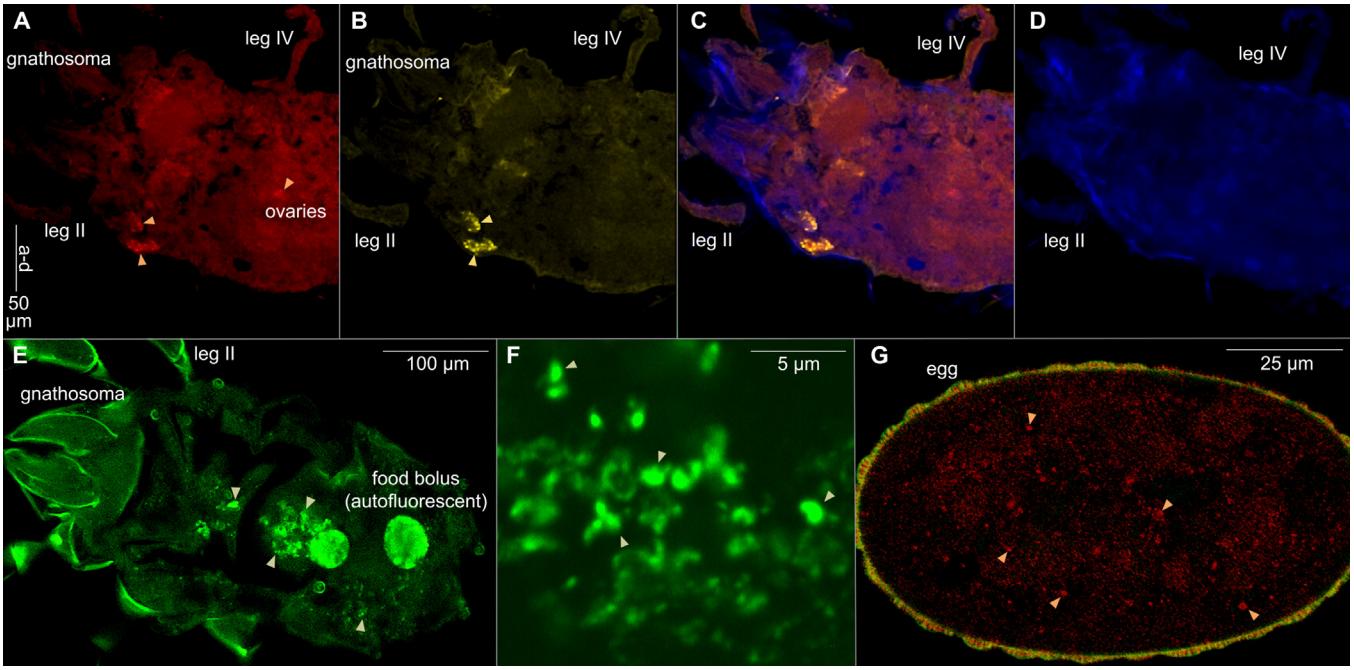

Fig. 3. Bacterial endosymbionts of *Tyrophagus putrescentiae* from UK and the Czech Republic visualized by different Fluorescence In Situ Hybridization (FISH) probes: (A, G) *Wolbachia*-specific (red channel), (B) *Rickettsia*-specific (yellow channel), (C) *Wolbachia* + *Rickettsia*, (E, F) Eubacterial (green channel), and (D) control. *Wolbachia* and *Rickettsia* are localized (A, B, arrowheads), while *Wolbachia* is also found in the area of the ovaries (A, arrowhead), in culture from a female from the UK. (E) Strong eubacterial signal was detected in the digestive tract, and parenchymal tissue bacteriocytes, from a female from the Czech Republic; two food boluses exhibit autofluorescence. (F) Enlarged view of E, showing fluorescent bacterial cells. (G) *Wolbachia* (arrowheads) in the mite's egg, in the UK culture (egg membranes showing autofluorescence and not signal in the red and green channels). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Horizontal gene transfers (HGTs) detected in acariform mites using the *Tyrophagus putrescentiae* genome (GenBank accession number GCA_012066115). HGT analyses were run using two protein subsets extracted from the Uniref50 database (all_no_Metazoa versus Metazoa_no_Arthropoda). For the source, the best nucleotide-to-protein search (tblastx) matches for the National Center for Biotechnology (NCBI) non-redundant (nr) database (no Metazoa) are given; for the target, the best tblastx matches for the NCBI whole genome shotgun (wgs) database (Endeostigmata + Trombidiformes + Oribatida) are given. More detail is given in Supplementary Data S2.

id	Locus	Query (TP) [define]	Source	Total score	Perc. ident	GenBank accession number	Target	Total score	Perc. ident	GenBank accession number
1	Nitroreductase	GMOD_00004316-RA	Bacteria: Verrucomicrobia	180	45.05	MBS0604115.1	Acariformes/ Parasitiformes	589	50.92	JAEMBT020000006.1
2	NADPH dehydrogenase/ NADH:flavin oxidoreductase	GMOD_00003087-RA	Bacteria: Cyanobacteria	373	53.37	WP_250121213.1	Acariformes/ Parasitiformes	1945	65.65	JAEMBT020000008.1
3	Glycoside hydrolase family 28/ polygalacturonase	GMOD_00002110-RA	Bacteria: Bacteroidetes	275	43.96	WP_130856424.1	Acariformes/ Parasitiformes	2232	54.81	JAEMBT020000008.1
4	Glucan *endo*-1,3-beta-glucosidase A1-like/ Glycoside hydrolase family 16	GMOD_00001330-RA	Eukaryota: Amoebozoa	259	51.38	PRP81173.1	Oribatida	2032	57.71	JAEMBT020000001.1
5a	Discoidin domain-containing protein / mycodextranase	GMOD_00003189-RA	Bacteria: Actinobacteria	631	58.61	WP_067367716.1	Oribatida	2877	69.95	CAJPVJ010009338.1
5b	copy 2	GMOD_00003959-RA	Bacteria: Actinobacteria	504	60.71	MBD0736812.1	Oribatida	2445	65.84	JAEMBT020000001.1
7a	Heparinase II/III-like protein	GMOD_00002564-RA	Bacteria: Bacteroidetes	291	42.33	WP_168862112.1	Acariformes	2179	66	JAEMBT020000009.1
7b	copy 2	GMOD_00003781-RA	Bacteria: Bacteroidetes	169	44.20	WP_113615130.1	Acariformes	1405	65.82	JAEMBT020000009.1
8a	NAD-dependent 5,10-methylenetetrahydrafolate dehydrogenase	GMOD_00004475-RA	Eukaryota: Glomeromycetes	277	54.47	CAG8533486.1	Acariformes	942	65.25	CAEY01000550.1
8b	copy 2	GMOD_00003902-RA	Eukaryota: Glomeromycetes	278	54.47	CAG8533486.1	Acariformes	950	65.25	CAEY01000550.1
9	D-Ala-D-Ala dipeptidase	JAAALH010000366.1:11632–12279	Bacteria: Alphaproteobacteria	134	40.30	WP_231555892.1	Acariformes/ Parasitiformes	323	59.09	LBFO01075982.1

TP, *Tyrophagus putrescentiae*; Total score, total bitscore; Perc. ident, percent identity.

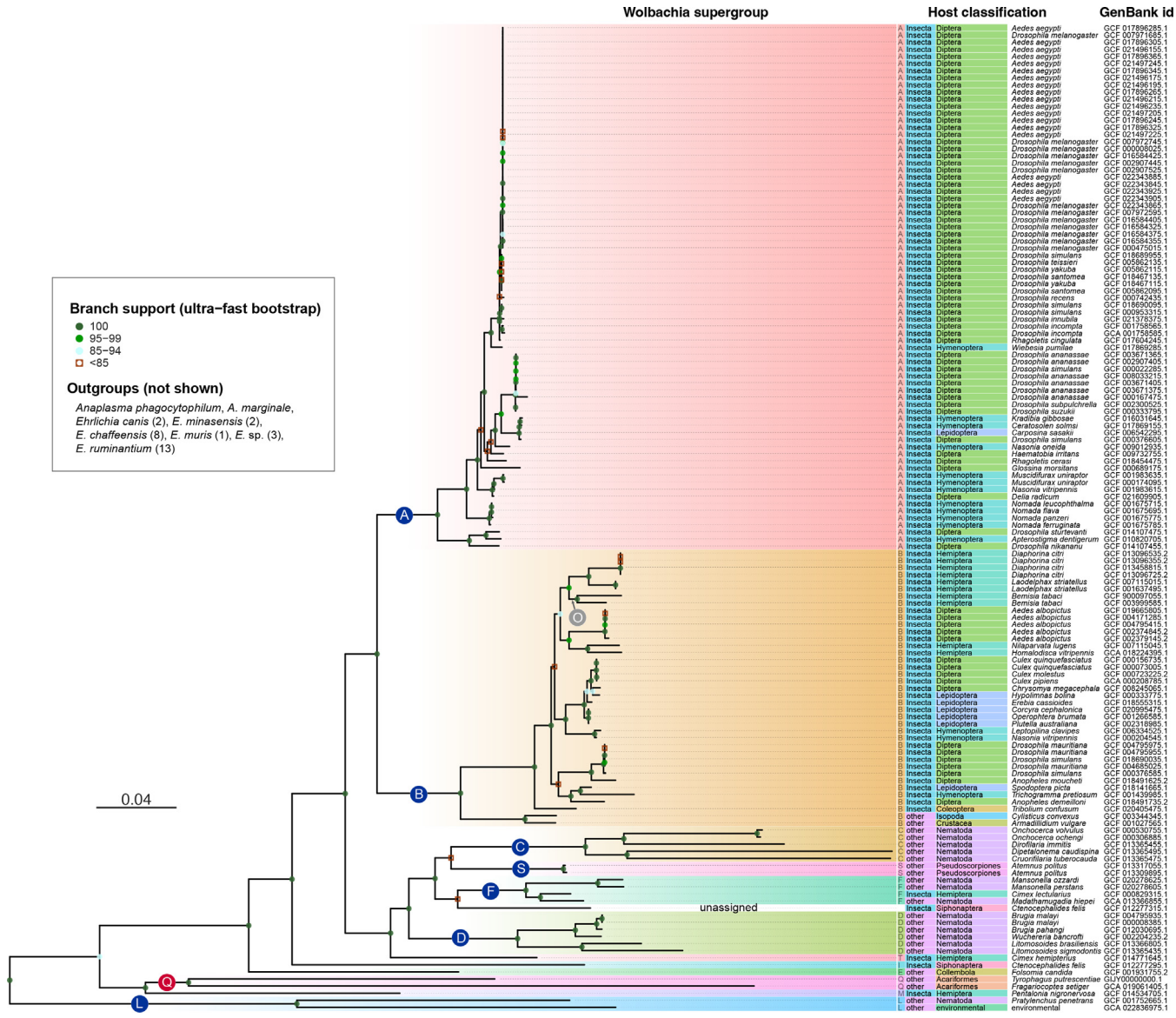

Fig. 4. Maximum likelihood phylogenomic tree of endosymbiotic bacterial genus *Wolbachia*. The mite-specific *Wolbachia* Supergroup Q is emphasized in red.

Rhagoletis zephyria GCF_001687245 (Dowle et al., 2020) containing about 100 MB of *T. putrescentiae* DNA (Table 1). These contaminating sequences, which were previously reported by us (Hubert et al., 2019), are now flagged and were removed by GenBank. For the contaminated sequences deposited into GenBank as insects and fungi, it is very likely that laboratory cultures of these organisms were infested by the mite, and this was unnoticed by the researchers. Suspect sequences of other organisms may result from inadvertent laboratory contamination. For example, the laboratory that deposited sequences of *T. putrescentiae* as sequences of a tree species (*Intsia palembanica*) also published sequences of *T. putrescentiae*, so spill-over contamination from mite cultures maintained in the same laboratory is possible. Contaminated sequences deposited as a mouse, bird, and worm (Table 1) may have resulted from environmental contaminations as the mite is ubiquitous and readily reproduces in many humid environments (Barker, 1967). This contamination is not surprising because fungal/insect/cell culture infestation by the mite was well known from the literature prior to the advent of molecular techniques (Amon et al., 1977). In addition, the case of the phytoseiid *Neoseiulus cucumeris* (Table 1) may be attributable to *T. putrescentiae* being used as the food for the predatory mite, which is a common practice in mass production of phytoseiids for biological control applications (Pekas et al., 2017).

Our metagenomic profiling of two independent mite samples reveals completely different mite microbiomes: in the Mexican sample, the most abundant bacterial species were *Alcaligenes faecalis* (58% of all Bacteria) and *Pseudomonas aeruginosa* (9%) (Table 2, Fig. 2A), while in the European sample, *Escherichia coli* (36%), *Bacillus* spp. (18%), and *Wolbachia* (15%) were dominant (Table 2). The two divergent mite microbiomes suggest the importance of local factors (such as food type, habitat, and available bacterial communities) in forming the microbiomes of this globally distributed mite species. The same effect has been observed previously in mite populations living in close geographic proximity but feeding on different food types – they harbored different microbiomes, except for sharing a single taxon, a *Solitalea*-like bacterium (Erban et al., 2016a; Lee et al., 2019). Because our two microbiomes do not display commonalities in taxonomic composition, it is likely that the mite can opportunistically recruit available local bacterial species

having a chitinolytic activity and/or other useful properties, rather than form permanent specialized associations with a fixed set of bacterial species. Several bacteria identified by us display chitinolytic properties, i.e. *Stenotrophomonas maltophilia* (abundance: 3% of all bacterial species) and *Serratia liquefaciens* (2%) from the Mexican sample; and *Bacillus cereus* (5%) from the European sample (Smrz and Catska, 2010; Brzezinska et al., 2014; Erban et al., 2016b). These bacteria have been isolated from *T. putrescentiae* previously and their chitinolytic properties were demonstrated experimentally (Smrz and Catska, 2010; Erban et al., 2016b). Furthermore, *Alcaligenes faecalis*, the most common bacterium in the Mexican sample, was also found to be an effective chitinase-producing bacterium on marine waste (Annamalai et al., 2011). These bacterial chitinolytic features allow metabolism of chitin from fungal cell walls; when their acarine hosts feed on fungus-rich diets, these bacteria probably cooperate with the mites, forming a nutritional symbiosis (Smrz and Catska, 2010). In this system, the mite, through its normal feeding activities, shreds the fungal mycelium (thus making it available for the microorganisms) and disperses both bacteria and fungi (Van Asselt, 1999; Smrz and Catska, 2010; Smrz et al., 2016), possibly promoting the spread of genetic variants and increasing recombination rates of these microorganisms on a local scale (Brasier, 1978). Several bacteria associated with *T. putrescentiae* are implicated in hospital acquired infections (Peleg and Hooper, 2010; Brooke, 2012), such as *Pseudomonas aeruginosa* and *Escherichia coli* (Table 2). These bacterial taxa are the first and second bacterial species most frequently isolated in hospital settings in the US (Lockhart et al., 2007). All these bacteria can rapidly develop resistance to multiple classes of antibiotics (Lister et al., 2009; Peleg and Hooper, 2010; Lambert et al., 2011; Nathwani et al., 2014), leading to high morbidity and mortality among hospitalized patients, particularly in intensive care units (Giske et al., 2008; Peleg and Hooper, 2010; Lambert et al., 2011; Brooke, 2012). The mite itself has been reported in healthcare settings as a single 1.27 Kb sequence originally identified as the bacterium "*Shinella* sp." (Brooks et al., 2017), but having a 100% match with *T. putrescentiae* (Table 1). As there is only one record in the literature, further studies on the role of this mite in dispersing antibiotic-resistant bacteria are needed.

A very divergent *Wolbachia* species (wTp) was found in the European sample and in the contaminated '*Rhagoletis zephyria*' genome that originated from the US. On our tree, wTp and the *Wolbachia* from the gall mite *F. setiger* formed a basal monophyletic lineage, supergroup Q (Fig. 4), suggesting that this lineage may be specific to acariform mites. It is possible that wTp may cause cytoplasmic incompatibility in its host, explaining the results of early breeding experiments that demonstrated large-scale reproductive incompatibility between morphologically similar populations of *T. putrescentiae* (Griffiths, 1979). However, an alternative explanation of these experiments could be the presence of two sibling mite species, *T. putrescentiae* and *Tyrophagus fanetzhangorum*, which are separated by large genetic distances and probably cannot interbreed (Fan and Zhang, 2007; Klimov and OConnor, 2009a; Murillo et al., 2018). *Wolbachia* can manipulate its host microbiome via pathogen blocking, which limits the ability of many pathogenic viruses, bacteria, and nematodes to grow in the host (Hedges et al., 2008; Kambris et al., 2009). For example, this system has recently gained medical relevance due to its ability to affect the transmission of human pathogens by blocking dengue virus within mosquitoes (Walker et al., 2011). Several hypotheses have been proposed to explain the mechanism of pathogen blocking. Among them, the lipid perturbations hypothesis, suggesting that *Wolbachia* may outcompete pathogens for lipids, a critical nutritional resource, seems to be better supported by experimental evidence (Geoghegan et al., 2017). A strong pathogen blocking effect has been observed when naturally uninfected mosquitoes were transinfected with *Drosophila*-specific strains of *Wolbachia* (McMeniman et al., 2009; Walker et al., 2011; Diaz-Nieto et al., 2021). wTp has also been observed to affect the associated microbiomes in its own mite host, *T. putrescentiae* (Hubert et al., 2019). Here, by analogy with the *Drosophila*-specific *Wolbachia* exhibiting a strong pathogen blocking effect in an unnatural host (mosquitoes), coupled with the ability of wTp to modulate the host's microbiomes, we suggest that further research needs to be done to elucidate whether wTp can be used control pathogens vectored by ticks and other parasitic Acari.

Despite the presence of diverse bacterial communities in the mould mite, we did not detect any recent horizontal gene transfer events in the *T. putrescentiae* genome. All potential HGT events occurred prior to the origin of Astigmata (Fig. 1C), in the common ancestor of either acariform or oribatid mites (Table 3). This includes D-alanyl-D-alanine dipeptidase HGT, which was previously suggested to occur within Astigmata (Xiong et al., 2022), however, we found this gene in Endeostigmata and Oribatida (Table 3, id = 9), indicating an earlier origin. Given that all detected putative HGTs were ancient, we suggest that they do not have immediate relevance to the mite's ability to be a widespread nutritional generalist.

In conclusion, we show that the mould mite *T. putrescentiae* is a significant contaminant in laboratory and industrial settings. Microbiome profiles of the samples from Europe and Mexico were completely different, suggesting that mite-bacterial symbiotic associations are formed via opportunistic recruitment of locally available bacterial species by the acarine host. We also found that the mould mite is a potential carrier of several antibiotic-resistant bacteria. Finally, based on a whole transcriptome sequence of a novel, mite-specific *Wolbachia* from *T. putrescentiae*, we identified it as part of a basal, mite-specific *Wolbachia* lineage (supergroup Q). These findings provide a unique insight into the early evolution and host associations of this bacterial genus. Based on the previously known blocking/microbiome modifying effect of *Wolbachia* from *T. putrescentiae*, we believe that this bacterium may be potentially useful for disease/pest control applications. We expect further discoveries of *Wolbachia* diversity associated with acariform mites.

CRediT authorship contribution statement

Pavel B. Klimov: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jan Hubert:** Resources, Writing – review & editing. **Tomas Erban:** Resources, Writing – review & editing. **M. Alejandra Perotti:** Formal analysis, Investigation, Resources, Visualization, Writing – review & editing. **Henk R. Braig:** Funding acquisition, Methodology, Resources, Writing – review & editing. **Alex Flynt:** Resources, Writing – review & editing. **Qixin He:** Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Yubao Cui:** Funding acquisition, Resources, Supervision, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2024.07.001.

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