Reclassification of *Wolbachia persica* as *Francisella persica* comb. nov. and emended description of the family *Francisellaceae*

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The taxonomic status of the bacterium *Wolbachia persica* is described, and based on the evidence presented, transfer of this species to the genus *Francisella* as *Francisella persica* comb. nov. is proposed. This reclassification is supported by data generated from genomic comparisons of *W. persica* ATCC VR-331T (≡FSC845T=DSM 101678T) to other near neighbours, including *Francisella tularensis* subsp. novicida. The full-length 16S rRNA gene sequence of strain ATCC VR-331T had 98.5% nucleotide identity to the cognate gene in *F. tularensis*, with the highest similarity to subspecies novicida. Phylogenetic trees of full-length 16S rRNA gene, gyrA and recA sequences from species of the genera *Wolbachia* (class Alphaproteobacteria) and *Francisella* (class Gammaproteobacteria) indicated that *W. persica* ATCC VR-331T was most closely related to members of the genus *Francisella* and not *Wolbachia*. Local collinear blocks within the chromosome of strain ATCC VR-331T had considerable similarity with *F. tularensis* subsp. novicida, but not with any *Wolbachia* strain. The genomes of strain ATCC VR-331T and *F. tularensis* subsp. novicida Utah 112T (≡ATCC 15482T) contained an average nucleotide identity mean of 88.72% and median of 89.18%. Importantly, the genome of strain ATCC VR-331T contained one *Francisella* Pathogenicity Island, similar to *F. tularensis* subsp. novicida, as well as the *Francisella*-specific gene *fopA1* and *F. tularensis*-specific genes *fopA2* and *lpnA* (also referred to as *tul4*). In contrast to the obligate intracellular genus *Wolbachia*, strain ATCC VR-331T and facultative intracellular *Francisella* can replicate in specialized cell-free media. Collectively, these results demonstrate that *Wolbachia persica* should be reclassified in the genus *Francisella* as *Francisella persica* comb. nov. The type strain of *Francisella persica* comb. nov. is ATCC VR-331T (≡FSC845T=DSM 101678T). An emended description of the family *Francisellaceae* is also provided.

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Abbreviations: ANI, average nucleotide identity; FPI, *Francisella* Pathogenicity Island; IS elements, insertion sequence elements; LCB, local collinear block.

The GenBank/EMBL/DDBJ accession number for the whole genome sequence of strain ATCC VR-331T is CP012505. A supplementary figure and two supplementary tables are available with the online Supplementary Material.
The genus Wolbachia was named after Simeon Burt Wolbach in collaboration with Marshall Hertig, during their study of Rickettsia-like microbes in insects. Species of the genus Wolbachia are closely related to the genera Rickettsia, Ehrlichia and Anaplasma, which are found in insects or are transmitted by arthropods such as ticks (O’Neill et al., 1992; Roussel et al., 1992). Wolbachia persica ATCC VR-331T was the first tick endosymbiont isolated, and was extracted from the malpighian tubules of the soft tick Argus (Persicargas) arboreus (previously referred to as Argas persicus) that was feeding on a heron in a rookery near Cairo, Egypt (Suitor & Weiss, 1961). The classification of strain ATCC VR-331T was originally based on (i) morphological similarity via light microscopy to the arthropod endosymbiont Wolbachia pipientis and other transovarially transmitted bacteria; (ii) the inability to grow on standard cell-free media; (iii) the predilection for reproductive tissues; and (iv) the lack of reaction in serological tests with known tick-borne pathogens (Suitor, 1964; Suitor & Weiss, 1961; Weiss et al., 1984). Later studies have shown that the partially sequenced 16S rRNA gene from strain ATCC VR-331T shared considerable similarity to the related gene in the genus Francisella (Dumler et al., 2001; Forsman et al., 1994; Niebylski et al., 1997; Noda et al., 1997; O’Neill et al., 1992; Weisburg et al., 1989). These phylogenetic analyses showed that strain ATCC VR-331T was closely related to the genus Francisella within the class Gammaproteobacteria (family Franciselaceae) and not the genus Wolbachia, a member of the class Alphaproteobacteria (family Anaplasmataceae). However, no reclassification has been published.

In the current study, the genome of strain ATCC VR-331T was sequenced, assembled to completion, and then analysed for content and relatedness to other bacteria. The data we obtained and present herein demonstrate that strain ATCC VR-331T belongs to the genus Francisella and not the genus Wolbachia. Therefore, we propose that type strain ATCC VR-331T (=FSC845=DSM 101678T) is reclassified as representing a novel species of the genus Francisella, named Francisella persica comb. nov., replacing the former species name Wolbachia persica.

Strain ATCC VR-331T was obtained from the American Type Culture Collection (ATCC) and grown axenically in supplemented tick cell culture medium referred to as modified Leibovitz’s L-15 medium (L-15B), as was previously described (Munderloh & Kurtti, 1989). Genomic DNA from strain ATCC VR-331T was extracted using standard procedures and sequenced by a combination of Illumina HiSeq deep sequencing and 454 pyrosequencing. The resulting Illumina 100 bp paired-end data were assembled using the Velvet program version 1.2.04 (Zerbino & Birney, 2008), and the 8 kb and 20 kb reads obtained from 454 pyrosequencing were assembled using Newbler (version 2.8). Whole-genome mapping of NcoI- and NheI-digested genomic DNA from strain ATCC VR-331T was performed as recommended by the manufacturer (OpGen) to produce a contiguous restriction map of the actual chromosome for comparison to the in silico sequence assembly. The assembled DNA sequence data for strain ATCC VR-331T was imported into MapSolver software (OpGen) and converted into in silico maps using the same restriction enzyme that was used to generate the whole-genome map. In-house Python scripts were then utilized to compare the data obtained from whole-genome mapping with the assembled sequences. To validate the accuracy of the completed genome of strain ATCC VR-331T, selected regions were PCR-amplified using a high fidelity Taq DNA polymerase and the resulting amplicons were sequenced using the Sanger method. The fully sequenced genome for strain ATCC VR-331T was deposited in the GenBank database. For functional annotation of the chromosome of strain ATCC VR-331T, the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html) was utilized; however, pseudogenes and disrupted open reading frames were manually curated.

Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990) assessment of the full-length 16S rRNA gene from strain ATCC VR-331T Utah 112T (=ATCC 15482T) indicated that the related gene in F. tularensis subsp. novicida shared the highest similarity (1507/1530, 98.5%). To further evaluate the genetic relationship of strain ATCC VR-331T with the genera Francisella and Wolbachia, phylogenetic trees were reconstructed using full-length gene sequences encoding 16S rRNA gyrA and recA, which were retrieved from the GenBank database. The corresponding gene in Coxiella burnetii RSA 493 was used as the outlier for each tree. CLUSTAL Omega software (McWilliam et al., 2013) was used for the multiple sequence alignments, and tree topology was assessed using unrooted neighbour-joining clustering and unweighted pair group method of arithmetic means algorithms with 500 bootstraps. MEGA software version 6.06 (Tamura et al., 2013) was used to visualize the phylogenetic trees. Both algorithms produced similar overall topologies for all three of these conserved bacterial genes and consistently depicted strain ATCC VR-331T clustering with members of the genus Francisella and not the genus Wolbachia. Fig. 1 shows the 16S rRNA gene phylogenetic tree and Fig. S1a, b (available in the online Supplementary Material) shows the gyrA and recA phylogenetic trees, respectively, using neighbour-joining with the bootstrap values denoted at each node. Further, these analyses consistently placed strain ATCC VR-331T nearest to the F. tularensis clade with F. tularensis subsp. novicida as the closest relative.

To compare the overall genomic structure and content of strain ATCC VR-331T with the near neighbour F. tularensis subsp. novicida, local collinear blocks (LCBs) were obtained using the progressiveMAUVE tool (Darling et al., 2010). These comparisons revealed considerable conservation of the LCBs, with numerous rearrangements and nucleotide polymorphisms typically found in Francisella interspecies comparisons (Fig. 2a). In contrast, LCB
comparisons between strain ATCC VR-331\textsuperscript{T} and the sequenced Wolbachia genomes did not show any observed similarity. Fig. 2b shows a comparison of the LCBs in strain ATCC VR-331\textsuperscript{T} with a representative strain of the type species of the genus Wolbachia, specifically Wolbachia pipientis wRi, an endosymbiont of Drosophila simulans with a 1.5 Mbp genome.

Average nucleotide identity (ANI) was recently shown to be one of the most robust measurements available to determine genomic relatedness for prokaryotes (Kim et al., 2014). Therefore, pairwise genome comparisons of strain ATCC VR-331\textsuperscript{T} with W. pipientis wRi and F. tularensis subsp. novicida Utah 112\textsuperscript{T} (=ATCC 15482\textsuperscript{T}) were performed. The ANI of strain ATCC VR-331\textsuperscript{T} with W. pipientis wRi
did not provide meaningful values since there were an insufficient number of nucleotide matches. In contrast, the ANI with *F. tularensis* subsp. *novicida* Utah 112T was determined to have a mean of 88.72 % and median of 89.18 %. The high ANI values obtained for the genomic comparisons between strain ATCC VR-331T and *F. tularensis* subsp. *novicida* Utah 112T, along with the high nucleotide identity for the 16S rRNA gene, demonstrated relatedness and demarked an interspecies relationship within the genus *Francisella*.

Prior to the genomic sequencing of strain ATCC VR-331T, *Francisella guangzhouensis* contained the smallest genome in the genus *Francisella*. The current study revealed that the chromosome of strain ATCC VR-331T was approximately 1.5 Mbp in size, which is 0.1 Mbp shorter in length than that of *F. guangzhouensis* and 0.4 Mbp shorter in length than the 1.9 Mbp genomes of *F. tularensis*. Next, the overall genomic features for strain ATCC VR-331T and *F. tularensis* subsp. *novicida* Utah 112T were compared, revealing a similar G+C content of 31.39 mol % and 32.48 mol %, respectively (Table S1). The smaller genome of strain ATCC VR-331T contained substantially fewer genes, but more pseudogenes and disrupted open reading frames than the chromosome of *F. tularensis* subsp. *novicida* Utah 112T (Table S1). Only one intact transposase gene was found in the genome of strain ATCC VR-331T, which encoded the insertion sequence (IS) element ISFtu3, whereas the chromosome of *F. tularensis* subsp. *novicida* Utah 112T contained 22 full-length transposase genes (Table S1). Two and four IS element remnants were observed in the genomes of strain ATCC VR-331T and *F. tularensis* subsp. *novicida* Utah 112T, respectively.

Interestingly, the chromosome of strain ATCC VR-331T contained a single *Francisella* Pathogenicity Island (FPI), similar to the genome of *F. tularensis* subsp. *novicida* Utah 112T (Tables S1 and S2). Of the 17 virulence genes in the FPI, 16 were present in the chromosome of strain ATCC VR-331T with only the absence of the gene encoding the pathogenicity determinant protein PdpD (Table S2). Nucleotide identity between the FPI genes in strain ATCC VR-331T and *F. tularensis* subsp. *novicida* Utah 112T ranged from 65.0 % to 83.2 %. Also noteworthy, the *Francisella*-specific gene fopA1 and *F. tularensis*-specific gene lpnA (also referred to as tul4) were identified in the genome of strain ATCC VR-331T. Further analysis of these membrane proteins revealed that fopA1 and lpnA sequences in strain ATCC VR-331T shared approximately 91 % (868/958) and 85 % (382/450) nucleotide identity, respectively, to the associated gene in *F. tularensis* subsp. *novicida*.

![Fig. 2. Local collinear block (LCB) comparisons between the aligned chromosomes of *Francisella persica* ATCC VR-331T and (a) *Francisella tularensis* subsp. *novicida* Utah 112T or (b) *Wolbachia pipientis* wRi, a strain of the type species of the genus *Wolbachia*. Bacterial strains are identified above the respective chromosomal size marker and the length is in base pairs. Associated LCBs are indicated with the same colour and unfilled regions denote nucleotide polymorphisms.](http://ijs.microbiologyresearch.org)
Utah 112T. The truncated fopA2 gene that is uniquely present in the chromosome of all four subspecies of *F. tularensis* (*tularensis, holarctica, mediasiatica* and *novicida*) was also present in the genome of strain ATCC VR-331T.

Previous phenotypic and electron microscopy evaluations showed that strain ATCC VR-331T was a Gram-negative, non-motile cocccobacillus bacterium with a diameter of 0.3–0.5 × 0.7–1.0 μm (Sui & Weiss, 1961). Similarly, the Gram-negative, non-motile cocccobacillus bacterium *Francisella* is 0.2–0.5 × 0.7–1.0 μm (Iwen, 2013). Unlike species of the genus *Wolbachia*, which are obligate intracellular bacteria, strain ATCC VR-331T and *F. tularensis* are considered fastidious organisms that can replicate in cell-free specialized media (Noda et al., 1997; Ragon et al., 2006; SJöstedt, 2007).

Bacterial symbionts that commonly infect ticks were determined to replicate intracellularly within putative endosomes or phagosomes (Burgdorfer et al., 1973; Cowdry, 1925; Hayes & Burgdorfer, 1981; Sui & Weiss, 1964; Yano et al., 1993). In congruence with these characteristics, both strain ATCC VR-331T and species of the genus *Francisella* are capable of infecting ticks and replicating intracellularly (Petersen et al., 2009; SJöstedt, 2007; Sui & Weiss, 1961). Previous animal studies showed that strain ATCC VR-331T was slightly to moderately pathogenic for guinea pigs, mice and newborn chickens, but not for rats, rabbits or adult chickens (Sui & Weiss, 1961). Similarly, *F. tularensis* subsp. *novicida* can cause disease, albeit rare, and differs in this respect to the other more virulent subspecies that often cause tularemia in numerous hosts (SJöstedt, 2007). The interaction between bacteria and host is complex and can range from symbiotic mutualism to pathogenic. Perhaps strain ATCC VR-331T is the bridge organism between *Francisella*-like endosymbionts found in ticks and the facultative intracellular pathogen *F. tularensis*, particularly the opportunistic subspecies *F. tularensis* subsp. *novicida*. Nevertheless, the findings presented in this current study support the proposal that strain ATCC VR-331T should be reclassified as representing a novel species of the genus *Francisella* and does not warrant designation as a species of the genus *Wolbachia*. The name proposed for the novel species is *Francisella persica* comb. nov. The type strain is ATCC VR-331T (≡FSC845T = DSM 101678T) and was originally isolated from the malpighian tubules of the soft tick *Argas (persicargas) arboreus* that was removed from a buff-backed heron (*Bubulcus ibis*) in Egypt, a former part of the United Arab Republic. The complete genome sequence for the type strain is available in the GenBank database under accession number CP012505.

**Emended description of family Francisellaceae**

**SJöstedt 2005**

The species *Francisella persica* comb. nov. is placed inside the family *Francisellaceae*. This family forms a highly monophyletic group, according to the 16S rRNA gene sequence phylogeny. *Francisella persica* comb. nov. can be axenically grown in modified Leibovitz's L-15 medium (L-15B), which is supplemented with trace minerals, vitamins, amino acids, α-ketoglutaric acid and glucose.

**Etymology:** L. fem. adj. *persica*, Persian; here, named after *Argas persicus*, the reputed host tick.

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**References**


**Description of Francisella persica** comb. nov.

**Basonym:** Wolbachia persica Sui & Weiss 1961.

The description for the species, including the morphological and biochemical properties, remains as was described by Sui & Weiss (1961).