SUPPLEMENTAL MATERIALS from Matz *et al.*, Challenges of *Francisella* classification exemplified by an atypical clinical isolate.

FIGURE S1

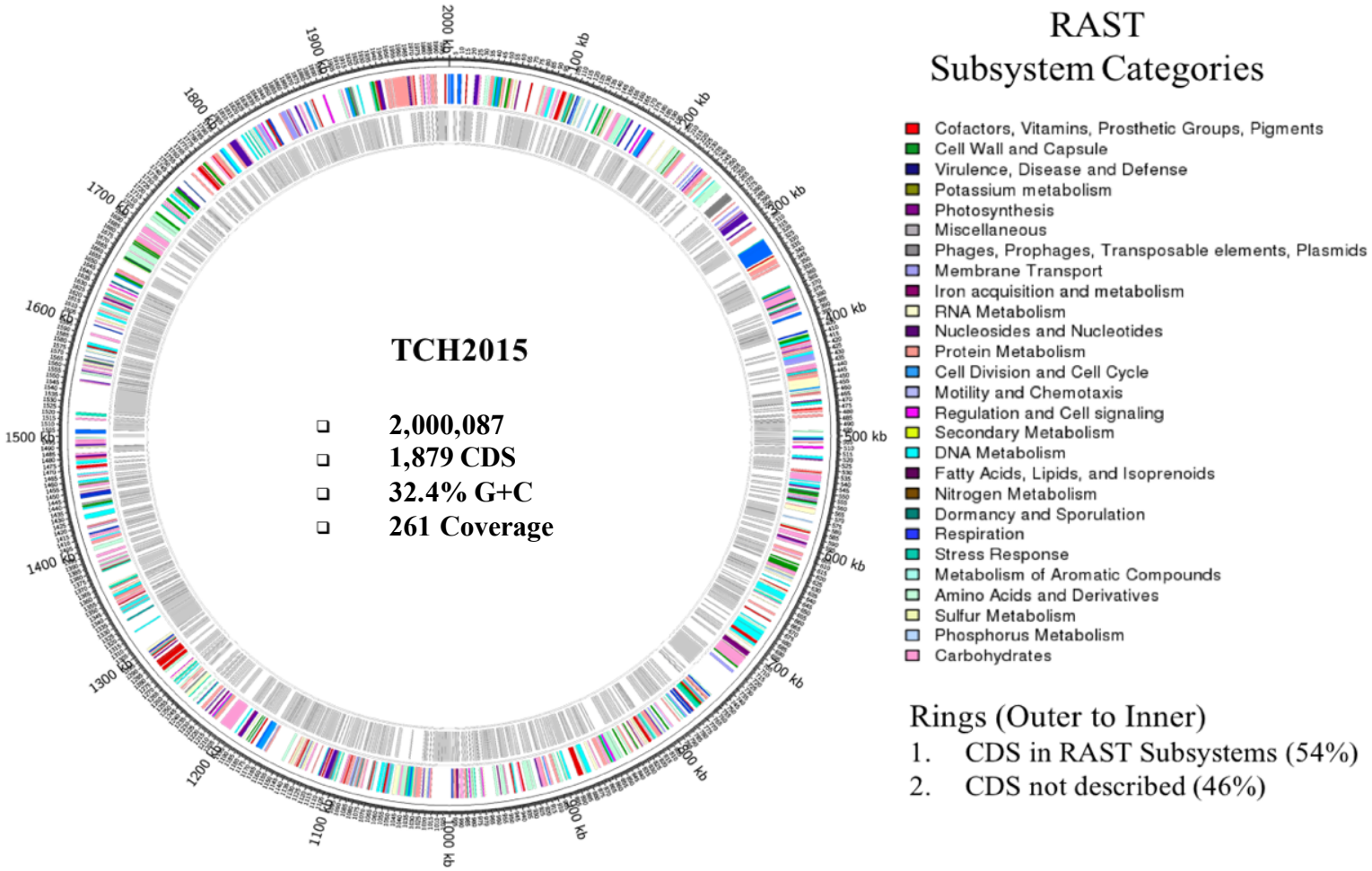
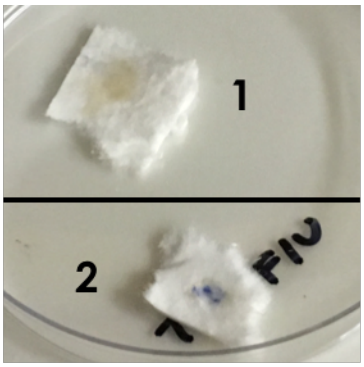


Figure S1. Genome topology of strain TCH2015. A Circos plot shows the distribution of genes across the 2 Mbp genome. CDS colors correspond to RAST subsystems. Gray rings in the inner circle represent undefined CDS. The post-filter N_{50} read length was 33,049 base pairs with a quality value of 0.85.

FIGURE S2

Figure S2. TCH2015 shares similar genomic content with *F. novicida* U112. Initial assessment of the TCH2015 assembly using BLAST® found *F. novicida* type strain U112 (NZ_CP009633) as one of the best hits. Start positions for both strains were set at the same position to analyze overall genome consistency. Nucleotide alignments were generated using Mauve 2.3.1 and visualized as a Circos plot. Five rings are shown. The outer and inner-most rings represent the genomic alignments of TCH2015 and U112, respectively. Matching colored regions representing areas of significant homology between both genomes. The second-most outer and inner rings are bound to their respective genomic rings and reflect extra content which is not present in the other genome. The center ring (black bars) shows ORF locations within the extra genomic content observed in TCH2015 (See Table 1).

FIGURE S3



OxiDrop™

1: *F. novicida* U112 (negative control)
2: *F. novicida* TCH2015

Figure S3. Oxidase test results for *F. novicida* strains U112 (top) and TCH2015 (bottom). OxiDrop™ reagent (Hardy Diagnostics) was applied to filter paper, and a bacterial colony was rubbed over the reagent. The dark blue dot indicates a positive result.

FIGURE S4

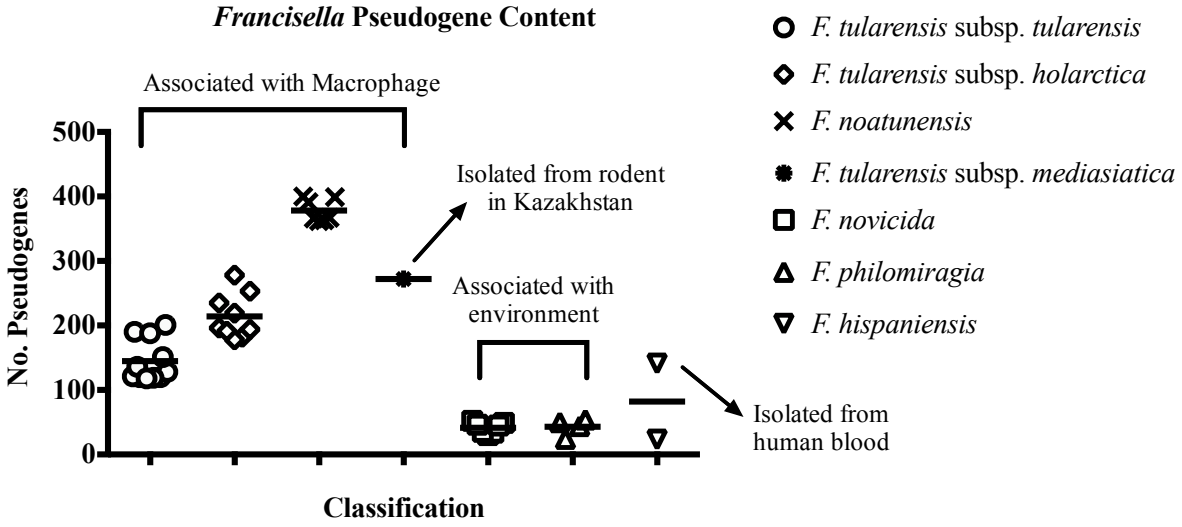


Figure S4. Estimated pseudogene content in *Francisella* genomes. While there is no distinguishing number of pseudogenes that are required to constitute virulence, avirulent strains possess fewer pseudogenes on average compared to virulent ones due to niche restriction (Rohmer *et al.*, 2007; Titball and Petrosino, 2007). NCBI annotations were queried for pseudogene numbers from several *Francisella* species or subspecies. Arrows indicate special cases (Larsson *et al.*, 2009; Huber *et al.*, 2010; Escudero *et al.*, 2010).

CASE REPORT

A 6-year-old male who resides in Guatemala presented to Texas Children's Cancer Center with a 5-week history of left cervical lymphadenopathy and was noted by his family to have painless left neck swelling while at home. The patient was active in water sports at the beach as well as canals around his home and was reported to drink bottled water but bathe in unfiltered tap water. No recent infections or unusual animal exposures were reported. Past medical history was significant for neutropenia during infancy, which was diagnosed as benign autoimmune neutropenia and resolved without intervention. The patient did not have a history of frequent, severe, or unusual infections, and growth was appropriate for the patient's age. The family denied fever, respiratory symptoms, night sweats, weight loss, rash, or other areas

of swelling. The primary care physician in Guatemala measured the node to be 2.5 by 2.0 cm. Complete blood count and erythrocyte sedimentation rate were within normal ranges, and Epstein-Barr virus serology and tuberculosis screening were negative. The patient completed a 10-day course of Amoxicillin with no change in lymphadenopathy and was referred to Texas Children's Hospital for further evaluation.

Upon referral, the patient was afebrile with left cervical lymphadenopathy that was firm and non-tender. Other lymphadenopathy, hepatosplenomegaly, petechiae or purpura, and testicular mass were not present on examination. A computed tomography scan of the neck showed a mildly enhancing, enlarged lymph node along the posterior margin of the left sternocleidomastoid muscle, measuring 1.9 by 1.3 cm, and two adjacent, non-enlarged, mildly enhancing nodes. Scattered, non-enlarged mildly enhancing nodes were present along the left jugulodigastric chain extending to the left supraclavicular region and were more numerous than on the right side of the neck. An excisional biopsy was performed and showed necrotizing lymphadenitis with multiple ill-defined aggregates of neutrophils and surrounding palisading histiocytes. Bacterial infiltrate on histopathology was not observed.

Immediately post-biopsy, the patient had markedly reduced but still palpable left cervical lymphadenopathy. However, the lymphadenopathy fully resolved without additional intervention by two months. Due to resolution of lymphadenopathy after lymph node excision, additional antibiotics were not given and the patient continues to do well. Due to the finding of *F. novicida* and prior history including neutropenia in infancy, concern for an innate immune defect led to a comprehensive immunologic evaluation. The patient was found to have decreased natural killer cell function (NK Lytic Units at 0.2; reference range > 2.6), and trio whole exome sequencing revealed compound heterozygous variants of uncertain clinical significance in the *RTEL1* gene. Telomere lengths were not diagnostic of a telomere disorder but further workup is in progress.

SUPPLEMENTAL REFERENCES

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