IJSEM Letters to the Editor

# Objections to the transfer of *Francisella novicida* to the subspecies rank of *Francisella tularensis*

We disagree with a recent proposal by Huber *et al.* to transfer *Francisella novicida* to the subspecies rank of *Francisella tularensis* (Huber *et al.*, 2010). We believe that the proposal is not appropriate in light of all currently available knowledge.

In 1989, Hollis *et al.* (1989) argued that *F. novicida* and *F. tularensis* could be considered to be one species as judged from DNA–DNA hybridization experiments (Hollis *et al.*, 1989). Their publication was not valid according to the requirements outlined in the Bacteriological Code (Lapage *et al.*, 1992; Tindall *et al.*, 2006). As a result, the proposed elimination of the species *F. novicida* and its demotion to a biogroup of *F. tularensis* was not included among prokaryotic names with standing in nomenclature. Notably, earlier publications considered *F. novicida* and *F. tularensis* to be separate species based on differences in phenotype including chemotaxonomic markers, distinct ecological roles, different clinical and epidemiological characteristics, and differing abilities and modes of invasion and mechanisms of tissue damage in mammals (Larson *et al.*, 1955; Olsufiev *et al.*, 1959; Skerman *et al.*, 1980).

From a practical standpoint, separate species names are useful in a microbiological laboratory or a clinical setting and also as a basis for regulations governing the handling of medically important organisms. For example, laboratory handling of *F. tularensis*, but not *F. novicida*, is associated with a high risk of airborne laboratory-acquired infection. Importantly, it is fairly easy to distinguish *F. novicida* and *F. tularensis* on the basis of their different growth and metabolic requirements on artificial media. Indeed, in Table 2 of Huber *et al.* (2010) data are provided that contradict their own proposal by presenting 11 metabolic reactions that are distinct between *F. novicida* and *F. tularensis* (Huber *et al.*, 2010).

Perhaps most importantly, recent findings from the analysis of multiple genome sequences of *F. tularensis* versus *F. novicida* have indicated that the increased host-association of *F. tularensis* is tied to evolution as a population lineage disconnected from *F. novicida*, even though genome-wide average nucleotide identities exceeded 97 % (Larsson *et al.*, 2009). We propose that different population structures and otherwise disparate evolutionary patterns in *F. tularensis* and *F. novicida* should be considered as arguments for retaining separate species names. A comparison of 17 genomes of members of the genus *Francisella* has shown that the emergence of *F. tularensis*, in an evolutionary and population genetic framework, was a speciation event with no signs of reversals. For example, there were no traces of genetic exchange between *F. tularensis* and *F. novicida*. The analysis provided genetic information that was more precise than crude DNA–DNA hybridization values for defining the genetic relationships between *F. tularensis* and *F. novicida*. Recent intense efforts, including evolutionary and population criteria, have provided a useful theoretical framework for defining prokaryotic species (Achtman & Wagner, 2008; Gevers *et al.*, 2005; Koeppel *et al.*, 2008). We believe that such a framework should be taken into consideration in the taxonomy of the genus *Francisella*.

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# Objections to the transfer of *Francisella novicida* to the subspecies rank of *Francisella tularensis* – response to Johansson *et al.*

The description of novel species requires the careful selection and use of a wide variety of methodologies. As pointed out by Tindall *et al.* (2010), experience gained over the past six decades has continued to demonstrate the value of comparing different datasets and also of basing the description and delineation of taxa on as wide a dataset as possible. A combination of data acquired from DNA-based methods (DNA–DNA hybridization, gene sequences, genomic fingerprints) and phenotyping (chemotaxonomic, physiological and morphological traits) provides a sound basis for the taxonomy of the prokaryotes (Tindall *et al.*, 2010). The decision as to whether two bacteria are members of a single species is still based on the results from DNA–DNA hybridizations (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002). In general, two bacterial strains are assigned to the same species if their DNAs reassociate at levels greater than 70 % and 5 % or less $\Delta T_m$ (Wayne *et al.*, 1987), but the latter criterion is only rarely applied. In addition, Wayne *et al.* (1987) pointed out '*Subspecies designations can be used for genetically close organisms that diverge in phenotype*'.

Our proposal to transfer *Francisella novicida* as a novel subspecies to *F. tularensis* subsp. *novicida* is in agreement with the above-mentioned recommendations. As demonstrated by the results from DNA–DNA reassociation experiments, *F. novicida* is genetically close to *F. tularensis* (Hollis *et al.*, 1989) and the phenotypic differences observed (Huber *et al.*, 2010) are in agreement with the subspecies concept. Another important point supporting this taxonomic rearrangement is the acceptance of the new combination within the scientific community. The use of this not yet validly published new combination may be related to the fact that in *Bergey's Manual of Systematic Bacteriology* (often erroneously considered as the 'bible' of bacterial systematics by those interested in bacterial taxonomy), the transfer of *F. novicida* to *Francisella tularensis* subsp. *novicida* was recommended in the chapter dealing with the genus *Francisella* (Sjöstedt, 2005). Although this proposal was never formally recognized, numerous microbiologists are already using the name. An online search survey in 'Pubmed' (http://www.ncbi.nlm.nih.gov/sites/entrez?db=PubMed) indicates that in recent years there is no significant difference in the frequencies of the use of the names *F. novicida* and *F. tularensis* subsp. *novicida*.

From our point of view, it is not consistent to have a species *F. tularensis* with three subspecies supported by DNA–DNA relatedness data but distinguishable by phenotypic traits and a separate species *F. novicida* that also shares high DNA–DNA relatedness values (>85 %) but which is phenotypically distinguishable. Based on the results from the literature and the results from our investigations, but also for sake of consistency, it is obvious that our proposal to assign *F. novicida* to *F. tularensis* as a novel subspecies is well supported.

Below are some additional replies to certain arguments proposed by Johansson *et al.* (2010) to support their stance against the reclassification of *F. novicida*.

It is argued, that:

> 'From a practical standpoint, separate species names are useful in a microbiological laboratory or a clinical setting and also as a basis for regulations governing the handling of medically important organisms. [...] Importantly, it is fairly easy to distinguish *F. novicida* and *F. tularensis* on the basis of their different growth and metabolic requirements on artificial media'.

In contrast to tularaemia caused by *F. tularensis* subsp. *tularensis* or *F. tularensis* subsp. *holarctica*, human or animal infections with strains of *F. tularensis* subsp. *novicida* are extremely rare and there are very few publications reporting the isolation of this facultative pathogen. Most of these reports have shown that it was very difficult to distinguish those isolates from strains of *F. tularensis*, not only for routine clinical laborat-