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; Koeppl et al., 2008). We believe that such a framework should be taken into consideration in the taxonomy of the genus Francisella.

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 ΔTm (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* subspp. 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* subspp. 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* subspp. 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 the 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 tularemia caused by *F. tularensis* subspp. *tularensis* or *F. tularensis* subspp. *holarctica*, human or animal infections with strains of *F. tularensis* subspp. *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-