Huber, B. E., Escudero, R., Busse, H. J., Seibold, E., Scholz, H. C., Anda, P., Kämpfer, P. & Splettstoesser, W. D. (2010). Description of *Francisella hispaniensis* sp. nov., isolated from human blood, reclassification of *Francisella novicida* (Larson *et al.* 1955) Olsufiev *et al.* 1959 as *Francisella tularensis* subsp. *novicida* comb. nov., and emended description of the genus *Francisella*. *Int J Syst Evol Microbiol* **60**, 1887–1896.

Koeppel, A., Perry, E. B., Sikorski, J., Krizanc, D., Warner, A., Ward, D. M., Rooney, A. P., Brambilla, E., Connor, N. & other authors (2008). Identifying the fundamental units of bacterial diversity: a paradigm shift to incorporate ecology into bacterial

systematics. *Proc Natl Acad Sci U S A* **105**, 2504–2509.

Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria* (1990 Revision). *Bacteriological Code*. Washington, DC: American Society for Microbiology.

Larson, C. L., Wicht, W. & Jellison, W. L. (1955). A new organism resembling *P. tularensis* isolated from water. *Public Health Rep* **70**, 253–258.

Larsson, P., Elfmark, D., Svensson, K., Wikström, P., Forsman, M., Brettin, T., Keim, P. & Johansson, A. (2009). Molecular evolutionary consequences of niche restriction in *Francisella*

tularensis, a facultative intracellular pathogen. *PLoS Pathog* **5**, e1000472.

Olsufiev, N. G., Emelyanova, O. S. & Dunayeva, T. N. (1959). Comparative study of strains of *B. tularensis* in the old and new world and their taxonomy. *J Hyg Epidemiol Microbiol Immunol* **3**, 138–149.

Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.

Tindall, B. J., Kämpfer, P., Euzéby, J. P. & Oren, A. (2006). Valid publication of names of prokaryotes according to the rules of nomenclature: past history and current practice. *Int J Syst Evol Microbiol* **56**, 2715–2720.

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 ΔT_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-

ories, but also for a reference laboratory (Birdsell *et al.*, 2009). We have the impression that the majority of routine laboratories will not be able to distinguish between the two subspecies on the basis of phenotypic characteristics or metabolic requirements.

It is also difficult to understand why only separate species are useful in a microbiological laboratory or a clinical setting but not separate subspecies.

The same is true for the basis of regulations governing the handling of medically important organisms. In fact, there is a clear example showing that subspecies differentiation can be applied for different classification of medically important bacteria. According to EU guideline 2000/54/EC, which had to be translated into national law in 27 European countries, *F. tularensis* subsp. *tularensis* [*Francisella tularensis* (Type A)] is classified as a BSL 3 pathogen, while *F. tularensis* subsp. *holarctica* [*Francisella tularensis* (Type B)] is classified as a BSL 2 pathogen (European Communities, 2000; EU directive 2000/54/EC).

It is argued, that:

‘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)’.

It is common knowledge that a bacterial species is composed of individuals that are not necessarily identical in their phenotypic traits. For instance, in the entry for *Pseudomonas fluorescens* in *Bergey’s Manual of Systematic Bacteriology* (Palleroni, 2005), among 139 traits listed, fewer than 50% (62 traits) are identical among the different biovars of this species and 18 traits are listed in which biovar I strains are variable. Hence, we do not think that 11 traits distinguishing *F. tularensis* subsp. *novicida* among 98 traits tested contradict our proposal. In this context, it has also to be mentioned here that the type strain of *Francisella tularensis* subsp. *holarctica* differs from the other two subspecies in seven traits and in an additional

four traits from one or the other subspecies (Huber *et al.*, 2009).

It is argued that:

‘In 1989, Hollis *et al.* (1989) showed that *F. novicida* and *F. tularensis* could be considered 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 the demotion of it to a biogroup of *F. tularensis* was not included among prokaryotic names with standing in nomenclature’.

In the paper of Hollis *et al.* (1989), the authors presented data on which they proposed the transfer of *Yersinia philomiragia* to the genus *Francisella* as *Francisella philomiragia* comb. nov. Furthermore, based on results from DNA–DNA hybridizations (>85% reassociation) they also proposed to transfer *Francisella novicida* to the species *Francisella tularensis* as the third biogroup *Francisella tularensis* biogroup *novicida* in addition to the biogroups *tularensis* and *palaeartica*. It is not clear why Hollis *et al.* (1989) did not apply the taxonomy proposed by Olsufjev & Meshcheryakova (1983), who had subdivided *F. tularensis* into three subspecies, *Francisella tularensis* subsp. *tularensis*, *Francisella tularensis* subsp. *holarctica* and *Francisella tularensis* subsp. *mediasiatica*, or why they did not refer to the paper of Olsufjev & Meshcheryakova (1983). In Validation List No. 32 (Hollis *et al.*, 1990), the name *Francisella philomiragia* comb. nov. was validly published whereas the name *F. tularensis* biogroup *novicida* was not. This was because the Bacteriological Code does not cover infraspecific names (e.g. biogroups, biovars, pathovars etc.) other than subspecies names.

Hence, there is no scientific argument to state that the paper of Hollis *et al.* (1989) was not valid according to the requirements of the Bacteriological Code. Only the proposed name *F. tularensis* biogroup *novicida* could not

be validly published according to the requirements of the Bacteriological Code. In fact the term ‘valid’ can only be applied to the valid publication of the name. This does not mean that the scientific conclusions of the paper of Hollis *et al.* (1989) are incorrect.

It is argued that:

‘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; Olsufjev *et al.*, 1959; Skerman *et al.*, 1980)’.

Microbiologists, including bacterial taxonomists, have always made use of the tools that are available to them at the time. Unfortunately, the paper of Olsufjev *et al.* (1959) was not accessible to us, but neither Larson *et al.* (1955) nor Skerman *et al.* (1980) mention any chemotaxonomic markers that they had examined. To the best of our knowledge, studies in bacterial taxonomy in the 1950s were restricted to ecological, physiological, morphological and serological examinations. Analyses of low-molecular mass cell components such as fatty acids, polar lipids, quinones (chemotaxonomic markers) or DNA-based studies were not yet established, and hence it also appears likely that Olsufjev *et al.* (1959) did not examine chemotaxonomic markers. In the following decades, with the introduction of novel tools, new knowledge has been gained which has led to numerous reclassifications of established taxa. For instance, species assigned to the genus *Pseudomonas* in *Bergey’s Manual of Systematic Bacteriology* (Palleroni, 1984) have since been shown to be distributed over the three classes *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. As a result, species phylogenetically only distantly related to the genus *Pseudomonas* have been reclassified as species of other genera. A similar fate has befallen numerous other taxa. Hence, there is

no reason why taxonomic conclusions concerning the genus *Francisella* proposed decades ago should remain untouchable when new findings suggesting reclassification are reported.

We agree with the authors of the letter that genomic data can provide an enormous amount of information. We think that bacterial taxonomy may undergo significant changes on the basis of the understanding of genomic and proteomic information. However, for the time being, we are far from able to interpret this information well enough to draw decisive conclusions. There are numerous open questions, e.g. 'Which genes belong to the conserved genome core considered useful to define a taxon and which belong to accessory dispensable genetic elements?' The impact of processes such as lateral gene transfer, gene duplication, recombination and rearrangements of genes in the genome is not clear and it may be very different in different lineages! For instance, the sizes of *Escherichia coli* genomes range from 4.6 to 5.7 Mb. This observation indicates that strains of a single species may lose or acquire 20–25 % of their genome from other sources without affecting their taxonomic status. So, for the time being, it is more appropriate to rely on the generally applied approaches for delineating taxa including species and subspecies (for a review see Tindall *et al.*, 2010). Reclassification of *F. novicida* as *F. tularensis* subsp. *novicida* is in agreement with these recommendations and also avoids confusion among those interested in bacterial taxonomy. Adhering to the species *F. novicida* might raise the question of why the other subspecies of *F. tularensis* have not been elevated to species level, though at least some arguments for the species *F. novicida* could also apply to the three recognized subspecies of *F. tularensis*.

Hans-Jürgen Busse,¹ Birgit Huber,¹
Pedro Anda,² Raquel Escudero,²

Holger C. Scholz,³ Erik Seibold,³ Wolf
D. Splettstoesser³ and Peter Kämpfer⁴

¹Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

²Laboratorio de Espiroquetas y Patógenos Especiales, Centro Nacional de Microbiología Instituto de Salud Carlos III, Madrid, Spain

³Bundeswehr Institute of Microbiology, German Reference Laboratory for Tularemia, Neuherbergstr. 11, D-80937 Munich, Germany

⁴Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

Correspondence: Hans-Jürgen Busse (hans-juergen.busse@vetmeduni.ac.at)

Birdsell, D. N., Stewart, T., Vogler, A. J., Lawaczek, E., Diggs, A., Sylvester, T. L., Buchhagen, J. L., Auerbach, R. K., Keim, P. & Wagner, D. M. (2009). *Francisella tularensis* subsp. *novicida* isolated from a human in Arizona. *BMC Res Notes* 2, 223.

European Communities (2000). Directive 2000/54/EC of The European Parliament and of The Council. *Off J Eur Communities* L262, 21–45.

Hollis, D. G., Weaver, R. E., Steigerwalt, A. G., Wenger, J. D., Moss, C. W. & Brenner, D. J. (1989). *Francisella philomiragia* comb. nov. (formerly *Yersinia philomiragia*) and *Francisella tularensis* biogroup *novicida* (formerly *Francisella novicida*) associated with human disease. *J Clin Microbiol* 27, 1601–1608.

Hollis, D. G., Weaver, R. E., Steigerwalt, A. G., Wenger, J. D., Moss, C. W. & Brenner, D. J. (1990). *Francisella philomiragia* comb. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 32. *Int J Syst Bacteriol* 40, 105–106.

Huber, B. E., Escudero, R., Busse, H.-J., Seibold, E., Scholz, H. C., Anda, P., Kämpfer, P. & Splettstoesser, W. D. (2010). Description of *Francisella hispaniensis* sp. nov., isolated from human blood, reclassification of *Francisella novicida* (Larson *et al.* 1955) Olsufiev *et al.* 1959 as *Francisella tularensis* subsp. *novicida* comb. nov., and emended description of the genus *Francisella*. *Int J Syst Evol Microbiol* 60, 1887–1896.

Johansson, A., Celli, J., Conlan, W., Elkins, K. L., Forsman, M., Keim, P. S., Larsson, P., Manoil,

C., Nano, F. E. & other authors (2010).

Objections to the transfer of *Francisella novicida* to the subspecies rank of *Francisella tularensis*. *Int J Syst Evol Microbiol* 60, 1717–1718.

Larson, C. L., Wicht, W. & Jellison, W. L. (1955). A new organism resembling *P. tularensis* isolated from water. *Public Health Rep* 70, 253–258.

Olsufiev, N. G., Emelyanova, O. S. & Dunayeva, T. N. (1959). Comparative study of strains of *B. tularensis* in the old and new world and their taxonomy. *J Hyg Epidemiol Microbiol Immunol* 3, 138–149.

Olsufiev, N. G. & Meshcheryakova, I. S. (1983). Subspecific taxonomy of *Francisella tularensis* McCoy and Chapin 1912. *Int J Syst Bacteriol* 33, 872–874.

Palleroni, N. J. (1984). Genus 1. *Pseudomonas* Migula 1894 237^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 141–199. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Palleroni, N. J. (2005). Genus I. *Pseudomonas* Migula 1894 237^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 323–379. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Sjöstedt, A. B. (2005). Genus I. *Francisella* Dorofe'ev 1947, 176^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 200–210. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* 30, 225–420.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rossello-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* 52, 1043–1047.

Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 60, 249–266.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 37, 463–464.