Gibbsiella papilionis Kim *et al.* 2013 is a later heterotypic synonym of *Gibbsiella dentisursi* Saito *et al.* 2012

Carrie Brady[1], Tomohiro Irisawa[2*], Takao Iino[2], Moriya Ohkuma[2], Dawn Arnold[1] and Sandra Denman[3]

[1] Centre for Research in Bioscience, Faculty of Health and Life Sciences, University of the West of England, Frenchay Campus, Bristol, United Kingdom

[2] Japan Collection of Microorganisms (RIKEN-BRC JCM), RIKEN Bioresource Centre, Tsukuba, Ibaraki 305-0074, Japan

[3] Forest Research, Centre for Ecosystems, Society and Biosecurity, Alice Holt Lodge, Surrey, United Kingdom

[*] Present address: Department of Animal Science, Tokyo University of Agriculture, Atsugi, Kanagawa 243-0034, Japan

Correspondence:
email: carrie.brady@uwe.ac.uk
tel: +44117 32 83647

Running title: Synonymy of *Gibbsiella dentisursi* and *Gibbsiella papilionis*

Contents category: New Taxa (Proteobacteria)

Keywords: *Gibbsiella*, *Enterobacteriaceae*, synonym

Word count: 1538

Note: The GenBank/EMBL accession numbers for sequences generated in this study are KT036410-KT036411 (16S rRNA).

Summary

Synonymy of *Gibbsiella dentisursi* DSM 23818^T (= NUM 1720^T) and *Gibbsiella papilionis* JCM 18389^T (= LEN 33^T) was suspected following multilocus sequence analysis (MLSA) of both type strains in a previous classification study, where they were found to share >99.6 % gene sequence similarity. The taxonomic relationship between these two strains was re-examined here using a polyphasic approach. A DNA-DNA similarity value of 98 % confirmed that the two type strains belong to a single taxon, while the phenotypic profiles were found to be nearly identical. Therefore we propose *Gibbsiella papilionis* as a later heterotypic synonym of *Gibbsiella dentisursi*.

In 2010 *Gibbsiella* was proposed as a novel genus in the family *Enterobacteriaceae* with a single species, *Gibbsiella quercinecans*, to house bacterial strains isolated from oak tissue showing symptoms of Acute Oak Decline (AOD) (Brady *et al*., 2010). Phylogenetic trees based on 16S rRNA-, *gyrB*- and *rpoB*-gene sequences placed these strains in a single well-supported cluster with *Serratia* and *Edwardsiella* as the closest phylogenetic neighbours. In 2012 a second *Gibbsiella* species was proposed, *G. dentisursi*, for a single strain isolated from the oral cavity of a bear in Japan (Saito *et al*., 2012). The description was supported by 16S rRNA gene sequencing as well as *gyrB* and *rpoB* sequences, which all showed *G. dentisursi* as a close phylogenetic relative of *G. quercinecans*. This was reflected in the DNA-DNA similarity value of 63.8 % obtained after hybridization of the type strains of *G. dentisursi* and *G. quercinecans*. Additionally, *G. dentisursi* shared the phenotypic characteristics and fatty acid profile of the genus *Gibbsiella*. Several months later, a third species, *G. papilionis*, was proposed for a single strain isolated from the intestine of a butterfly in Korea (Kim *et al*., 2013). As with *G. quercinecans* and *G. dentisursi*, 16S rRNA-, *gyrB*- and *rpoB*-gene sequencing was used to determine the phylogenetic position of *G. papilionis*. In the 16S rRNA gene phylogenetic tree, each *Gibbsiella* species was situated on a separate branch, all with high bootstrap support. However, the *gyrB* and *rpoB* phylogenetic trees both revealed a much closer relationship between *G. dentisursi* NUM 1720^{T} and *G. papilionis* LEN 33^{T}, with little or no sequence variation evident between the two strains (Kim *et al*., 2013). Additionally, DNA-DNA hybridization was only carried out between the type strains of *G. papilionis* and the type species *G. quercinecans*, this value was given as 41±2 %. Two years later in 2014, a fourth *Gibbsiella* species, *G. greigii*, was proposed for several strains isolated from symptomatic oak in the USA (Brady *et al*., 2014). In this study, multilocus sequence analysis (MLSA) based on partial sequences of *gyrB*, *rpoB*, *infB* and *atpD* was performed on the oak isolates as well as the type strains of the three known *Gibbsiella* species: *G. quercinecans*, *G. dentisursi* and *G. papilionis*. Phylogenetic analysis based on the concatenated partial gene sequences placed *G. dentisursi* and *G. papilionis* in the same cluster and suggested that these two species belong to single taxon (Brady *et al*., 2014). In the present study, the taxonomic position of *G. dentisursi* and *G. papilionis* is re-evaluated using data generated from 16S rRNA gene sequencing, MLSA, DNA-DNA hybridization and phenotypic characteristics.

The 16S rRNA genes of *G. dentisursi* DSM 23818^T (= NUM 1720^T) and *G. papilionis* JCM 18389^T (= LEN 33^T) were sequenced using the primers and methodology previously described (Coenye *et al*., 1999). Alignment of the trimmed sequences (1344 bp), based on secondary structure, and phylogenetic analysis were carried out as published (Brady *et al*., 2014). The 16S rRNA gene pairwise sequence similarity between *G. dentisursi* DSM 23818^T and *G. papilionis* JCM 18389^T is 99.3 %, while both strains exhibit > 98.0 % sequence similarity to *G. quercinecans* LMG 25500^T and *G. greigii* FRB 224^T. In the 16S rRNA gene maximum likelihood phylogenetic tree (Suppl. Fig. 1), DSM 23818^T and JCM 18389^T cluster together with little branch length deviation and high bootstrap support of 96 %. This cluster is situated in the *Gibbsiella* clade with *G. quercinecans* and *G. greigii*.

As mentioned above, MLSA was previously performed on *G. quercinecans* LMG 25500^T, *G. dentisursi* DSM 23818^T and *G. papilionis* JCM 18389^T in an earlier taxonomic study. Sequences used for the phylogenetic tree construction are from Brady *et al*. (2013) and Brady *et al*. (2014). Accession numbers are listed in Suppl. Table S1 and the sequences can be downloaded from Genbank. The sequence similarity between *G. dentisursi* DSM 23818^T and *G. papilionis* JCM 18389^T for each of the housekeeping genes (*gyrB, rpoB, infB* and *atpD*) is 99.6 – 99.8 %. In contrast, the sequence similarity between these two strains and *G. quercinecans* LMG 25500^T and *G. greigii* FRB 224^T is 98.2 – 98.3 % and 96.9 – 97.0 %, respectively. These similarities are reflected in the clustering of the four *Gibbsiella* species in a maximum likelihood phylogenetic tree based on the concatenated partial gene sequences of the four housekeeping genes (Fig. 1). *G. dentisursi* and *G. papilionis* are contained in a strongly supported cluster with no branch length deviation, which is on the border of the *G. quercinecans* cluster, while the *G. gregii* cluster is further removed. The topology of the MLSA phylogenetic tree strongly suggests that *G. dentisursi* and *G. papilionis* are in fact the same species.

To further test this hypothesis, the DNA similarity amongst the type strains of the four *Gibbsiella* species was determined by fluorometric DNA-DNA hybridization using photobiotin-labelled DNA probes (Ezaki *et al*., 1989). The hybridization temperature used was 45 °C and reciprocal reactions were performed for each pairing. The DNA similarity between *G. dentisursi* JCM 17291^T (= NUM 1720^T = DSM 23828^T) and *G. papilionis* JCM 18389^T was found to be 98 %, confirming that these two species belong to the same taxon. Values of 20 – 44 % were observed when both of these type strains were hybridized to

G. quercinecans FRB 97^T and *G. greigii* FRB 224^T. The DNA-DNA hybridization data also confirmed Δ*T*m results obtained between the four *Gibbsiella* species (Brady *et al*., 2014).

Biolog GN2 microplate assays were performed on *G. dentisursi* DSM 23818^T and *G. papilionis* JCM 18389^T in triplicate, along with *G. quercinecans* FRB 97^T as a positive control, according to the manufacturer's instructions. Plates were incubated at 28 °C and scored after 6, 24 and 48 h. It was observed previously that the Biolog profile obtained for the type strain of *G. papilionis* in the *G. gregii* study (Brady *et al*., 2014) differed considerably from that reported by Kim *et al*. (2013), with 12 less substrates utilized. The results obtained in triplicate for *G. papilionis* in the present study agree with those published in the *G. greigii* study. The Biolog profiles for *G. dentisursi* and *G. papilionis* were found to be nearly identical and differed only in their utilization of α-ketobutyric acid, L-alanyl-glycine and glycyl-L-glutamic acid. This is probably due to variation within the species as DSM 23818^T and JCM 18389^T were isolated from two diverse sources.

The 16S rRNA gene sequence similarity, MLSA data, DNA hybridization values and phenotypic data all indicate that *G. dentisursi* and *G. papilionis* belong to the same taxon as a single species. We propose that *G. papilionis* is a later heterotypic synonym of *G. dentisursi* with the type strain as NUM 1720^T (= DSM 23818^T = JCM 17201^T).

Emended description of *Gibbsiella dentisursi* Saito *et al*. 2012

Gibbsiella dentisursi (den.tis.ur'si. L. gen. n. dentis of the tooth, L gen. n. ursi of the bear, N.L. gen. n. dentisursi from the tooth of a bear).

The description is based on Saito *et al*. (2012), Kim *et al*. (2013) and this study.

Gram-negative, non-motile rods (0.5 – 1.5 x 3.0 – 6.0 μm) occurring singly. Colonies are circular, convex, opaque and cream in colour with smooth edges on trypticase soy agar. Facultative anaerobic, oxidase negative and catalase positive. Growth occurs at temperatures of 4 – 37 ºC, 0 – 5 % (w/v) NaCl and pH 5 – 9, with optimal growth at 30 – 37 ºC, pH 8 – 9 and 1 % (w/v) NaCl. Positive for β-galactosidase, citrate utilization and acetoin production. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S,

urease, tryptophan deaminase, indole and gelatinase production. Nitrate is reduced to nitrite. Acid is produced from: glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-mannitol, D-sorbitol, methyl-αD-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-melibiose, D-saccharose, D-trehalose, D-raffinose, gentiobiose, D-turanose, D-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. α-Cyclodextrin, dextrin, glycogen, tweens 40 and 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-cellobiose, D-fructose, L-fucose (weak), D-galactose, gentiobiose, α-D-glucose, inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, α-ketoglutaric acid, D,L-lactic acid, succinic acid, bromosuccinic acid, L-alaninamide, L-alanine, L-aspargine, L-aspartic acid, L-glutamic acid, D-serine, L-serine, inosine, uridine, thymidine, 2,3-butanediol, glycerol, D,L,α-glycerol phosphate, α-D-glucose-1-phosphate and α-D-glucose-6-phosphate are oxidized. Reactions to α-ketobutyric acid (type strain is weakly positive), L-alanyl-glycine (type strain is negative) and glycyl-L-glutamic acid (type strain is negative) are variable. Positive for activity of esterase, leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Major fatty acids include $C_{14:0}$, $C_{16:0}$ and $C_{17:0}$ cyclo and the DNA G + C contenst of NUM 1720^T and LEN 33^T are 55.0 and 58.7 mol %, respectively.

The type strain is NUM 1720^T (= DSM 23818^T = JCM 17201^T), isolated from the oral cavity of a bear.

Acknowledgements

This study was funded by the Forestry Commission, Woodland Heritage and the Monument Trust of the United Kingdom.

References

Brady, C., Denman, S., Kirk, S., Venter, S., Rodríguez-Palenzuela, P. & Coutinho, T. (2010). Description of *Gibbsiella quercinecans* gen. nov., sp. nov., associated with Acute Oak Decline. *Syst Appl Microbiol* **33,** 444-450.

Brady, C., Cleenwerck, I., Venter, S., Coutinho, T. & De Vos, P. (2013). Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, *E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. Syst. Appl. Microbiol. 36, 309-319.

Brady, C., Hunter, G., Kirk, S., Arnold, D. & Denman, S. (2014). *Gibbsiella greigii* sp. nov., a novel species associated with oak decline in the USA. *Syst Appl Microbiol* **37,** 417-422.

Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J.R.W., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49,** 405-413.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39,** 224-229.

Kim, P.S., Shin, N., Kim, J.Y., Yun, J., Hyun, D. & Bae, J. (2013). *Gibbsiella papilionis* sp. nov., isolated from the intestinal tract of the butterfly *Mycalesis gotama*, and emended description of the genus *Gibbsiella*. *Int J Syst Evol Microbiol* **63,** 2607-2611.

Saito, M., Shinozaki-Kuwahara, N. & Takada, K. (2012). *Gibbsiella dentisursi* sp. nov., isolated from the bear oral cavity. *Microbiol Immunol* **56,** 506-512.

Figure 1: Maximum likelihood tree based on concatenated partial *gyrB*, *rpoB*, *atpD* and *infB* gene sequences of all validly described species of the genus *Gibbsiella* and closest phylogenetic neighbours. Only MLSA sequences generated from the same strain are used in the tree construction. Accession numbers are listed in Suppl. table S1 and sequences can be downloaded from Genbank. Bootstrap values after 1000 replicates are expressed as percentages. *Xenorhabdus nematophila* ATCC 19061^{T} (NC_014228) is included as an outgroup. The scale bar indicates the fraction of substitutions per site.