

Taxonomic Description

***Ferranicluibacter rubi* gen. nov., sp. nov., a new member of family *Rhizobiaceae* isolated from stems of elmleaf blackberry (*Rubus ulmifolius* Schott) in Northwest Spain.**

1.1 Author names

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ABSTRACT

The strain CRRU44^T was isolated from the stems of *Rubus ulmifolius* plants growing in Salamanca (Spain). The phylogenetic analysis of the 16S rRNA gene sequence places this strain within the family *Rhizobiaceae* showing that it is equidistant to the type species of several genera from this family with similarity values ranging from 91.0% to 96.3%. The strain CRRU44^T formed a divergent lineage which clustered with *Endobacterium cereale* RZME27^T, *Neorhizobium galegae* HAMBI540^T and *Pseudorhizobium pelagicum* R1-200B4^T. The phylogenomic analysis showed that the strain CRRU44^T was equal or more distant to the remaining genera of the family *Rhizobiaceae* than other genera among them. The calculated ANIb and AAI values with respect to the type species of all genera from the family *Rhizobiaceae* were lower than 78.5 and 76.5%, respectively, which are the currently cut-off values proposed to differentiate genera within this family. All these results together with those from phenotypic and chemotaxonomic analyses support that the strain CRRU44^T represents a novel species of a novel genus within the family *Rhizobiaceae*, for which the name *Ferrancluibacter rubi* gen. nov., sp. nov. is proposed (type strain CRRU44^T=CECT 30117^T=LMG 31822^T).

INTRODUCTION

Study of plant-microbe interactions has revealed a huge diversity of microorganisms associated with endophytic environments [1]. In last decades, the point of view about plant microbial diversity has changed, discovering how plants are able to modulate the microbial rhizospheric populations and consequently the endophytic ones [2]. Since some plant endophytic bacteria are able to promote the plant growth [3, 4], the search of these bacteria associated with different plants is a reliable practice to allow the development of new biofertilizers within the framework of sustainable agriculture. To date, these bacteria have been isolated more from roots than from stems and from herbaceous plants than from shrubs despite the benefits of the fruits produced by some of shrubby plants in nutrition and human health [5]. This is the case of blackberries produced by *Rubus fruticosus* and *Rubus ulmifolius*, which contain several valuable nutrients and bioactive compounds, particularly flavonoids compounds [6,7]. Although the bacterial endophytes of these two plants have been scarcely studied to date, several strains of rhizobia have been found in the endophytic communities of roots and stems of *R. fruticosus* and *R. ulmifolius*, respectively [8,9]. For instance, several strains of genus *Rhizobium* have been isolated from roots of *R. fruticosus* [8], whereas the strains isolated from

stems of *R. ulmifolius* belonged to genera *Neorhizobium* and *Mesorhizobium* and one strain named CRRU44^T formed a new lineage within the family *Rhizobiaceae* [9]. This family currently encompasses more than twenty genera (<https://lpsn.dsmz.de/family/rhizobiaceae>), some of them originally isolated from the inner of plants such as *Liberibacter*, whose type species *Liberibacter crescens* was isolated from expressed sap of a Babaco papaya [10] and *Endobacterium*, whose type species *Endobacterium cereale* was isolated from a maize root [11]. Endophytic species, some of them recently described, are also encompassed by other genera of this family, such as *Agrobacterium* [12,13,14], *Neorhizobium* [15], *Pararhizobium* [16] and *Rhizobium* [17,18,19].

In the present work, we characterized the strain CRRU44^T isolated from stems of elmleaf blackberry (*R. ulmifolius*) and the obtained results from phylogenetic, phenotypic and chemotaxonomic analyses support the classification of this strain in a new genus and species of family *Rhizobiaceae* for which we propose the name *Ferranicluibacter rubi* gen. nov., sp. nov.

Resources and isolation

The strain CRRU44^T was isolated from a stem of *R. ulmifolius* growing in a soil from Ciudad Rodrigo in Salamanca (Salamanca, NW Spain, 40°35'02.6"N 6°31'56.5"W), where this plant is commonly associated with agricultural and abandoned lands. For isolation, stems were surface-disinfected with 70% ethanol (1 min), 2% NaClO (3 min) and then, they were rinsed ten times with sterile distilled water and crushed in 10 ml of sterile PBS pH7. Serial decimal dilutions were preformed and aliquots of 0.1 ml from each dilution were inoculated on YMA [20] for 48h. In parallel, as control for complete disinfected stem surface, several samples of stem (1 cm) were incubated in the same medium in order to ensure their complete external disinfection. No growth was observed around these stems.

16S RNA phylogeny

The 16S rRNA gene was amplified and sequenced in the Sequencing DNA Service (NUCLEUS) of the University of Salamanca (Spain) as was previously described [21]. The obtained sequence was compared with those from GenBank through the EzBioCloud database [22]. The sequences were aligned using the Clustal W program [23]. The phylogenetic distances were calculated according to Kimura's two-parameter model [24]. The phylogenetic trees were inferred using the neighbour joining and maximum likelihood models [25,26] and MEGA 7 [27] was used for all phylogenetic analyses.

The analysis of the 16S rRNA gene of strain CRRU44^T showed that this strain belongs to the family *Rhizobiaceae*. Its closest related type strain is *Endobacterium cereale* RZME27^T with 96.86% similarity, whereas values lower than 96% were found for the type strains of the type species of the remaining genera currently included in the family *Rhizobiaceae*. Also, the strain CRRU44^T showed similarity values ranging from 96% to 98% with the type strains of many non-type species from the genera *Rhizobium*, *Allorhizobium*, *Neorhizobium*, *Pseudorhizobium* and *Mycoplana* (data not shown). The phylogenetic analysis of this gene showed that the strain CRRU44^T clustered with *E. cereale* RZME27^T, *N. galegae* HAMBI540^T and *P. pelagicum* R1-200B4^T (Fig. 1). Nevertheless, this strain could belong to a new genus since several genera of the family *Rhizobiaceae* were more closely related among them than the strain CRRU44^T with respect to its closest relatives (Fig. 1).

The 16S rRNA gene sequence of this strain showed similarity values higher than 99% with respect to those available in Genbank for some uncultured bacteria and for several strains isolated from diverse sources that could belong to the new genus (Fig. S1).

Four these strains, Leaf371, Leaf383, 9140 and NFR03, were included in the recent work of Kuzmanović *et al.*, who showed that they belong to a phylogenetic group closely related to the genus *Xaviernesmea* [28]. Our results showed that this phylogenetic group corresponds to the new genus found in the present study for which we propose the name *Ferrancluibacter* gen. nov. (Fig. S1). Bacteria from this genus are widely distributed in very different ecosystems, since they have been found in soil and water related sources, in leaves of several plants, such as *Arabidopsis thaliana*, *Galium album* and *Fraxinus excelsior*, in shoots of switchgrass and *Rubus ulmifolius* (strain CRRU44^T), in hyphae of Ascomycetous fungi, in vegetable pickles, such as Chinese paocai, and even in human skin (Fig. S1).

Genome Features

The genomic DNA from a pure culture of the strain CRRU44^T was extracted using the DNeasy UltraClean Microbial DNA Isolation Kit (Qiagen) following the manufacturer's protocol. Sequencing, upon preparation of pair-end libraries, was performed on Illumina MiSeq sequencing platform (2x250bp). Sequencing data was assembled using Velvet 1.2.10 [29]. Annotation was done using RAST 2.0 (Rapid Annotation using Subsystem Technology) [30,31] and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [32,33]. The draft genome sequence of *F. rubi* CRRU44^T (42 contigs) was deposited in DDBJ/EMBL/GenBank under the Bioproject PRJNA610165 (accession number JAANCM000000000). The circular genome map of the strain CRRU44^T and the schematic map of

BLAST comparison among the genomes of this strain and the type strains of its closest type species was generated using the CGView server (<http://cgview.ca/>) [34,35]. The phylogenomic analysis was conducted with the UBCG tool (www.ezbiocloud.net/tools/ubcg), which uses 92 single-copy protein encoding sequences [36] to reconstruct a phylogenomic tree. Some external programs are installed for the UBCG pipeline: HMMER3 is used for gene finding [37] while *hmmsearch* and *FastTree* are used for identifying UBCG genes and drawing an approximate ML tree [38], respectively. Average amino acid identity (AAI) was calculated with the online server ANI/AAI-Matrix [39] with default settings, which uses *MMSeqs2* for protein comparisons, a minimum query coverage of 50% and a minimum identity of 40% for AAI calculations. The ANIb values were calculated with JSpeciesWS Online Service, which is based on BLAST+ (version 2.2.29+) [40]. Carbohydrate-active enzyme analysis was performed using dbCAN2 meta server (<http://cys.bios.niu.edu/dbCAN2>) [41]. Digital DNA–DNA hybridization (dDDH) values were calculated using the genome-to-genome distance calculator website service from DSMZ (GGDC 2.1) [42,43]. Comparative genome-wide analysis of orthologous clusters and gene ontology analysis among all predicted protein-coding genes was performed using OrthoVenn2 (<https://orthovenn2.bioinfotoolkits.net/home>) [44]. The genomes of phylogenetically related type strains were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genome/>).

The genome characteristics of the strain CRRU44^T and those of the type strains of its closest phylogenetic type species are recorded in Table S1 and Fig. S2. The G+C content of strain CRRU44^T was calculated from the genome data being 62.8%, which falls within the range reported for the family *Rhizobiaceae* [45].

The results of the phylogenomic analysis of 92 core genes showed that the strain CRRU44^T clustered with the type strains of *Xaviernesmea oryzae* 1.7048^T, *Pararhizobium giardinii* H152^T, *Ensifer adhaerens* Casida A^T and *Sinorhizobium fredii* USDA205^T (Fig. 2). In the phylogram, the strain CRRU44^T occupied a branch which was equal or more distant to the remaining genera of this family than other genera among them (Fig. 2). These results and those obtained after the 16S rRNA gene analysis support that the strain CRRU44^T can represent a new genus within the family *Rhizobiaceae*. The calculated ANIb and AAI values between the strain CRRU44^T and the type strains of the type species of each one of the remaining genera of the family *Rhizobiaceae* were lower than 78.5 and 76.5%, respectively (Table 1). Identical results were found between the strain CRRU44^T and the type strains of non-type species from several genera showing 16S rRNA gene values higher than 96% (Table S2). Until now it had been considered that different genera of family *Rhizobiaceae* showed ANIb values lower than 76% [11,46]. Nevertheless, recently Kuzmanovic et al. [28] proposed that the threshold values of ANIb and AAI are 78.5% and 76.5%, respectively, for genera differentiation

within the family *Rhizobiaceae* supporting the classification of the strain CRRU44^T in a new genus within this family.

The whole genomes of several non-type strains with 16S rRNA gene sequences closely related to that of the strain CRRU44^T are available in Genbank (Fig. S1). The calculated ANIb and AAI values among the genomes of these strains and with respect to that of the strain CRRU44^T were higher than 78.5% and 76.5%, respectively, showing that all of them also belong to the genus *Ferranicluiibacter* (Tables S3 and S4). However, only five of these strains belong to the same species *F. rubi* as the strain CRRU44^T because their genomes presented dDDH and ANIb values higher than 70% and 95-96%, respectively (Table S3), which are the threshold value recommended for species differentiation [47]. These strains formed a clade after the phylogenomic analysis, which showed that the remaining strains of genus *Ferranicluiibacter* belong to a second clade formed by three strains and five phylogenetic lineages (Fig. 2) These clades and lineages correspond to seven different species taking into account the values of ANIb, dDDH and AAI (Tables S3 and S4).

The comparison between the genome of the strain CRRU44^T and those of the type strains of *X. oryzae*, *P. giardinii*, *E. adhaerens* and *S. fredii* is depicted in Fig. S2A, which showed that the genome of *F. rubi* CRRU44^T is closer to that of the type strain of *X. oryzae* than to those of the type strains of *P. giardinii*, *E. adhaerens* and *S. fredii*. This comparison showed the absence in these four last genomes of important regions present in that of the strain CRRU44^T (Fig. S2A). For example, the genome of the strain CRRU44^T contains a phage HNH homing endonuclease, other phage proteins and a large number of CDS that do not show homology with proteins of bacterial origin. It also contains numerous CDS annotated as phage head terminase, phage portal protein and phage major capsid protein, which are not found in the genomes of its closest relatives. We also found in the genome of strain CRRU44^T genes related to the synthesis of exopolysaccharides, some of them related with three insertion elements, such as ISR1 transposase (*Rhizobium* class IV strains). These elements are located in regions of the genome of the strain CRRU44^T whose sequences are highly divergent to those of its closest type strains and are associated with highly mutagenic elements in *Rhizobium* [48]. Other regions found in the genome of CRRU44^T and absent in those of its closest relatives *X. oryzae*, *P. giardinii*, *E. adhaerens* and *S. fredii* are the *fhuABCD* operon involved in ferrichrome/hydroxamate synthesis and transport, the *entB* gene (enterobactin synthase) and *dhbCE* gene. The analysis of the genes encoding carbohydrate hydrolytic enzymes showed that the strain CRRU44^T has a narrower profile than *E. adhaerens* Casida A^T, *S. fredii* USDA205^T and *P. giardinii* H152^T and broader than *X. oryzae* 1.7048^T except in the carbohydrate binding module. This profile mainly comprises glycosylhydrolases and glycosyltransferases that are the main carbohydrate active enzymes annotated in the genomes of the analysed strains (Fig. S2B). Moreover, genes involved in

legume nodulation (nod) or nitrogen fixation (nif) have not been found in the genome of the strain CRRU44^T. These genes have not been found either in the genomes of *E. adhaerens* [49] and *X. oryzae* [50], but they are present in the genomes of *P. giardinii* and *S. fredii*, which are able to induce legume nodules where they carry out the fixation of atmospheric nitrogen [51,52]. Comparison of the pangenome of the strain CRRU44^T with respect to the type strains of the closest species showed that they had a core pangenome formed by 2381 clusters of orthologs. From them, 32 orthologous clusters were only present in strain CRRU44^T whereas 18, 89, 62 and 76 were only present in *X. oryzae* 1.7048^T, *E. adhaerens* Casida A^T, *S. fredii* USDA205^T and *P. giardinii* H152^T, respectively (Fig S2C).

Physiology and Chemotaxonomy

In this study, the phenotypic characterization was performed using the API ID32GN and API 20NE systems (BioMérieux, France) in the conditions indicated by the manufacturer and the results were read after 72h of incubation at 28°C. Growth temperature range was determined by incubating cultures in YMA medium [20] at 4, 15, 28 and 37°C. Growth pH range was determined in the same medium with final pH 5, 6, 7, 8 and 9. PCA buffer (Na₂HPO₄ 0.4M and citric acid 0.2M) was used to adjust the pH from 5 to 6, phosphate buffer (Na₂HPO₄ 0.2M and NaH₂PO₄ 0.2M) was used for pH 7 and TE buffer 0.2M was used for pH 8 and 9. Salt tolerance was tested in the same medium containing 0.5, 1, 1.5 and 2% (w/v) NaCl. Catalase production was assayed by using 0.3 % hydrogen peroxide with one colony taken from the TY plates. Oxidase activity was detected by using N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride. The flagella type was determined by electron microscopy after 48 h of incubation in nutrient agar at 22 °C. Cells were gently suspended in sterile water and then stained with 0.2% uranyl acetate and examined at 80 kV with a Zeiss EM 209 transmission electron microscope. Strain CRRU44^T is motile by means of polar and subpolar flagella (Fig. S3).

Phenotypic characteristics of the strain CRRU44^T are included in the protologue and the differences with respect to its closely related type species of the family *Rhizobiaceae* are recorded in Table 2. The strain CRRU44^T differs from these species in nitrate reduction, in the growth in different conditions of temperature, pH and ClNa concentrations and in the assimilation of several carbon sources. (Table 2).

Cellular fatty acids were analysed by using the Microbial Identification System (MIDI; Microbial ID) Sherlock 6.1 and the library RTSBA6 according to the technical instructions provided by this system

[55]. The strain CRRU44^T was cultured aerobically on TY plates [56] at 28°C and cells were collected during the late-exponential phase of growth. The obtained results showed that the major fatty acids of *F. rubi* are those from summed feature 8 (C_{18:1} ω6c/C_{18:1} ω7c) as occurs in their closest relative genera of the family *Rhizobiaceae* (Table 3). The main difference was observed in the percentage of C_{18:1}ω7c 11-methyl, which is the second major fatty acid in the strain CRRU44^T (Table 3).

Protologue

Description of *Ferrancluibacter* gen. nov.

Ferrancluibacter (Fer.ran.i.clu.i.bac'ter. N.L. masc. n. *bacter*, a rod N.L. masc. n. *Ferrancluibacter*, a rod named to honour Jaime Ferrán i Clúa, a leading Spanish bacteriologist of the 19th century)

Aerobic, Gram negative, motile and non-sporulating rods forming white colonies on YMA. Optimal growth at 28°C and pH7. Catalase and oxidase are positive. Nitrate reduction, glucose fermentation and arginine dihydrolase are negative. Esculin hydrolysis and production of urease and β-galactosidase are positive. Production of indole and H₂S is negative. The main fatty acids are C_{18:1} ω6c/C_{18:1} ω7c. Delineation of the genus was determined by the phylogenetic information from 16S rRNA gene sequence and by the analysis of the complete genome sequence. The type species is *Ferrancluibacter rubi*.

Description of *Ferrancluibacter rubi* sp. nov.

Ferrancluibacter rubi (ru'bi. L. masc. n. *rubus*, a blackberry, and also the generic name of blackberry (*Rubus*); L. gen. masc. n. *rubi*, of blackberry, of *Rubus*).

Cells are Gram-negative, aerobic, motile by polar or subpolar flagella, non-sporulating and rod-shaped. Colonies on YMA are cream/white, circular and convex with diameter of 0,5 mm within 48 h at 28°C. Catalase and oxidase positive. It grows from 5°C to 37°C and optimally at 28°C and from pH 5 to 8 with optimum growth at pH 7. It grows up to 2% NaCl. Nitrate reduction, gelatinase and arginine dihydrolase are negative. Urease and β-galactosidase are positive. Esculin hydrolysis is positive. Assimilation of D-glucose, L-arabinose, L-rhamnose, D-ribose, D-mannose, mannitol, N-acetyl-glucosamine, inositol, sucrose, maltose, malate, melibiose, salicin, L-fucose and D-sorbitol is positive. Assimilation of citrate, gluconate, caprate, adipate, phenylacetate, itaconate, suberate, malonate, propionate, valerate, glycogen, L-alanine, L-serine, L-histidine 3-hydroxi-butyrate, 2 and 5 keto-gluconate, 3 hydroxi-benzoate and L-proline is negative. Acetate, D, L-lactate and 4 hydroxi-benzoate are weakly assimilated. The major fatty acids are those from summed feature 8 (C_{18:1} ω6c/C_{18:1} ω7c) and C_{16:0}. G+C content was 62.8 mol %. The type strain CRRU44^T (= CECT 30117^T=LMG 31822^T) was isolated from a stem of *Rubus ulmifolius* Schott. The draft genome sequence was deposited in DDBJ/EMBL/GenBank the under the Bioproject PRJNA610165 (accession number JAANCM000000000) and the 16S rRNA gene under the accession number MT146893.

AUTHOR STATEMENTS

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Conceptualization: RR; Data curation: JDFF; Formal Analysis: JDFF, RRC, EV; Funding acquisition: RR ; Investigation RRC, JDFF; Methodology JDFF, JMI; Project administration RR; Supervision: JDFF, RR; Writing – original draft RRC; Writing – review & editing JDFF, JMI, EV, RR.

1.7 Conflicts of interest

The authors declare that there are no conflicts of interest.

1.8 Funding information

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ABBREVIATIONS

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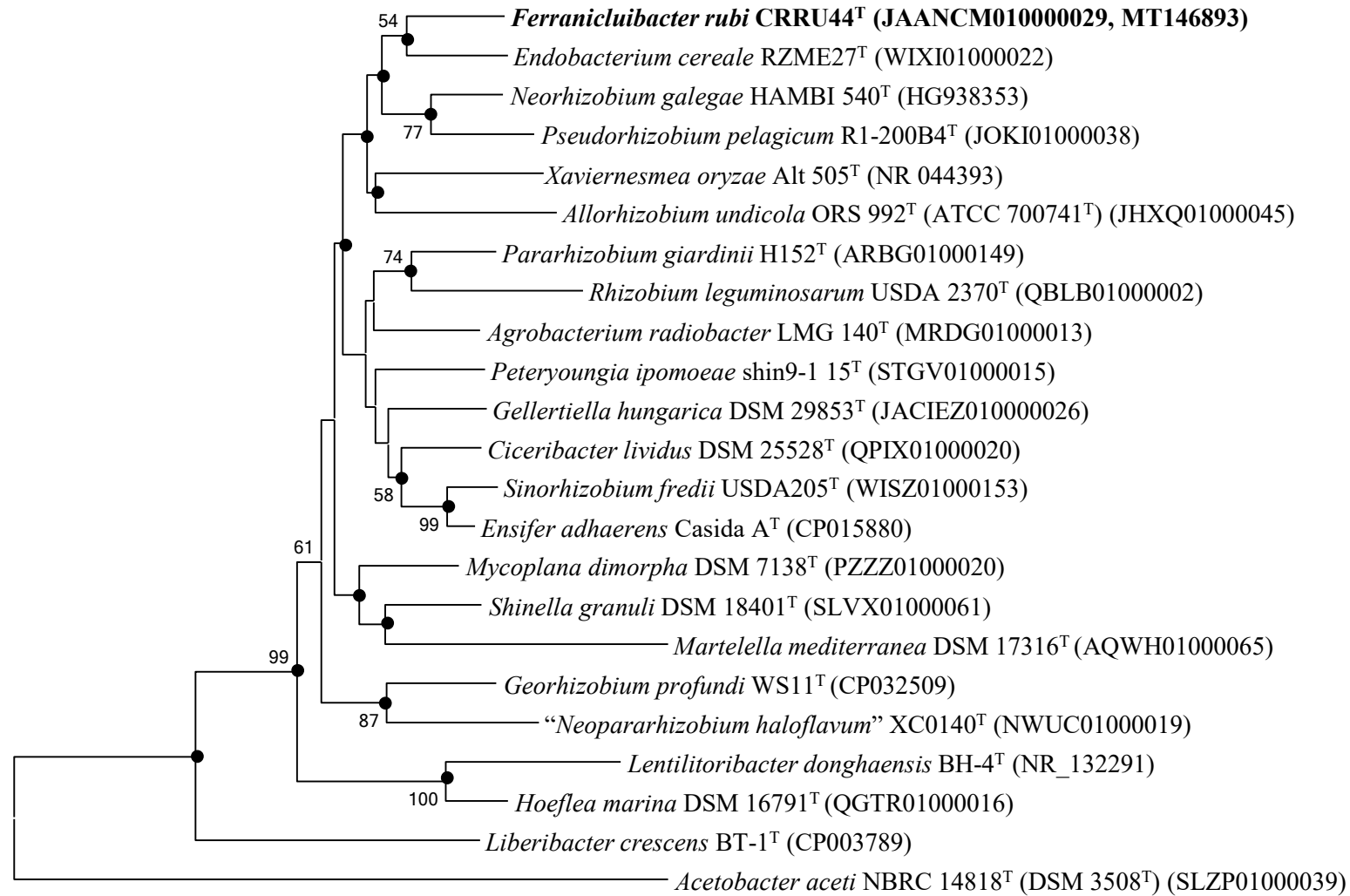
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FIGURES AND TABLES

Figure 1. Neighbour joining phylogenetic rooted tree based on 16S rRNA gene sequences (1310 nt from the end of the insert present in *Gellertiella hungarica*) showing the phylogenetic position of *F. rubi* CRRU44^T within the family *Rhizobiaceae*. Bootstrap values calculated for 1000 replications are indicated. Bar, 5 nt substitution per 1000 nt. Accession numbers from Genbank are given in brackets. The nodes marked with filled circles were also obtained with maximum likelihood algorithm.

Figure 2. Phylogenomic tree generated after the analysis of 92 core genes for *F. rubi* CRRU44^T and the type strains of the type species from other genera of the family *Rhizobiaceae*. Tree inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5 . The numbers above branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 54,7 %.



0.02

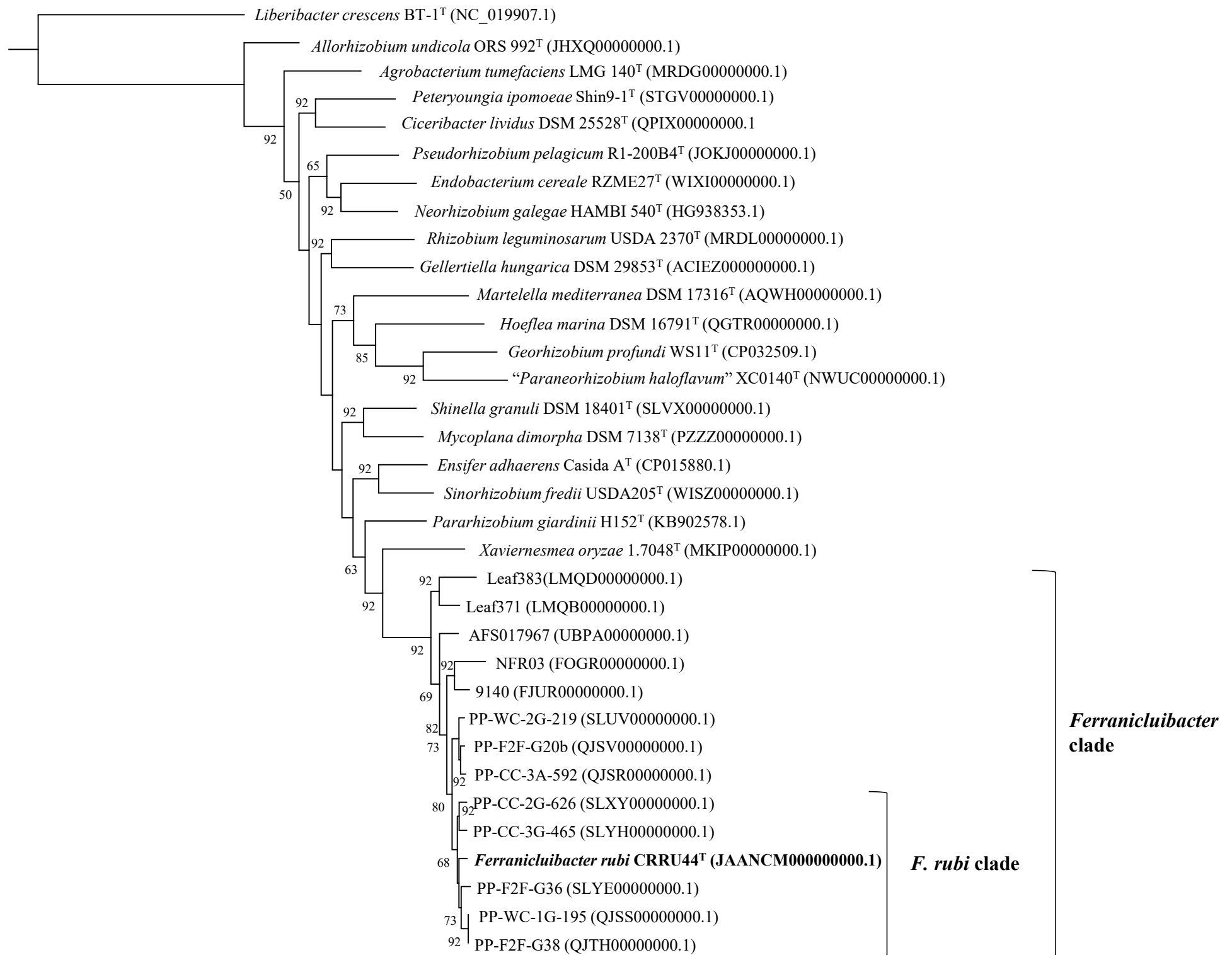


Table 1. Pairwise ANI and AAI values between the genomes of *Ferrancluibacter rubi* CRRU44^T and those of the type strains of other type species of the family *Rhizobiaceae*. Strains: 1, *Ferrancluibacter rubi* CRRU44^T (JAANCM000000000.1); 2, *Endobacterium cereale* RZME27 (WIXI000000000.1); 3, *Neorhizobium galegae* HAMB1540^T (HG938353.1); 4, *Rhizobium leguminosarum* USDA 2370^T (QBLB01000000.1); 5, *Pseudorhizobium pelagicum* R1-200B4^T (JOKJ000000000.1); 6, *Gellertiella hungarica* RAM11^T (JACIEZ010000000.1); 7, *Peteryoungia ipomeae* shin9-1^T (STGV000000000.1); 8, *Ciceribacter lividus* DSM 25528^T (QPIX01000000.1); 9, *Georhizobium profundi* WS11^T (CP032509.1); 10, *Pararhizobium giardinii* H152^T (ARBG01000149.1); 11, *Shinella granuli* DSM 18401^T (SLVX01000000.1); 12, *Allorhizobium undicola* ORS 992^T (JHXQ0100000.1); 13, *Agrobacterium radiobacter* DSM 30147^T (ASXY01000000.1); 14, *Ensifer adhaerens* Casida A^T (CP015880.1); 15, *Hoeflea marina* DSM 16791^T (QGTR000000000.1); 16, *Liberibacter crescens* BT-1^T (NC_019907.1); 17, *Marteella mediterranea* DSM 17316^T (AQWH000000000.1); 18, *Mycoplana dimorpha* DSM 7138^T (PZZZ000000000.1); 19, *Paraneorhizobium haloflavum* XC0140^T (NWUC000000000.1); 20, *Sinorhizobium fredii* USDA205^T (WISZ000000000.1); 21 *Xaviernesmea oryzae* 1.7048^T (MKIP000000000.1). Data are in percentages. ANI values are presented on the bottom left and AAI values are presented in the top right.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*	64.88	65.43	66.58	64.91	64.03	65.29	65.42	59.67	71.77	66.98	64.82	65.39	68.92	60.15	55.55	59.31	66.86	60.17	68.97	60.50
2	72.49	*	71.29	66.31	69.74	63.33	65.90	66.15	58.94	64.79	64.71	65.72	68.51	64.35	58.58	55.68	59.31	64.42	58.75	64.26	70.18
3	73.02	75.61	*	68.46	72.41	64.08	66.96	67.39	59.87	65.89	64.90	66.39	68.70	65.05	59.48	56.06	60.16	65.12	59.54	65.39	63.47
4	72.95	73.34	74.49	*	65.88	65.16	66.29	67.25	59.08	69.23	66.31	67.06	67.25	66.35	59.49	56.90	59.47	66.48	59.36	67.89	63.46
5	73.34	75.04	75.90	73.89	*	64.79	68.01	68.08	60.49	65.19	65.83	66.50	68.06	64.98	60.41	56.16	60.46	66.10	60.42	65.25	65.04
6	72.86	72.78	73.05	73.47	72.99	*	66.05	66.97	59.92	65.10	66.19	64.93	64.32	65.72	60.19	56.40	59.59	66.43	60.01	65.77	63.79
7	72.47	73.29	72.91	73.15	73.13	72.50	*	71.47	60.36	66.03	66.48	68.83	67.44	66.02	60.62	55.69	60.90	65.64	60.72	65.62	63.99
8	73.31	73.82	74.08	74.16	74.19	73.33	74.11	*	60.80	66.99	67.10	69.79	67.95	66.32	61.56	56.87	60.97	66.85	61.26	66.91	64.40
9	71.27	70.91	70.90	71.03	71.04	70.95	70.58	71.03	*	60.20	61.05	59.90	59.64	60.43	61.19	53.22	57.21	61.37	72.14	60.32	65.21
10	75.11	72.90	73.17	74.56	72.72	72.57	72.34	72.97	70.70	*	70.36	65.99	66.37	72.97	60.98	56.17	59.06	69.97	60.75	74.05	59.80
11	74.17	73.14	73.36	74.18	73.39	73.55	72.68	73.70	71.08	75.25	*	65.52	66.11	70.76	61.45	55.75	59.37	74.20	61.13	69.77	68.90
12	72.28	72.97	72.70	72.81	72.64	72.50	73.23	73.27	69.94	72.43	72.75	*	67.57	64.85	59.85	56.45	60.45	65.14	59.90	65.48	66.12
13	72.26	74.30	73.77	73.29	73.38	72.26	72.59	73.11	70.10	72.86	73.20	72.76	*	65.98	59.34	56.97	60.01	65.52	59.20	65.97	65.04
14	74.32	72.75	73.20	73.64	72.98	72.82	72.52	73.04	70.87	75.67	76.03	71.84	72.40	*	60.87	56.20	58.76	70.57	60.48	77.52	64.86
15	71.36	70.91	71.23	71.32	71.45	71.22	70.60	71.37	71.12	71.68	72.60	70.53	70.52	71.69	*	53.15	57.31	61.86	61.86	60.75	66.75
16	64.82	63.60	64.63	64.68	64.36	64.91	64.67	64.45	64.11	64.71	64.60	64.56	64.60	64.63	64.60	*	53.47	56.07	52.93	56.24	59.68
17	71.03	71.12	71.09	71.18	71.27	71.39	70.69	71.45	69.98	71.22	71.59	70.94	70.90	70.99	70.46	63.49	*	59.85	57.17	58.88	55.47
18	74.33	73.32	73.77	74.49	73.98	73.59	72.78	73.99	71.55	75.37	78.27	72.40	72.97	76.01	72.48	63.27	71.92	*	61.54	70.17	59.41
19	70.90	70.30	70.30	70.69	70.59	70.70	69.96	70.65	73.89	70.76	71.66	69.78	70.01	71.13	70.79	63.33	69.96	71.31	*	60.88	66.14
20	74.19	72.40	73.17	74.21	73.06	72.83	72.23	73.18	71.25	76.08	75.44	71.83	72.45	78.17	71.45	65.11	71.28	74.98	70.51	*	66.98
21	74.66	71.82	72.12	72.42	72.82	72.5	72.35	72.82	71.12	73.96	73.62	72.54	72.38	73.46	71.22	64.8	71.28	73.99	70.96	73.56	*

Table 3. Cellular fatty acid composition of *F. rubi* CRRU44^T and those of the type strains of its closest related type species of the family *Rhizobiaceae* after the 16S rRNA gene and genome analyses. Strains: 1, *F. rubi* CRRU44^T; 2, *E. cereale* RZME27^T; 3, *N. galegae* HAMB1540^T; 4, *P. pelagicum* R1-200B4^T; 5, *Xaviernesmea oryzae* Alt 505^T; 6, *Pararhizobium giardinii* H152^T; 7, *Ensifer adhaerens* Casida A^T; 8; *Sinorhizobium fredii* USDA 205^T., Fatty acids present in amounts lower than 1% in all species are not shown. nd: not detected. Data are from this study and from Menéndez *et al.* [11], Kimes *et al.* [53], Delajudie *et al.* [54] and Yan *et al.* [57]

Fatty acid	1	2	3	4	5	6	7	8
C _{16:0}	8.7	8.9	11.6	7.7	28.7	6.6	2.9	10.0
C _{17:0}	0.5	0.1	1.9	nd	nd	nd	nd	0.5
C _{18:0}	1.8	2.1	2.3	2.3	2.3	0.6	4.0	5.0
C _{16:0} 3OH	1.8	2.1	2.5	nd	nd	nd	1.7	0.6
C _{18:0} 3OH	0.5	1.3	1.6	1.5	nd	2.3	2.5	2.0
C _{17:0} cyclo	nd	nd	nd	nd	3.8	nd	nd	1.0
C _{18:1} ω7c 11-methyl	20.9	0.4	0.5	3.0	9.5	nd	2.1	0.2
C _{19:0} cyclo ω8c	nd	nd	10.8	4.4	nd	nd	0.4	15.0
Summed feature 2	1.2	5.2	4.6	4.4	6.3	8.8	6.0	6.9
Summed feature 3	5.5	1.8	0.6	1.5	nd	6.9	0.9	0.8
Summed feature 8	53.4	77.7	61.1	73.8	32.9	74.9	79.3	54.9

Summed feature 2: (C_{14:0} 3OH/ C_{16:1} iso I)

Summed feature 3: (C_{16:1} ω7c / C_{16:1} ω6c)

Summed feature 8: (C_{18:1} ω7c/ C_{18:1} ω6c)

Table 2. Phenotypic differences between *Ferranicluiabacter rubi* CRRU44^T and the type strains of its closely related type species of the family *Rhizobiaceae* after the 16S rRNA gene and genome analyses. Strains: 1, *F. rubi* CRRU44^T; 2, *E. cereale* RZME27^T; 3, *N. galegae* HAMB1540^T; 4, *P. pelagicum* R1-200B4^T; 5, *X. oryzae* Alt 505^T; 6, *P. giardinii* H152^T; 7, *E. adhaerens* Casida A^T; 8, *S. fredii* USDA 205^T. +: positive, -: negative, w:weak, nd: no data. Data are from this study and from Menéndez *et al.* [11], Kimes *et al.* [53], Delajudie *et al.* [54], and Peng *et al.* [50].

Characteristics	1	2	3	4	5	6	7	8
Colony colour (on YMA)	Ivory	white	white-pink	white	cream	white-cream	white	white-cream
Nitrate reduction	-	+	-	+	nd	-	+	nd
Growth in 1% NaCl	+	+	+	+	+	+	+	+
Growth in 7% NaCl	-	-	-	+	-	-	-	-
Growth at 37°C	+	w	+	+	+	w	+	-
Growth at pH 5	+	-	+	-	+			+
Assimilation of:								
Gluconate	-	+	+	+	+	-	-	-
Malate	+	+	+	-	+	+	+	+
L-rhamnose	+	+	-	+	+	+	+	+
Inositol	+	+	+	-	+	+	+	+
Melibiose	+	+	+	-	+	+	+	+
5-keto-gluconate	-	+	+	+	nd	-	-	-
Propionate	-	+	-	-	nd	-	+	-
3-hydroxybutyrate	-	-	-	-	+	-	-	-
L-histidine	-	+	+	-	+	+	+	+
L-alanine	-	w	+	w	+	-	+	-
L-serine	-	-	-	+	+	-	+	-

***Ferranicluibacter rubi* gen. nov., sp. nov., a new member of family *Rhizobiaceae* isolated from stems of elmleaf blackberry (*Rubus ulmifolius* Schott) in Northwest Spain.**

1.1 Author names

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1.3 Keywords

Ferranicluibacter, *Ferranicluibacter rubi*, berries, *Rubus*, endophytic, Spain

1.4 Repositories:

BioProject: PRJNA610165 BioSample: SAMN14278655 Genome Accession: JAANCM000000000 16S rRNA gene Accession: MT146893

Table S1. Genome properties of *Ferranicluiibacter rubi* and its close related species after the genome comparison analysis. Strains: 1, *Ferranicluiibacter rubi* CRRU44^T (JAANCM000000000.1); 2, *Xaviernesmea oryzae* 1.7048 (MKIP00000000.1); 3, *Ensifer adhaerens* Casida A^T (CP015880.1); 4, *Sinorhizobium fredii* USDA205^T (WISZ00000000.1); 5, *Pararhizobium giardinii* H152^T (ARBG00000000.1).

Attributes	1	2	3	4	5
Sequence size (bp)	5,477,819	5,391,466	7,267,502	7,152,016	6,810,126
Number of contigs	42	59	3	255	190
GC content (%)	62,83	62,80	62.80	63,00	60,80
Longest contig size	807,428	494,935	-	311,002	709,858
Shortest contig size	408	519	-	1,100	965
N50 value	381,396	350,529	-	74,219	311,841
L50 value	5	7	-	28	8
Total number of genes	5,164	4,777	6,939	6,904	6,287
Total CDSs	5,108	4,717	6,720	6,833	5,983
Number of coding sequences/CDSs (with protein)	4,983	4,628	6,584	6,426	5,929
Pseudogenes	125	89	136	407	304
Number of RNAs (tRNAs, rRNAs, ncRNAs)	56 (48, 4, 4)	60 (51, 5, 4)	83 (64, 15, 4)	71 (52, 15, 4)	54 (46, 4, 4)

Table S2. Pairwise ANI and AAI values between the genomes of *Ferranicluiacter rubi* CRRU44^T and those other type strains from non-type species of the family *Rhizobiaceae* with similarity in the 16S rRNA gene above 96%. Strains: 1, *Ferranicluiacter rubi* CRRU44^T (JAANCM000000000.1); 2, *Endobacterium cereale* RZME27^T (WIXI000000000.1); 3 *Rhizobium altiplani* BR 10423^T (LNCD000000000.1); 4, *Rhizobium azooxidifex* DSM 100211^T (JACIEE000000000.1); 5, *Rhizobium borbori* DSM 26385^T (JACIDU000000000.1); 6, *Ensifer morelense* Lc04^T (JABEKT000000000.1); 7, *Rhizobium soli* AS3.12^T (JACHBU000000000.1); 8, *Rhizobium subbaronis* JC85^T (OBQD000000000.1); 9, *Rhizobium viscosum* DSM 7307^T (JADBEC000000000.1); 10, *Rhizobium halotolerans* AB21^T (CABFWE000000000.1); 11, *Rhizobium esperanzae* CNPSo 668^T (MXPU000000000.1); 12, *Rhizobium flavum* YW14^T (MUXO000000000.1); 13, *Rhizobium changzhiense* WYCCWR 11279^T (JACGBJ000000000.1); 14, *Rhizobium mesosinicum* CCBAU 25010^T (JAEUAK000000000.1); 15 *Pseudorhizobium banfieldiae* NT-26^T (FO082820.1); 16, *Rhizobium grahamii* CCGE 502^T (AEYE000000000.1); 17, *Rhizobium favelukesii* LPU83^T (ATTO000000000.1); 18, *Neorhizobium huautlense* S02^T (PVBM000000000.1); 19, *Rhizobium tibeticum* CF048^T (JUHN000000000.1); 20, *Neorhizobium vignae* CCBAU 05176^T (JNNU000000000.1). Data are in percentages. ANI values are presented on the bottom left and AAI values are presented in the top right.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*	64.89	66.00	67.24	63.75	69.00	65.52	66.76	65.72	65.07	66.31	65.35	66.24	65.49	65.30	65.88	66.28	65.34	66.57	65.29
2	72.49	*	65.99	64.50	64.97	64.39	71.72	63.84	66.42	70.12	66.70	70.16	66.88	65.98	69.81	66.28	66.23	71.27	65.99	71.40
3	72.30	72.59	*	66.85	64.67	66.94	66.57	66.07	76.30	66.61	76.65	67.27	76.34	76.10	66.46	87.80	86.34	67.65	87.28	67.22
4	74.25	72.87	73.08	*	64.21	70.12	65.52	81.73	66.91	66.52	67.13	66.88	66.80	66.53	66.94	66.97	67.01	66.43	67.18	65.41
5	72.23	73.05	71.91	72.67	*	63.88	66.37	64.62	64.93	66.55	65.39	66.98	65.05	65.14	66.26	64.74	64.62	65.96	64.61	65.97
6	73.88	72.52	72.56	74.65	71.63	*	65.16	70.08	66.06	65.81	66.57	66.06	66.90	66.29	66.20	67.40	66.80	65.72	67.34	65.52
7	72.57	76.19	72.74	73.11	72.67	72.62	*	65.03	66.53	71.68	66.77	71.63	67.12	66.61	71.63	66.47	65.91	74.47	66.39	75.13
8	73.60	72.66	72.71	80.66	72.62	74.76	72.32	*	65.99	65.78	66.34	67.62	66.27	65.93	66.01	66.30	66.47	66.19	66.25	64.77
9	72.57	73.15	76.86	73.12	72.11	72.73	72.72	72.81	*	66.86	80.14	67.03	80.90	91.08	67.13	76.36	77.06	68.59	76.96	68.10
10	72.52	74.47	72.63	73.19	72.29	72.78	74.27	73.15	72.76	*	66.96	95.85	66.78	67.11	95.75	66.74	66.55	74.17	66.75	73.24
11	73.29	73.84	77.60	74.18	72.63	73.66	73.23	73.65	79.98	72.94	*	67.45	88.39	80.26	67.39	76.54	77.14	68.92	77.10	68.11
12	72.69	74.28	72.70	73.39	72.66	72.67	74.21	74.24	73.08	94.9	73.33	*	66.99	67.03	95.23	67.20	66.76	73.86	66.96	73.44
13	73.31	73.85	77.35	74.02	72.62	73.70	73.38	73.64	80.18	72.85	85.82	72.91	*	80.48	67.08	76.42	77.36	68.98	77.71	68.93
14	72.51	73.10	76.66	73.07	72.15	72.78	72.71	72.73	88.63	72.41	79.45	72.29	79.92	*	67.19	76.28	76.53	68.49	76.98	67.96
15	72.69	74.57	72.64	73.51	72.33	72.84	74.37	72.91	72.99	94.53	73.43	94.58	73.48	72.89	*	66.78	66.59	73.98	66.77	73.49
16	72.19	72.67	84.52	72.82	71.62	72.78	72.15	72.54	76.69	71.98	76.99	72.03	77.16	76.38	72.05	*	86.44	67.98	86.82	67.41
17	72.65	73.16	84.38	73.07	71.85	73.04	72.19	72.68	77.17	72.29	77.33	72.24	77.78	76.76	72.34	83.39	*	67.94	94.37	67.38
18	72.41	75.14	73.17	73.14	72.33	72.53	75.4	72.76	73.52	74.45	73.89	74.57	73.97	73.38	74.32	72.85	73.14	*	67.91	81.76
19	72.54	72.88	84.71	73.01	71.63	72.98	72.25	72.82	76.94	72.12	77.31	72.14	77.67	76.65	72.30	83.59	94.17	72.62	*	67.73
20	72.80	75.69	73.06	73.31	72.51	73.01	76.37	72.87	73.87	74.46	74.20	74.62	74.65	73.76	74.52	72.76	72.99	79.04	73.1	*

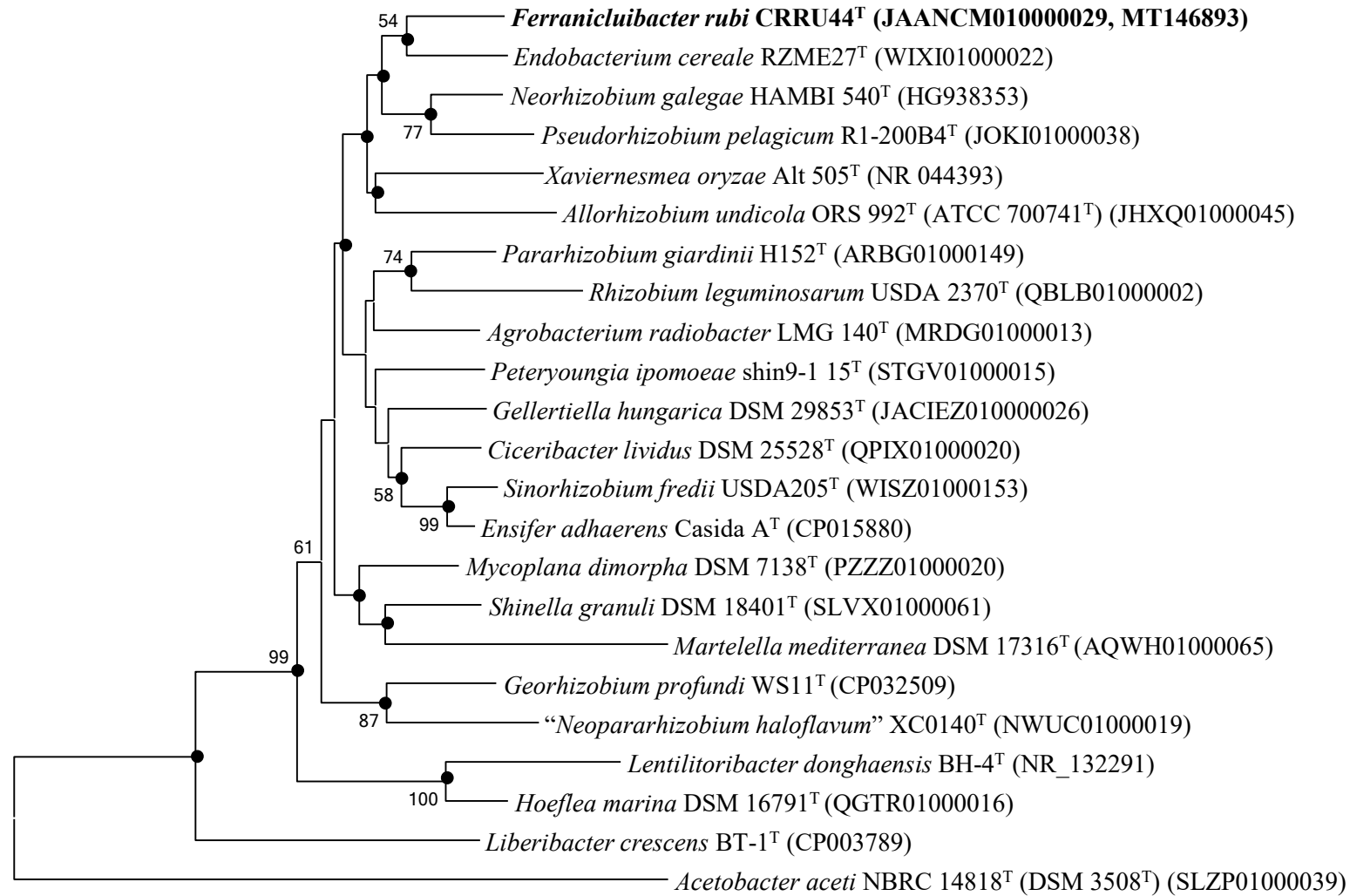
Table S3. Calculated ANIb and dDDH values for the *Ferrancluibacter rubi* CRRU44^T, those of the type strains of other type species of the family *Rhizobiaceae* and other available genomes of related strains. 1, *Ferrancluibacter rubi* CRRU44^T (JAANCM000000000.1); 2, AFS017967 (UBPA00000000.1); 3, Leaf371 (LMQB00000000.1); 4, Leaf383(LMQD00000000.1); 5, 9140 (FJUR00000000.1); 6, PP-CC-3A-592 (QJSR00000000.1); 7, PP-WC-1G-195 (QJSS00000000.1); 8, PP-F2F-G20b (QJSV00000000.1); 9, PP-F2F-G38 (QJTH00000000.1); 10, PP-CC-2G-626 (SLXY00000000.1); 11, PP-WC-2G-219 (SLUV00000000.1); 12, PP-F2F-G36 (SLYE00000000.1); 13, PP-CC-3G-465 (SLYH00000000.1); 14, NFR03 (FOGR00000000.1); 15, *Endobacterium cereale* RZME27^T (WIXI00000000.1); 16, *Neorhizobium galegae* HAMBI540^T (HG938353.1); 17, *Rhizobium leguminosarum* USDA 2370^T (QBLB01000000.1); 18, *Pseudorhizobium pelagicum* R1-200B4^T (JOKJ00000000.1); 19, *Gellertiella hungarica* RAM11^T (JACIEZ00000000.1); 20, *Peteryoungia ipomeae* shin9-1^T (STGV00000000.1); 21, *Ciceribacter lividus* DSM 25528^T (QPIX00000000.1); 22, *Georhizobium profundi* WS11^T (CP032509.1); 23, *Pararhizobium giardinii* H152^T (ARBG00000000.1); 24, *Shinella granuli* DSM 18401^T (SLVX00000000.1); 25, *Allorhizobium undicola* ORS 992^T (JHXQ00000000.1); 26, *Agrobacterium radiobacter* DSM 30147^T (ASXY01000000.1); 27, *Ensifer adhaerens* Casida A^T (CP015880.1); 28, *Hoeflea marina* DSM 16791^T (QGTR00000000.1); 29, *Liberibacter crescens* BT-1^T (NC_019907.1); 30 *Martelella mediterranea* DSM 17316^T (AQWH00000000.1); 31, *Mycoplana dimorpha* DSM 7138^T (PZZZ00000000.1); 32, *Paraneorhizobium haloflavum* XC0140^T (NWUC00000000.1); 33, *Sinorhizobium fredii* USDA205^T (WISZ00000000.1); 34, *Xaviernesmea oryzae* 1.7048 (MKIP00000000.1). ANIb values are in the bottom-left and dDDH values in the top-right. Data are in percentages.

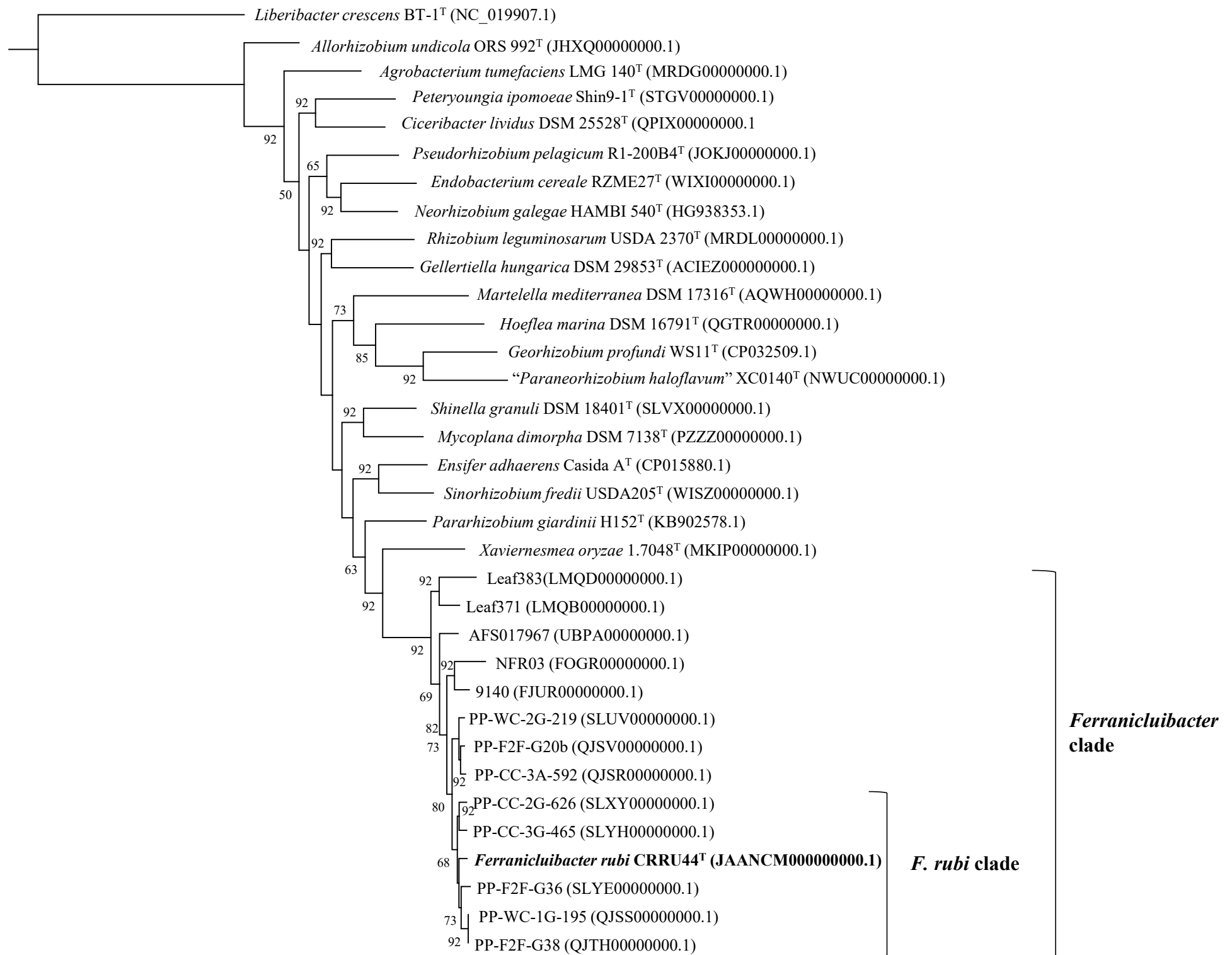
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	*	42.20	31.60	28.20	47.30	59.60	79.70	60.10	79.30	78.30	60.80	75.50	78.60	34.80	23.20	20.70	20.50	20.60	20.30	20.40	20.80	20.10	21.50	21.40	20.00	20.50	21.50	20.10	44.30	19.60	20.80	19.00	21.10	21.60
2	90.40	*	31.30	28.20	37.60	42.10	42.10	42.00	42.30	42.10	42.50	42.10	42.10	32.80	22.70	20.70	20.60	20.60	20.70	20.50	16.00	14.20	21.70	21.40	19.90	20.50	21.60	19.70	43.10	19.80	21.20	19.60	21.40	21.80
3	85.81	85.77	*	31.50	30.40	31.50	31.40	31.70	31.50	31.70	31.90	31.40	31.60	29.20	22.60	20.80	20.80	20.60	20.70	20.70	15.60	14.00	21.80	21.40	20.10	20.60	21.70	20.00	43.60	19.70	21.30	19.70	21.30	21.60
4	83.89	83.99	86.15	*	27.50	28.20	28.10	28.30	28.10	28.10	28.50	28.20	28.10	26.80	22.40	20.60	20.50	20.60	20.50	20.30	20.50	20.10	22.00	21.20	19.90	20.30	21.40	19.90	41.20	19.70	21.20	19.60	21.10	21.60
5	91.44	88.61	84.99	83.18	*	47.80	47.30	47.70	47.40	47.40	47.90	47.50	47.20	36.90	24.10	20.70	20.60	20.40	21.40	20.70	20.80	20.10	22.20	22.30	20.10	20.60	21.30	20.10	41.80	20.10	21.10	19.70	21.20	21.90
6	94.51	90.30	85.72	83.75	91.74	*	59.90	84.60	60.30	60.00	84.00	60.00	60.10	34.60	22.80	20.50	20.30	20.60	20.40	20.50	15.50	14.20	21.50	21.20	19.90	20.60	21.70	20.10	44.10	20.00	21.10	19.20	21.30	21.70
7	96.80	89.77	85.21	83.33	91.00	93.85	*	61.40	98.30	80.20	62.50	77.20	80.70	34.50	23.20	20.50	20.50	20.40	20.60	20.70	15.30	14.00	21.30	21.30	20.00	20.40	21.30	20.20	44.00	19.90	20.90	19.30	21.20	21.80
8	94.09	89.90	85.51	83.51	91.18	97.47	94.63	*	62.00	61.60	84.50	61.70	61.70	34.80	23.20	20.80	20.50	20.50	20.50	20.40	15.30	14.00	21.50	21.30	20.00	20.70	21.40	20.30	44.10	19.70	21.00	19.30	21.30	21.80
9	97.08	90.00	85.36	83.46	91.15	94.14	99.56	94.83	*	79.90	63.10	76.60	79.70	34.60	23.20	20.50	20.50	20.40	20.50	20.60	15.30	14.00	21.40	21.30	19.80	20.50	21.30	20.20	44.00	19.80	20.80	19.20	21.30	21.50
10	96.77	89.88	85.41	83.42	90.93	93.99	97.45	94.51	97.43	*	62.30	76.40	79.80	34.50	23.30	20.60	20.70	20.60	20.30	20.60	15.40	14.00	21.50	21.40	20.10	20.40	21.30	19.90	43.80	19.90	20.90	19.20	21.20	21.50
11	94.26	90.17	85.88	83.97	91.38	97.46	95.03	97.76	95.08	94.94	*	62.70	62.00	35.00	23.20	20.80	20.50	20.80	20.80	20.90	15.50	14.20	21.60	21.50	20.00	20.60	21.40	20.20	43.40	19.90	21.30	19.30	21.30	21.90
12	96.33	89.71	85.22	83.28	90.96	93.86	96.93	94.41	96.76	96.87	93.78	*	76.00	34.70	23.00	20.60	20.50	20.40	20.50	20.60	15.20	13.80	21.60	21.50	20.00	20.30	21.30	19.80	43.80	19.50	21.00	19.70	20.90	21.80
13	96.96	89.94	85.38	83.43	91.06	94.07	97.54	94.64	97.39	97.47	93.92	96.91	*	34.50	23.10	20.60	20.50	20.50	20.60	20.70	15.40	13.90	21.40	21.30	20.60	20.60	21.20	19.90	44.00	19.80	21.00	19.50	21.30	21.70
14	87.15	86.21	84.36	82.53	87.58	87.22	87.06	87.21	87.07	87.06	86.90	87.22	87.08	*	23.10	20.60	20.30	20.30	20.20	20.40	14.60	13.70	21.50	21.30	19.90	21.10	21.10	19.90	43.00	20.00	21.00	19.20	21.10	21.20
15	72.49	72.60	72.54	72.12	72.44	72.54	72.48	72.53	72.48	72.43	72.53	72.51	72.42	72.11	*	24.90	23.80	24.40	22.70	24.10	23.90	21.10	23.20	22.90	22.50	23.50	23.60	20.80	3.70	21.60	22.90	19.20	22.60	21.90
16	73.02	73.06	72.96	72.47	72.72	72.90	72.84	72.87	72.86	72.85	72.79	72.87	72.82	72.54	75.61	*	21.40	20.70	20.40	20.30	20.60	20.30	20.90	20.80	20.00	20.50	20.80	19.50	47.50	19.40	20.80	19.60	20.70	20.80
17	72.95	72.99	73.14	72.50	72.87	73.09	72.93	73.10	72.99	72.86	72.88	72.96	72.94	72.54	73.34	74.49	*	20.20	20.50	20.20	20.50	19.90	21.50	21.00	19.70	20.20	21.30	19.40	48.80	19.70	20.80	19.90	21.00	20.80
18	73.34	73.46	73.20	72.98	73.09	73.35	73.33	73.31	73.32	73.34	73.53	73.33	73.32	72.90	75.04	75.90	73.89	*	20.00	20.50	20.50	20.00	20.40	20.60	20.00	20.90	20.50	19.20	33.40	19.90	20.50	18.90	20.90	20.70
19	72.86	73.05	72.93	72.57	72.67	72.81	72.86	72.86	72.81	72.70	72.97	72.83	72.84	72.46	72.78	73.05	73.47	72.99	*	20.20	20.50	20.40	20.50	21.00	20.50	20.50	21.00	19.40	44.60	19.70	20.70	20.50	21.00	21.10
20	72.47	72.75	72.78	72.32	72.56	72.57	72.52	72.60	72.53	72.54	72.56	72.51	72.48	72.29	73.29	72.91	73.15	73.13	72.50	*	20.60	19.80	20.50	20.50	21.20	20.30	20.90	19.80	45.50	20.10	20.60	18.80	20.80	21.00
21	73.31	73.50	73.40	73.07	73.07	73.26	73.27	73.29	73.24	73.27	73.24	73.23	73.26	72.81	73.82	74.08	74.16	74.19	73.33	74.11	*	20.20	20.30	21.00	20.90	20.60	21.20	19.40	46.80	19.80	21.20	18.70	21.00	21.20
22	71.27	71.37	71.46	71.07	71.11	71.42	71.27	71.39	71.23	71.31	71.50	71.32	71.33	70.95	70.91	70.90	71.03	71.04	70.95	70.58	71.03	*	19.70	20.20	19.60	20.20	21.00	19.80	41.50	20.10	21.00	19.80	20.90	20.40

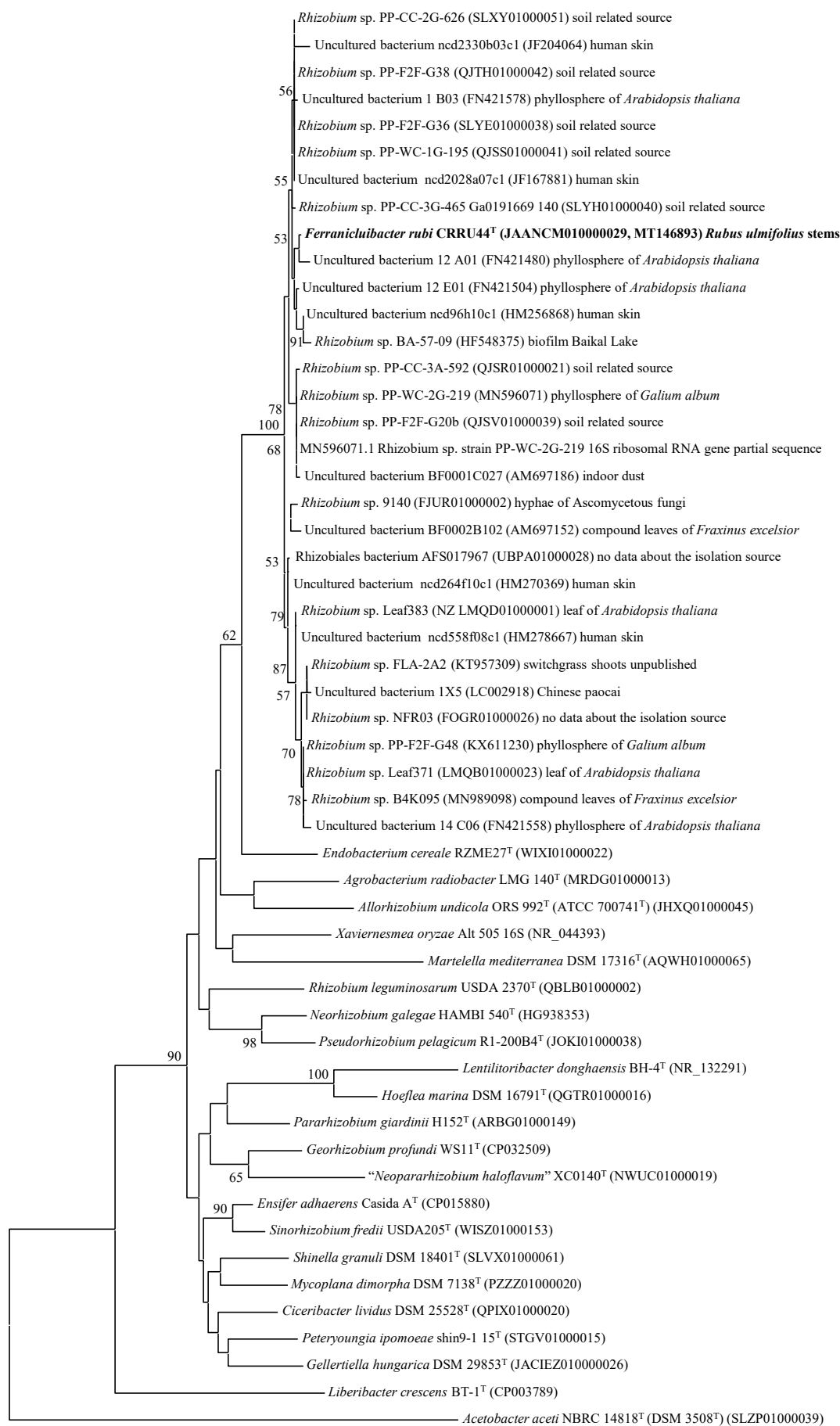
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24	74.17	74.43	74.34	74.45	74.35	74.28	74.23	74.34	74.20	74.11	74.30	74.09	74.16	73.55	73.14	73.36	74.18	73.39	73.55	72.68	73.70	71.08	75.25	*	20.50	20.60	22.30	19.90	45.60	19.90	23.00	19.50	22.10	21.80
25	72.28	72.35	72.32	71.84	72.26	72.29	72.27	72.25	72.25	72.20	72.37	72.18	72.18	71.88	72.97	72.70	72.81	72.64	72.50	73.23	73.27	69.94	72.43	72.75	*	20.80	20.40	46.30	19.70	19.70	21.20	19.20	20.30	21.00
26	72.26	72.58	72.54	72.06	72.31	72.37	72.28	72.40	72.30	72.34	72.50	72.30	72.30	72.14	74.30	73.77	73.29	73.38	72.26	72.59	73.11	70.10	72.86	73.20	72.76	*	20.70	19.10	42.60	19.70	21.20	19.20	20.40	20.80
27	74.32	74.41	74.36	74.37	74.29	74.31	74.21	74.24	74.26	74.27	74.44	74.31	74.25	73.76	72.75	73.20	73.64	72.98	72.82	72.52	73.04	70.87	75.67	76.03	71.84	72.40	*	20.50	45.50	19.60	20.50	19.60	23.70	21.60
28	71.36	71.72	71.68	71.31	71.27	71.45	71.37	71.50	71.37	71.38	71.52	71.42	71.34	71.05	70.91	71.23	71.32	71.45	71.22	70.60	71.37	71.12	71.68	72.60	70.53	70.52	71.69	*	41.40	20.20	22.10	19.90	20.10	20.50
29	64.82	64.95	64.89	64.75	65.05	65.14	64.94	65.30	64.93	64.79	65.18	64.93	65.02	64.69	63.60	64.63	64.68	64.36	64.91	64.67	64.45	64.11	64.71	64.60	64.56	64.60	64.63	64.60	*	44.60	45.50	41.50	47.70	48.10
30	71.03	71.25	71.24	71.02	70.95	71.05	70.99	71.00	71.01	70.90	71.29	70.96	70.98	70.73	71.12	71.09	71.18	71.27	71.39	70.69	71.45	69.98	71.22	71.59	70.94	70.90	70.99	70.46	63.49	*	20.30	19.80	20.30	20.50
31	74.33	74.79	74.70	74.15	74.28	74.55	74.43	74.46	74.41	74.43	74.52	74.40	74.36	73.91	73.32	73.77	74.49	73.98	73.59	72.78	73.99	71.55	75.37	78.27	72.40	72.97	76.01	72.48	63.27	71.92	*	19.60	22.20	21.40
32	70.90	71.03	71.13	70.71	70.82	71.02	70.86	70.87	70.89	70.96	71.04	70.86	70.85	70.52	70.30	70.30	70.69	70.59	70.70	69.96	70.65	73.89	70.76	71.66	69.78	70.01	71.13	70.79	63.33	69.96	71.31	*	20.20	20.10
33	74.19	74.49	74.42	74.28	74.34	74.23	74.15	74.22	74.18	74.11	74.24	74.07	74.14	73.74	72.40	73.17	74.21	73.06	72.83	72.23	73.18	71.25	76.08	75.44	71.83	72.45	78.17	71.45	65.11	71.28	74.98	70.51	*	21.40
34	*	42.20	31.60	74.34	74.71	59.60	79.70	60.10	79.30	78.30	60.80	75.50	78.60	34.80	23.20	20.70	20.50	20.60	20.30	20.40	20.80	20.10	21.50	21.40	20.00	20.50	21.50	20.10	44.30	19.60	20.80	19.00	21.10	21.60

Table S4. Calculated AAI values for the *Ferranichuibacter rubi* CRRU44^T, those of the other available genomes of close related strains. 1, *Ferranichuibacter rubi* CRRU44^T (JAANCM000000000.1); 2, AFS017967 (UBPA00000000.1); 3, Leaf371 (LMQB00000000.1); 4, Leaf383 (LMQD00000000.1); 5, 9140 (FJUR00000000.1); 6, PP-CC-3A-592 (QJSR00000000.1); 7, PP-WC-1G-195 (QJSS00000000.1); 8, PP-F2F-G20b (QJSV00000000.1); 9, PP-F2F-G38 (QJTH00000000.1); 10, PP-CC-2G-626 (SLXY00000000.1); 11, PP-WC-2G-219 (SLUV00000000.1); 12, PP-F2F-G36 (SLYE00000000.1); 13, PP-CC-3G-465 (SLYH00000000.1); 14, NFR03 (FOGR00000000.1). Data are in percentages.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	100													
2	93.68	100												
3	89.61	89.46	100											
4	88.09	88.58	90.47	100										
5	93.89	91.90	87.80	88.83	100									
6	96.20	93.65	89.83	88.38	94.40	100								
7	98.13	93.57	89.38	88.49	94.25	96.31	100							
8	96.57	93.49	89.70	88.44	93.99	98.37	96.60	100						
9	98.14	93.58	89.40	88.49	93.98	96.40	99.53	96.72	100					
10	97.81	93.47	89.49	88.18	93.58	96.32	98.17	96.56	98.15	100				
11	95.96	92.90	88.83	87.88	93.31	97.90	96.49	98.03	96.49	96.30	100			
12	97.95	93.51	89.33	88.44	94.24	96.28	98.21	96.61	97.93	97.81	96.46	100		
13	98.13	93.40	89.24	88.12	93.83	96.40	98.33	96.67	98.32	98.07	96.08	97.84	100	
14	91.27	90.72	88.98	88.02	91.17	91.74	91.35	91.61	91.16	91.02	90.82	91.33	90.99	100







Genus *Ferranicleuibacter*

Figure S1. Neighbour joining phylogenetic rooted tree based on 16S rRNA gene sequences (1310 nt from the end of the insert present in *Gellertiella hungarica*) showing the phylogenetic position of *F. rubi* CRRU44^T and other related uncultured bacteria and isolated strains whose 16S rRNA gene sequences are available in Genbank. Bootstrap values calculated for 1000 replications are indicated. Bar, 5 nt substitution per 1000 nt. Accession numbers from Genbank are given in brackets.