INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Pulido-Suárez *et al.*, *Int. J. Syst. Evol. Microbiol.* 2026;76:007059

DOI 10.1099/ijsem.0.007059

MICROBIOLOGY SOCIETY

Bradyrhizobium nivariense sp. nov., predominant species nodulating *Cytisus supranubius* in the high mountain ecosystem of Tenerife (Canary Islands)

Laura Pulido-Suárez[1,*,†], José David Flores-Félix[2,3,†], Sara Torronteras-Hernández[1], José M. Igual[4,5], Álvaro Peix[4,5], Encarna Velázquez[2,3,5] and Milagros León-Barrios[1]

Abstract

Bradyrhizobial strains nodulate the native legume *Cytisus supranubius* (*Genisteae*) in its natural habitat: the high-mountain ecosystem of Tenerife (Canary Islands), where nitrogen fixation is an essential activity in its N-poor soils. Three of these strains, SSUT18^T, SSUT77 and SSUT112, are representative of a major phylotype within the genus *Bradyrhizobium,* and the phylogenetic analysis of their 16S rRNA gene sequences showed that *Bradyrhizobium canariense* is their closest relative. Nevertheless, the multilocus sequence analysis and the genome analysis distinguished these strains from the type strain of *B. canariense* and from the remaining species of the genus *Bradyrhizobium*. The calculated Average Nucleotide Identity (blast) (ANIb) and digital DNA–DNA hybridization values between the genomes of the strains from the new species and *B. canariense* were also lower than the threshold values proposed for species differentiation. These data, together with differences observed in chemotaxonomic and phenotypic characteristics, support the classification of the strains SSUT18^T, SSUT77 and SSUT112 into a new species of *Bradyrhizobium*, for which we propose the name *Bradyrhizobium nivariense* sp. nov. (type strain SSUT18^T=CECT 30998^T=LMG 33353^T).

INTRODUCTION

The *Genisteae* tribe comprises a large group of mostly shrub legumes. This tribe shows the greatest diversity in the Mediterranean region [1], and it is also well represented in the Canary Islands (Spain), where it occupies a wide diversity of environments [2]. Studies on the diversity of rhizobia nodulating genistoid legumes in these islands have so far been limited to two herbaceous species of the genus *Lupinus* [3] and a few of the woody endemic species of the genera *Chamaecytisus*, *Teline* and *Adenocarpus* [4–6]. These previous studies have shown that these legumes can be nodulated by diverse strains of the genus *Bradyrhizobium*, predominantly *Bradyrhizobium canariense*, along with other, less predominant genotypes corresponding to *Bradyrhizobium japonicum* and two phylogenetic lineages closely related to this species [4, 6].

Within genistoid legumes, *Cytisus supranubius* (syn. *Spartocytisus supranubius*, 'Teide broom') is the most representative species in the high mountain ecosystem of Tenerife, Canary Islands, with most populations growing in the Teide National Park (TNP). Through the nitrogen-fixing symbiosis with rhizobia, this legume is the main input of nitrogen (N) in the otherwise N-poor

Author affiliations: [1]Departamento de Bioquímica, Microbiología, Biología Celular y Genética, Universidad de La Laguna, Tenerife, Spain; [2]Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain; [3]Instituto de Investigación en Agrobiotecnología (CIALE), Universidad de Salamanca, Salamanca, Spain; [4]Unidad Asociada Grupo de Interacción Planta-Microorganismo, Universidad de Salamanca-IRNASA-CSIC, Salamanca, Spain; [5]IRNASA-CSIC, Salamanca, Spain.

***Correspondence:** Laura Pulido-Suárez, slaurama@ull.edu.es

Keywords: *Bradyrhizobium*; *Cytisus supranubius*; legume root-nodule endophyte.

Abbreviations: ANI, Average Nucleotide Identity; BLAST, Basic Local Alignment Search Tool; COGs, Clusters of Orthologous Genes; dDDH, digital DNA–DNA hybridization; GBDP, Genome-BLAST Distance Phylogeny; GTDB, Genome Taxonomy Database; MLSA, Multilocus Sequence Analysis; NCBI, National Center for Biotechnology Information; TNP, Teide National Park; YMA, yeast mannitol agar.

The GenBank accession numbers for the genome sequences of strains *Bradyrhizobium nivariense* SSUT18^T, *B. nivariense* SSUT77 and *B. nivariense* SSUT112 are, respectively, NZ_JARWCR000000000, NZ_JARVWW010000000 and NZ_JARVWX010000000. The 16S rRNA gene sequences were deposited with accession numbers MT186800, OP976367 and OP976362. Accession numbers for the housekeeping genes are MT210564, OQ032979 and OQ032989 for *recA*; MT210556, OQ033003 and OQ033022 for *glnII*, and OQ266786, OQ266784 and NZ_JARVWX010000094 for *atpD*. Accession numbers for *nodC* are MT321501, OR209139 and OR209140. Accession numbers for *nodA* are NZ_JARWCR010000065, NZ_JARVWW010000078 and NZ_JARVWX010000129. For *nifH*, accession numbers are NZ_JARWCR010000003, NZ_JARVWW010000041 and NZ_JARVWX010000071.

†These authors contributed equally to this work

Eight supplementary figures and two supplementary tables are available with the online version of this article.

007059 © 2026 The Authors

soils of the TNP [7]. However, despite the importance of this symbiosis, there are few data about the *C. supranubius* nodulating rhizobia.

In a previous study, Jarabo-Lorenzo *et al*. [4] characterized five bradyrhizobial strains isolated from *C. supranubius* root nodules through 16S-RFPL profiling, showing that these strains belonged to a ribotype which was later described as *B. canariense* [4, 5]. More recent studies of a broader sample of *C. supranubius* root nodule isolates indicated a greater diversity of bradyrhizobia nodulating this genistoid legume [8, 9]. These studies showed that they belong to both the *B. japonicum* (superclade I) and *Bradyrhizobium elkanii* superclades defined by Ormeño-Orillo and Martinez-Romero [10], although most of them grouped within the superclade I, with *B. canariense* being their closest species [8, 9]. However, these strains have 16S rRNA, *recA* and *glnII* gene sequences divergent from those of *B. canariense* [8, 9], suggesting that they may represent a new species. We present here a phylogenetic, genomic, chemotaxonomic and phenotypic characterization of the representative strains of this group and propose their classification as a novel species, *Bradyrhizobium nivariense* sp. nov., with type strain SSUT18^T (CECT 30998^T=LMG 33353^T).

ISOLATION AND ECOLOGY

The strains included in the present study, SSUT18^T, SSUT77 and SSUT112, were isolated in a previous work [8, 9] from effective (reddish) root nodules of *C. supranubius* in the northeastern area of the TNP (28°17′ 15.90″ N; 16°30′ 40.54″ W, at 2,180 m a.s.l.) following standard protocols [11]. Previous studies have confirmed the reinfection capacity and symbiotic efficiency of the type strain SSUT18^T in its host legume [8, 12].

PHYLOGENETIC ANALYSES

In the previous work, it was shown that the analysed strains belong to the *Bradyrhizobium* superclade I [8]. In the present study, we have updated the 16S rRNA gene phylogeny, given that since our previous publication, several *Bradyrhizobium* species belonging to this superclade have been described. Results confirmed that the strains SSUT18^T, SSUT77 and SSUT112 nodulating *C. supranubius*, which have identical 16S rRNA gene sequences, formed a cluster with the type strain of *B. canariense*, divergent from the remaining *Bradyrhizobium* species within the superclade I (Figs 1 and S1, available in the online Supplementary Material).

To confirm the phylogeny based on the 16S rRNA gene, we performed a multilocus sequence analysis (MLSA) with concatenated partial sequences of *atpD, recA* and *glnII,* three of the housekeeping genes most used to analyse the phylogenetic relationships of species from the genus *Bradyrhizobium* [13]. In agreement with the 16S rRNA phylogenetic analysis, the MLSA also grouped the three strains included in this study in a cluster separated from other *Bradyrhizobium* type strains from clade I, with also *B. canariense* being their closest relative (Fig. 2).

GENOMIC ANALYSES

The genomic DNA of strains SSUT18^T, SSUT77 and SSUT112 was extracted and sequenced at MicrobesNG (Birmingham, UK) using Illumina MiSeq and paired-end libraries (2×250 bp). Raw reads were trimmed using Trimmomatic [14], and read quality was assessed using in-house scripts in SAMtools [15]. Sequences were then assembled with Unicycler [16], and annotation was performed with RAST 2.0 (Rapid Annotation using Subsystem Technology, https://rast.nmpdr.org/rast.cgi)[17] and PGAP (National Center for Biotechnology Information -NCBI- Prokaryotic Genome Annotation Pipeline, https://www.ncbi.nlm.nih.gov/refseq/annotation_prok/[18, 19]. Plasmid detection has been handled by PlasmidFinder [20] and VirSorter2 [21] combined with manual mining. Also, the quality and contamination level of genomes have been evaluated employing CheckM [22]. The draft genome sequences of strains SSUT18^T, SSUT77 and SSUT112 were deposited in DDBJ/EMBL/GenBank under the accession numbers JARWCR000000000, JARVWW010000000 and JARVWX010000000 for strains SSUT18^T, SSUT77 and SSUT112, respectively. The genome features of these strains are summarized in Table 1.

Strains belonging to the superclade I, as defined by the 16S rRNA phylogeny, were selected for genome-based phylogenetic analysis, and their corresponding reference genome sequences were retrieved from the NCBI Genome database (https://www.ncbi.nlm.nih.gov/genome/). Phylogenomic analyses were performed through the Type Strain Genome Server, which uses the Genome-BLAST (Basic Local Alignment Search Tool) Distance Phylogeny method (GBDP) to compare the nucleotides of whole-genomic sequences [23, 24]. The results of this analysis showed that the strains of *B. nivariense* clustered with the type strain of *B. canariense*, although they clearly distinguished both species (Fig. 3).

We compared the genomes of the *B. nivariense* strains with those of the Genome Taxonomy Database (GTDB) using the GTDB-Tk software toolkit [25]. The obtained results showed that *B. nivariense* strains are related to a group of *Bradyrhizobium* strains which contains the type strain of *B. canariense* BTA-1^T (accession number GCF_019402665.1) in agreement with the results of the phylogenetic analyses (Fig. S2).

To confirm that the strains SSUT18^T, SSUT77 and SSUT112 belong to a new *Bradyrhizobium* species, digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) were calculated using, respectively, the genome-to-genome distance

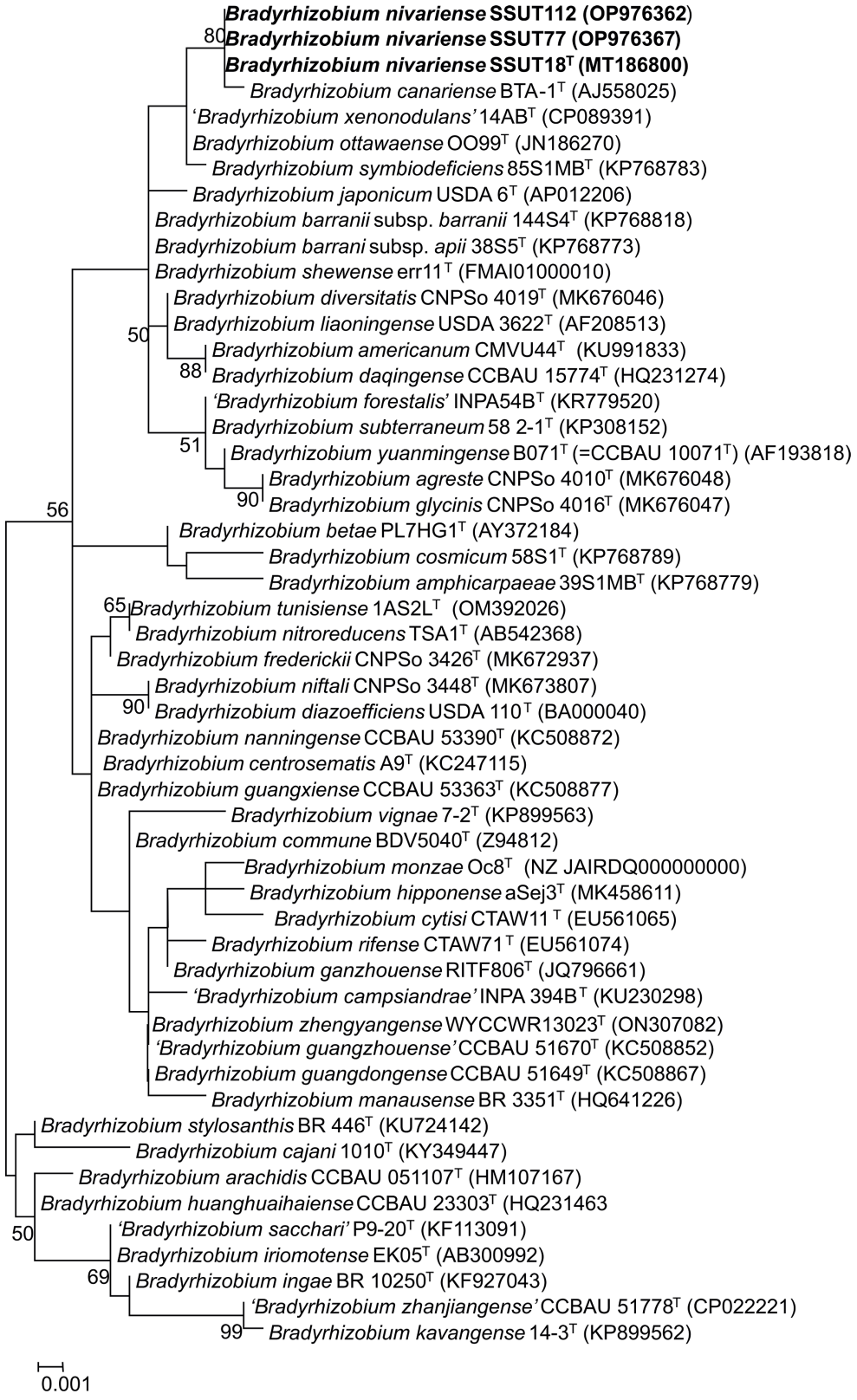


Fig. 1. Maximum-likelihood phylogenetic tree based on the 16S rRNA gene sequences (1,288 bp) showing the relationship between *Bradyrhizobium nivariense* strains and those of the *Bradyrhizobium* species from the *Bradyrhizobium* superclade I. Only bootstrap values >50% (500 replicates) are shown. Accession numbers are shown in parentheses.

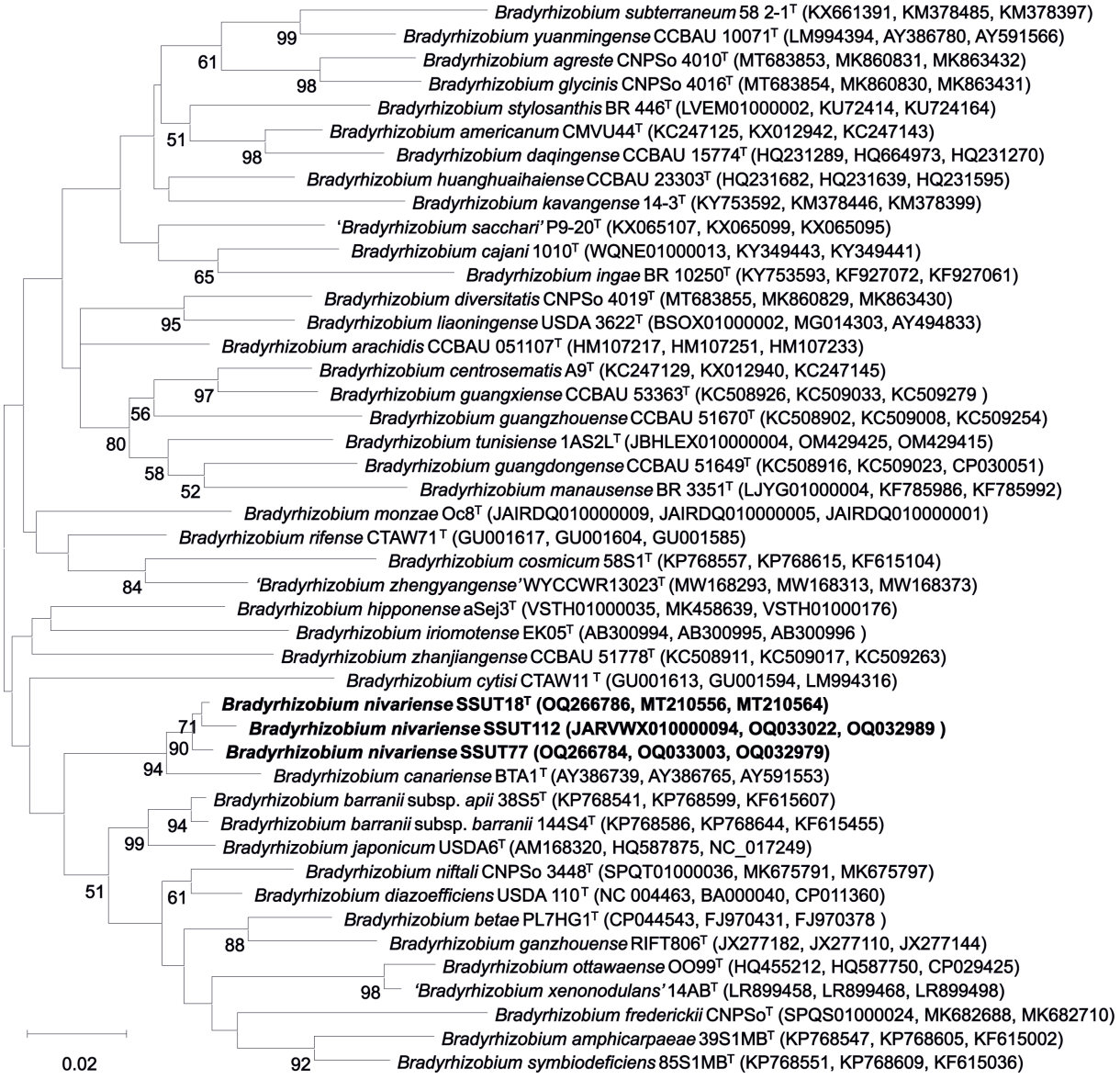


Fig. 2. Maximum-likelihood MLSA based on partial concatenated *atpD* (387 bp), *glnII* (426 bp) and *recA* (345 bp) gene sequences showing the relationships of strains from *Bradyrhizobium nivariense* and those of the *Bradyrhizobium* species from the superclade I. Only bootstrap values of 50% (500 replicates) are shown. Accession numbers are indicated in parentheses.

calculator online service from DSMZ, https://tygs.dsmz.de/ [23] and the JSpecies web service, https://jspecies.ribohost.com/jspeciesws/ [26]. For these analyses, the closest related species in the phylogram based on whole-genome analysis were selected. According to the current taxonomic thresholds for the definition of novel prokaryotic species, strains sharing >95–96% ANIb values and >70% dDDH correspond to the same species [13, 27]. The results, presented in Table 2, show that *B. nivariense* strains SSUT18^T, SSUT77 and SSUT112 share over 97% ANIb values among them, indicating that they are strains of the same species, whereas ANIb values with their closest bradyrhizobial strains are below 90%, lower than the accepted threshold. Similarly, the dDDH values with the type strains of described species are in all cases below 45%, whereas our strains share over 80%. All these values strongly support that these three strains belong to a novel species of the genus *Bradyrhizobium*.

The circular genome map and the schematic map of the BLAST comparison of the genomes of *B. nivariense* strains with that of the closest type strain, BTA-1^T, shown in Fig. S3, were generated using CGView, https://cgview.ca/ [28, 29]. Concerning the presence of plasmids in the genomes of the *B. nivariense* strains, the application of specialized plasmid detection tools did not yield conclusive results. Nevertheless, it is possible that these strains carry one or more plasmids, because their genomes contain a complete *repABC* operon and several additional repA units, which are essential for plasmid segregation and replication [30].

Table 1. Genome features of the strains from *B. nivariense*

Strains: 1, SSUT18^T (JARWCR000000000); 2, SSUT77 (JARVWW010000000); 3, SSUT112 (JARVWX010000000).

Attribute	1	2	3
Sequence size (bp)	9,582,934	8,989,714	9,741,229
Number of contigs	226	146	283
G+C content (%)	63.07	63.34	63.12
N50 value	95,410	212,706	173,804
L50 value	28	12	17
Total number of genes	9,197	8,617	9,377
Total CDSs	9,135	8,562	9,321
Number of coding sequences/CDSs	8,725	8,277	8,863
Pseudogenes	410	285	458
Number of RNAs (tRNAs, rRNAs, ncRNAs)	62 (55,3,4)	55 (48,3,4)	56 (49,3,4)
Coverage	67×	33×	48×
Completeness	99.53	99.87	99.53
Contamination	2.28	1.35	1.27

CDSs, Coding DNA sequences.

The comparative pangenome analyses of orthologous clusters and of gene ontology among predicted protein-coding genes were performed with the OrthoVenn2 webserver, https://orthovenn2.bioinfotoolkits.net/home [31]. The pangenome of *B. nivariense* SSUT18^T, SSUT77, SSUT112 and *B. canariense* BTA-1^T revealed a core genome (Fig. S4A) of 5,677 clusters of orthologous genes (COGs) between the four strains, 1,671 being exclusive to the three *B. nivariense* strains. The Venn diagram (Fig. S4B) highlighted intraspecific variability within *B. nivariense*. Fifty-two orthologous genes are solely present in SSUT18^T, 49 in SSUT112 and only 15 in SSUT77. At the same time, SSUT18 ^{T} and SSUT77 share 195 clusters, SSUT18 and SSUT112 share 501 and SSUT77 and SSUT112 share 614 orthologous. Outside the core genome, *B. canariense* BTA-1^T and SSUT18^T share 91 COGs, whereas SSUT77 and SSUT112 share 41 and 72 with BTA-1T, respectively.

The function-based comparison between the three *B. nivariense* strains and *B. canariense* BTA-1^T analysis performed with RAST (Fig. S5A) showed differences in some subsystems between the genomes of *B. nivariense* and *B. canariense* BTA-1^T. For example, in those involved in sulphur metabolism (organic sulphur assimilation), stress response, resistance to some toxic elements (e.g. Co-Zn and Cd resistance) and the nitrogen cycle. *B. nivariense* strains SSUT18^T, SSUT77 and SSUT112 have two ATP-Binding Cassete (ABC)-type transport systems related to organic sulphur assimilation absent in *B. canariense* BTA-1^T. This could be related to the isolation source of these strains since *C. supranubius* grows on naturally sulphur-rich volcanic soils. A glucan biosynthesis precursor involved in osmotic stress is also absent in the strain BTA-1^1 but present in the *B. nivariense* strains. Also, three proteins related to heavy metal resistance present in *B. canariense* BTA-1^T were not found in the genomes of *B. nivariense* strains. Finally, differences were also detected in the nitrogen cycle (Fig. S5B) since several genes involved in the nitrogen cycle, such as *napAB*, *nirK*, *norBC* and *nirK*, were not found in *B. nivariense* strains. Nevertheless, genes involved in nitrogen fixation, such as *nifHDK*, are found in the genomes of both *B. nivariense* and *B. canariense*. In any case, further studies are necessary to confirm the results of the functional analysis carried out with RAST. Also found in the analysis were the *nodABC* genes, involved in the infection process. A previous study [8] showed, based on *nodC* gene analysis (the most widely used marker for *Bradyrhizobium* symbiovar differentiation), that *B. nivariense* strains SSUT18^T, SSUT77 and SSUT112 belong to the symbiovar genistearum (Fig. S6). In the present study, the analysis of the *nodA* and *nifH* genes showed that the three strains clustered with strains belonging to the symbiovar genistearum (Figs S7 and S8).

CHEMOTAXONOMIC AND PHENOTYPIC CHARACTERIZATION

For the fatty acid analysis, strains were grown as described in Pulido-Suárez *et al.* [32]. Whole-cell fatty acids of strain SSUT18^T were extracted and analysed according to the MIDI protocol [33]. The profiles were obtained with MIDI Sherlock 6.1 and the library RTSBA6, following the manufacturer's instructions. The type strain of *B. canariense* BTA-1^T was included in this analysis as reference. The results showed that the most abundant fatty acids in *B. nivariense* (Table S1) were those in summed feature 8

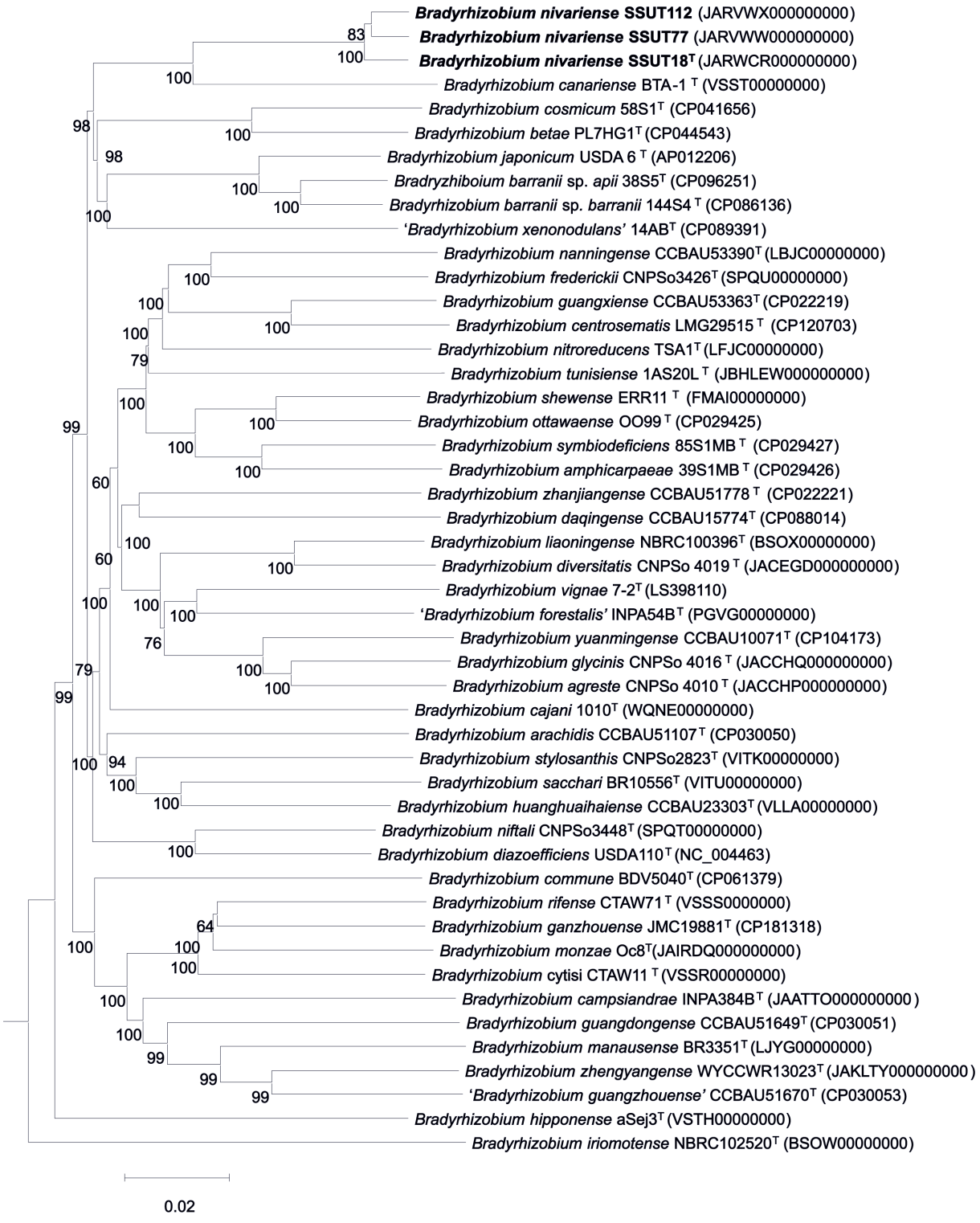


Fig. 3. Whole-genome sequence phylogram based on GBDP distances, showing the relationships of strains from *Bradyrhizobium nivariense* and type strains of the *Bradyrhizobium* superclade I. The numbers at the nodes are GBDP pseudo-bootstrap values >60% from 100 replications. Accession numbers are indicated in parentheses.

Table 2. Percentages of calculated dDDH (top diagonal) and ANIb values (bottom diagonal)

Strains: 1, SSUT18^T; 2, SSUT77; 3, SSUT112; 4, *B. canariense* BTA-1^T; 5, *B. cosmicum* 58S1^T; 6, *B. betae* PL7HG1^T; 7, *B. japonicum* USDA6 ^{T}; 8, *B. barranii* sp. *apii* 38S5^T; 9, *B. barranii* sp. *barranii* 144S4^T; 10, *B. 'xenonodulans'* 14AB^T (CP089391). Values marked in bold are those calculated for the strains of *B. nivariense* sp. nov.

	1	2	3	4	5	6	7	8	9	10
1		**83.50**	**86.00**	44.30	35.0	35.5	36.6	37.0	36.8	35.8
2	**97.29**		**87.10**	44.20	35.0	35.4	36.5	37.1	36.9	36.0
3	**97.13**	**97.29**		44.20	34.9	35.4	36.5	37.3	36.8	35.9
4	89.91	89.65	89.39		34.0	34.4	35.2	35.5	35.2	34.7
5	87.00	86.18	86.03	86.03		54.3	36.4	36.5	36.3	35.6
6	87.08	86.10	85.78	86.01	92.89		37.0	37.0	37.0	36.2
7	86.86	86.68	86.39	86.39	87.78	88.05		60.4	63.6	35.6
8	86.33	86.83	86.52	86.49	87.77	87.99	93.56		70.9	38.3
9	86.11	86.83	86.41	86.41	87.74	87.83	93.92	94.65		38.2
10	87.02	86.67	86.29	86.39	87.31	87.59	88.00	87.94	87.92	

Accession numbers: 1, SSUT18^T (JARWCR00000000); 2, SSUT77 (JARVWW01000000); 3, SSUT112 (JARVWX01000000); 4, *B. canariense* BTA-1^T (VSST00000000); 5, *B. cosmicum* 58S1^T (CP041656); 6, *B. betae* PL7HG1^T (CP04453); 7, *B. japonicum* USDA6^T (AP012206); 8, *B. baranii* sp. *baranii* 144S4^T (CP06136); 9, *B. baranii* sp. *baranii* 144S4^T (CP06136); 10, *B. xenondulans* 14AB^T (CP089391).

(18:1 ω7c, 18:1 ω6c) with 68.7% followed by C16:0 (19.1%), as occurs in other bradyrhizobial species [34]. The major differences with respect to *B. canariense* BTA-1^T were in the amounts of C16:1 ω5*c*, C19:0 cyclo ω8*c* and summed feature 3 (C16:1 *ω7c*/16 and 1 *ω6c*), which were, respectively, 0.7, 5.6 and 3.2% in *B. nivariense* SSUT18^T and 3.5, 2.5 and 1.6% in *B. canarisense* BTA-1^T (Tables 3 and S1).

The cell morphology was observed after Gram-staining performed as described by Doetsch [35]. The temperature range for strain growth was determined by incubating them at 5 °C, 10 °C, 28 °C, 37 °C and 40 °C. Salt tolerance was tested in yeast mannitol agar (YMA) [36] supplemented with 0.5, 1, 1.5, 2 and 2.5% (w/v) NaCl. The pH growth range was determined in YMA at pH 4.5, pH

Table 3. Phenotypic and chemotaxonomic differences between *B. nivariense* SSUT18^T (1) and its closest relative *B. canariense* BTA-1^T (2)

Characteristic	1	2
Growth at:		
pH 4.5	–	+
pH 6	w	+
37 °C	–	w
1% NaCl	–	w
Assimilation as C source:		
D-Galactose	w	+
D-Arabinose	w	+
Gluconate	–	+
Fatty acids:		
C16:1 ω5*c*	0.7	3.5
C19:0 cyclo ω8*c*	5.6	2.5
Summed feature 3 (C16:1 ω7*c*/16:1 ω6*c*)	3.2	1.6

+, positive; –, negative; w, weak. Data are from this study.

6 (adjusted with phosphate citric acid buffer, Na_2HPO_4 0.4 M and citric acid 0.2M), pH 7 (with Na_2HPO_4 0.2 M and NaH_2PO_4 0.2 M) and pH 9 (adjusted with TE buffer 0.2 M). API 20NE galleries were inoculated according to the manufacturer's instructions (BioMérieux, France), and they were incubated for 7 days at 28 °C. The assimilation of some carbon and nitrogen sources was also evaluated in Bergersen minimal medium [37]. D-mannitol, D-glucose, D-arabinose, D-galactose or D-mannose 10 g l^{-1} were added to the medium as the sole carbon source. The use of amino acids as the sole nitrogen source was tested by adding L-proline, L-glycine, L-aspartate, L-serine or L-glutamine to a final concentration of 0.5 g l^{-1}. These results were read after 14 days after inoculation. Oxidase activity was detected with a commercial test strip (Panreac, AppliChem) containing N,N,N′,N′-tetramethyl-1,4-phenylenediamine dihydrochloride. Catalase production was evaluated with 0.3% hydrogen peroxide in one colony taken from fresh YMA cultures. The type strain of *B. canariense* BTA-1^T was included in the phenotypic characterization as a reference. The results of the phenotypic tests showed a similar profile for the three strains of *B. nivariense* (Table S2).

The phenotypic differences between *B. nivariense* and its closest relative *B. canariense* BTA-1^T are recorded in Table 3. The main differences between them were the growth at pH 4.5 and in the assimilation of gluconate, negative in the case of *B. nivariense* and positive in the case of *B. canariense*, as well as in the percentage of the fatty acids C16:1 ω5*c* and C19:0 cyclo ω8*c,* previously described. Also, *B. nivariense* showed weak results in several tests which were positive for *B. canariense* BTA-1^T, such as growth at pH 6 and assimilation of D-galactose and D-arabinose. Growth at 37 °C and 1% NaCl was negative for *B. nivariense* but positive for BTA-1^T.

In summary, the results of this study support classifying strains SSUT18^T, SSUT77 and SSUT112 as a new species of the genus *Bradyrhizobium*, for which the name *B. nivariense* sp. nov. is proposed.

DESCRIPTION OF *BRADYRHIZOBIUM NIVARIENSE* SP. NOV.

Bradyrhizobium nivariense (ni.va.ri.en'se. L. neut. adj. *nivariense*, derived from *Nivaria*, the name ancient Romans gave to the island of Tenerife, from which this bacterium was isolated).

Cells are Gram-staining negative, non-spore-forming, aerobic rods. Colonies on YMA measure 0.5–1 mm after 7 days at 28 °C. Colonies are mucoid and round with entire margins, very mucous and whitish. *B. nivariense* strains grow within a pH range from 6 to 8 with optimal growth at pH 7. The growth at pH 4.5 is negative. They grow at temperatures from 10 °C up to 30 °C, with 28 °C being the optimal temperature. Growth is supported in the presence of up to 0.5% NaCl. Oxidase positive and catalase weak. Glucose fermentation is negative. The production of arginine dehydrolase, urease, β-galactosidase, gelatinase and the hydrolysis of esculin are negative. Assimilation of D-mannitol, D-glucose and D-mannose is positive, but it is weak for L- and D-arabinose and D-galactose. Assimilation of maltose, *N*-acetylglucosamine, gluconate, caprate, adipate, malate, citrate and phenylacetate is negative. Assimilation as a nitrogen source of L-proline, L-aspartate, L-serine and L-glutamine was positive, but negative for L-glycine. The most abundant fatty acids were those from summed feature 8 (C18:1 ω7c, C18:1 ω6c) and C16:0. The type strain SSUT18^T (=LMG 33353^T=CECT 30998^T) was isolated from an effective root nodule of *C. supranubius* in soils of the TNP (Tenerife, Canary Islands) and belongs to the symbiovar genistearum. Its genome has been deposited in DDBJ/EMBL/GenBank under the accession no. JARWCR000000000. The G+C content of the type strain SSUT18^T is 63.1 mol%, and the genome has ~9,582,934 bp.

Funding information
This work was supported by Fundación CajaCanarias-Caixa (grant 2017REC13) and Consejería de Economía, Conocimiento y Empleo del Gobierno de Canarias-Cofinanciación Programa Operativo FEDER Canarias 2014-2020 (grant ProID2020010103). LPS was awarded a contract from Fundación CajaCanarias-Caixa and an FPU fellowship from the Spanish Ministerio de Educación, Cultura y Deporte. The authors also thank the Strategic Research Programs for Units of Excellence CLU-2018-04 (University of Salamanca) and CLU-2019-05 (IRNASA/CSIC) co-funded by the Junta de Castilla y León and European Union (ERDF "Europe drives our growth").

Conflicts of interest
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Stępkowski T, Banasiewicz J, Granada CE, Andrews M, Passaglia LMP. Phylogeny and phylogeography of rhizobial symbionts nodulating legumes of the tribe genisteae. *Genes* 2018;9:163.
2. Acebes J, León M, Rodríguez MArcoMGarcía A. Ptridophyta, Spermatophyta. In: Arechavaleta M, Rodríguez N, Zurita N and García A (eds). *Lista de especies silvestres de Canarias. Hongos, plantas y animales terrestres*. 2010. pp. 119–172.
3. Jarabo-Lorenzo A, Pérez-Galdona R, Donate-Correa J, Rivas R, Velázquez E, *et al*. Genetic diversity of bradyrhizobial populations from diverse geographic origins that nodulate *Lupinus* spp. and *Ornithopus* spp. *Syst Appl Microbiol* 2003;26:611–623.
4. Jarabo-Lorenzo A, Velázquez E, Pérez-Galdona R, Vega-Hernández MC, Martínez-Molina E, *et al*. Restriction fragment length polymorphism analysis of 16S rDNA and low molecular weight RNA profiling of rhizobial isolates from shrubby legumes endemic to the Canary islands. *Syst Appl Microbiol* 2000;23:418–425.
5. Vinuesa P, Rademaker J, Bruijn FJ, Werner D. Genotypic characterization of bradyrhizobium strains nodulating endemic woody

legumes of the canary islands by PCR-restriction fragment length. *Appl Environ Microbiol* 1998;64:2096–2104.

6. Vinuesa P, León-Barrios M, Silva C, Willems A, Jarabo-Lorenzo A, *et al*. *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (Papilionoideae: *Genisteae*) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium genospecies* alpha and *Bradyrhizobium genospecies* beta. *Int J Syst Evol Microbiol* 2005;55:569–575.
7. Wheeler C, Dickson J. Symbiotic nitrogen fixation and distribution of *Spartocytisus supranubius* on Las Cañadas, Tenerife. *Vieraea* 1990;19:309–314.
8. Pulido-Suárez L, Notario Del Pino J, Díaz-Peña FJ, Perdomo-González A, González-Rodríguez ÁM, *et al*. High diversity of bradyrhizobial species fix nitrogen with woody legume *Spartocytisus supranubius* in a high mountain ecosystem. *Microorganisms* 2023;11:1–18.
9. Pulido-Suárez L, Díaz-Peña F, Pino JN, Medina-Cabrera A, León-Barrios M. Alteration of soil rhizobial populations by rabbit latrines could impair symbiotic nitrogen fixation in the insular alpine ecosystem of Teide National Park. *Appl Soil Ecol* 2021;160:103850.
10. Ormeño-Orrillo E, Martínez-Romero E. A genomotaxonomy view of the *Bradyrhizobium* genus. *Front Microbiol* 2019;10:1–13.
11. Somasegaran P, Hoben HJ. Counting rhizobia by a plant infection method. In: *Handbook for Rhizobia*. Springer-Verlag, 1994. pp. 58–64.
12. Pulido-Suárez L, González-Rodríguez ÁM, Cubas J, del Arco-Aguilar M, Martín-Esquivel JL, *et al*. Applying agronomic principles of rhizobial inoculation to the conservation of a keystone legume species in a high mountain ecosystem on an oceanic island. *Front Agron* 2021;3:660574.
13. de Lajudie PM, Andrews M, Ardley J, Eardly B, Jumas-Bilak E, *et al*. Minimal standards for the description of new genera and species of rhizobia and agrobacteria. *Int J Syst Evol Microbiol* 2019;69:1852–1863.
14. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
15. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, *et al*. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009;25:2078–2079.
16. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLOS Comput Biol* 2017;13:e1005595.
17. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, *et al*. The RAST server: rapid annotations using subsystems technology. *BMC Genom* 2008;9:1–15.
18. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, *et al*. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res* 2018;46:D851–D860.
19. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, *et al*. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44:6614–6624.
20. Carattoli A, Hasman H. PlasmidFinder and In Silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS). *Methods Mol Biol* 2020;2075:285–294.
21. Guo J, Bolduc B, Zayed AA, Varsani A, Dominguez-Huerta G, *et al*. VirSorter2: a multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. *Microbiome* 2021;9:37.
22. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
23. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
24. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
25. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. *Bioinformatics* 2022;38:5315–5316.
26. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
27. Riesco R, Trujillo ME. Update on the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2024;74:006300.
28. Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Research* 2008;36:W181–W184.
29. Petkau A, Stuart-Edwards M, Stothard P, Van Domselaar G. Interactive microbial genome visualization with GView. *Bioinformatics* 2010;26:3125–3126.
30. Sharma S, Sathyanarayana BK, Bird JG, Hoskins JR, Lee B, *et al*. Plasmid P1 RepA is homologous to the F plasmid RepE class of initiators. *J Biol Chem* 2004;279:6027–6034.
31. Xu L, Dong Z, Fang L, Luo Y, Wei Z, *et al*. OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res* 2019;47:W52–W58.
32. Pulido-Suárez L, Flores-Félix JD, Socas-Pérez N, Igual JM, Velázquez E, *et al*. Endophytic *Bosea spartocytisi* sp. nov. coexists with rhizobia in root nodules of *Spartocytisus supranubius* growing in soils of Teide National Park (Canary Islands). *Syst Appl Microbiol* 2022;45:126374.
33. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC News* 2001:1–6.
34. Tighe SW, de Lajudie P, Dipietro K, Lindström K, Nick G, *et al*. Analysis of cellular fatty acids and phenotypic relationships of *Agrobacterium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* species using the Sherlock Microbial Identification System. *Int J Syst Evol Microbiol* 2000;50:787–801.
35. Doetsch RN. Determinative methods of light microscopy. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW and Wood WA, et al (eds). *Manual of Methods for General Bacteriology*. Washington (DC): American Society for Microbiology; 1981. pp. 21–33.
36. Vincent J. *A Manual for the Practical Study of the Root-Nodule Bacteria*. Oxford, UK: Blackwell Scientific Publications; 1970.
37. Bergersen F. The growth of rhizobium in synthetic media. *Aust J Biol Sci* 1961;14:349–360.

The Microbiology Society is a membership charity and not-for-profit publisher.

Your submissions to our titles support the community – ensuring that we continue to provide events, grants and professional development for microbiologists at all career stages.

Find out more and submit your article at microbiologyresearch.org