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Bradyrhizobium algeriense sp. nov., a novel species isolated from effective nodules of *Retama sphaerocarpa* from Northeastern Algeria

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ABSTRACT

We have characterized genetic, phenotypic and symbiotic properties of bacterial strains previously isolated from nitrogen-fixing nodules of *Retama sphaerocarpa* from Northern Algeria. Phylogenetic analyses of 16S rRNA genes and three concatenated housekeeping genes, *recA*, *atpD* and *glnII*, placed them in a new divergent group that is proposed to form a new *Bradyrhizobium* species, *Bradyrhizobium algeriense* sp. nov. (type strain RST89^T, LMG 27618 and CECT 8363). Based on these phylogenetic markers and on genomic identity data derived from draft genomic sequences, *Bradyrhizobium valentinum* LmjM3^T, *Bradyrhizobium lablabi* CCBAU 23086^T, *Bradyrhizobium retamae* Ro19^T, and *Bradyrhizobium jicamae* PAC68^T are the closest relatives of *B. algeriense* RST89^T, with sequence identities of 92–94% and Average Nucleotide Identities (ANIm) under 90%, well below the 95–96% species circumscription threshold. Likewise, a comparison of whole-cell proteomic patterns, estimated by Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight (MALDI-TOF) mass spectrometric analysis, yielded almost identical spectra between *B. algeriense* strains but significant differences with *B. valentinum*, *Bradyrhizobium paxllaeri*, *Bradyrhizobium icense*, *B. lablabi*, *B. jicamae* and *B. retamae*. A phylogenetic tree based on symbiotic gene *nodC* revealed that the *B. algeriense* sequences cluster with sequences from the *Bradyrhizobium* symbiovar retamae, previously defined with *B. retamae* strains isolated from *Retama monosperma*. *B. algeriense* strains were able to establish effective symbioses with *Retama raetam*, *Lupinus micranthus*, *Lupinus albus* and *Genista numidica*, but not with *Lupinus angustifolius* or *Glycine max*.

Introduction

Retama spp. (tribe *Genisteae*, family *Fabaceae*) are shrubs native to the Mediterranean basin and adapted to grow under arid conditions. They are able to produce N_2-fixing root nodules, which makes them useful for restoration of arid or semiarid degraded ecosystems [30]. All endosymbiotic bacteria isolated from *Retama* nodules belong to the genus *Bradyrhizobium* [2]. Isolates from *Retama sphaerocarpa* growing in central Spain have been classified as *Bradyrhizobium canariense* [31]. Isolates from *R. sphaerocarpa* growing in Bouarfa (Morocco) and Ciudad Real (Spain), and isolates from *Retama monosperma* growing in Ras el Ma (Morocco) have been recently included in the *Bradyrhizobium retamae* species [14]. One hundred twenty-five isolates from root nodules of *Retama raetam* and *R. sphaerocarpa*, native of Northeastern Algeria, were described as *Bradyrhizobium* sp. [2]. Some of the *R. sphaerocarpa* isolates appeared to differ from the above-mentioned species, and have been characterized in this work by molecular, phenotypic and phylogenetic methods. These *R. spaherocarpa* strains are proposed to define the novel *Bradyrhizobium algeriense* sp. nov.

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Materials and methods

Bacteria and culture conditions

The isolates were obtained in a previous work [2] from surface-sterilized, effective nodules from *R. sphaerocarpa* plants growing in Algerian soils, and grown on Yeast Mannitol Agar (YMA [35]) at 28 °C. All pure cultures were tested for their ability to establish effective symbiosis with *R. sphaerocarpa* under controlled conditions (see below).

Phenotypic characterization

Cultural characteristics were assessed on YMA plates or YM Broth. Mean generation times were determined by spectrophotometric (600 nm) sampling of aerated (200 rpm orbital shaking) cultures. Growth temperature and pH range were determined by incubating cultures in YMA at 28 and 37 °C, and at pH of 4.0 or 10.0, respectively. Basal medium pH was adjusted with 1 M HCl to pH 7, and 0.05 g L^{-1} of bromothymol blue was added before sterilization. Salt tolerance was assayed by adding 1% (w/v) NaCl to the medium. GEN III MicroPlates (Biolog Inc.) were used to study assimilation of several compounds as carbon or nitrogen sources, using YNB (Becton Dickinson USA) as basal medium. Intrinsic antibiotic resistance was tested on YMA plates containing the following antibiotics: ampicillin (50 and 100 μg mL^{-1}), erythromycin (50 μg mL^{-1}), gentamycin (30 μg mL^{-1}), tetracycline (5 μg mL^{-1}), spectinomycin (50 μg mL^{-1}), kanamycin (50 μg mL^{-1}), streptomycin (10, 60 and 100 μg mL^{-1}), chloramphenicol (20 μg mL^{-1}) or rifampicin (5 μg mL^{-1}).

Protein composition was assessed by whole-cell matrix-assisted laser-desorption time-of-flight mass spectrometry (WC MALDI-TOF MS), using freshly-grown colonies analyzed as previously described [3,26].

Cellular fatty acid composition analyses were carried out at the Spanish Type Culture Collection (Colección Española de Cultivos Tipo, CECT, Paterna, Valencia). Cultures were grown aerobically on YM broth at 28 °C, and cells were collected at the late log phase of growth. Fatty acid methyl esters were prepared and resolved using the methods described by Sasser [34], and identified with the MIDI Sherlock Microbial Identification System (version 6.1), using the TSBA6 database.

Genotypic characterization

DNA fragment amplification and sequencing were performed with primers: 41F and 1488R for 16S rRNA [17]; atpD255F and atpD782R, recA41F and recA640R for partial sequences of *atpD* and *recA* respectively [13]; GSII-1 and GSII-4 for *glnII* [41]; nodCfor540 and nodCrev1160 for *nodC* [21]. Sequences were compared with those from GenBank using the BLASTN algorithm [1]. Sequences from *R. sphaerocarpa* isolates and from all the available *Bradyrhizobium* type species were aligned using the SINA alignment service from the SILVA database (http://www.arb-silva.de/aligner/) [27] for 16S rRNA genes, and ClustalX software [38] for housekeeping genes and symbiotic gene *nodC*. Most phylogenetic analyses were carried out with the software package MEGA 6.06 [37]. Kimura's two-parameter model was used to calculate distances [20], and phylogenetic trees were inferred using either the neighbor-joining (NJ [32]), maximum parsimony (MP [12]) and maximum likelihood (ML [11]) methods. Bootstrap analyses were based on 1000 subsets. For maximum likelihood trees, the PhyML 3.0 package [15,16] was used, and the best nucleotide substitution model for ML trees was determined by means of jModelTest 2.1.1 [7,16]. Robustness of ML tree topologies was inferred by nonparametric bootstrap tests based on 100 pseudoreplicates.

Table 1
Percentage Average Nucleotide Identity (ANIm) between *B. algeriense* strains RST89^T and RST91, and related *Bradyrhizobium* strains.

Strain	RST89^T	RST91
B. algeriense RST89^T	–	99.95%
B. algeriense RST91	99.74%	–
B. retamae Ro19^T	89.26%	89.27%
B. valentinum LmjM3^T	88.73%	88.70%
B. lablabi CCBAU 23086^T	88.48%	88.46%
B. jicamae PAC68^T	88.26%	88.28%
B. elkanii USDA 76^T	85.39%	85.42%
B. japonicum USDA 6^T	84.68%	84.66%
B. diazoefficiens USDA 110^T	84.63%	84.60%
B. sp. ORS278	84.32%	84.37%
B. sp. BTAi1	84.31%	84.40%

Draft genome sequences (Illumina HiSeq 2000, 500 bp paired-end libraries, 100 bp reads, 7 million reads), were obtained for *R. sphaerocarpa* strains and for type strains of closely-related *Bradyrhizobium* species by BGI (Hong Kong, China). These partial genomic sequences were assembled with SOAPdenovo2 [23] and used, together with available genome sequences from databanks, to calculate Average Nucleotide Identities (ANI). Genome sequences obtained in this work were deposited in Genbank as the following Bioprojects: *Bradyrhizobium lablabi* CCBAU 23086^T, PRJNA241376; *Bradyrhizobium jicamae* PAC68^T, PRJNA241374; *B. retamae* Ro19^T, PRJNA241375; *B. algeriense* RST89, PRJNA438070; and *B. algeriense* RST91, PRJNA438074. Pairwise genome comparisons were carried out with the JSpecies software package and the MUMMER option (ANIm) [29].

Plant tests

The plant symbiotic behavior of *B. algeriense* strains was tested in sterile Leonard jars filled with vermiculite containing Jensen's solution [35,42]. *B. algeriense* culture suspensions (2 mL, 10^8–10^9 cells mL^{-1}) were added onto seedlings of the legume to be tested (2–5-day-old) in the Leonard jars, and plants were grown in the greenhouse (25 °C) for a minimum of 3 and a maximum of 8 weeks, after which plants were examined for the presence of nodules, their number, size and appearance, as well as for appearance and weight of the aerial part. Tests were run in triplicate and compared with negative (uninoculated plants) and positive (plants inoculated with cognate symbionts) controls.

Results and discussion

R. sphaerocarpa root-nodule isolates were Gram-negative, non spore-forming rods, and extremely slow-growing bacteria with mean generation times >20 h in YMB. Non-mucous colonies (<2 mm) took at least 10 days to appear on YMA plates incubated at 28 °C and produced an alkaline reaction on YMA medium supplied with bromthymol blue, suggesting that isolates belong to the genus *Bradyrhizobium* [18]. Three related isolates from the Toudja maquis, RST88bis, RST89, and RST91, out of four belonging to IGS type 9 [2], were chosen for further work. RAPD-PCR analysis showed two band patterns for RST88bis and RST89/RST91, respectively (Supplementary Fig. S1). Further DNA sequence analyses (see Table 1 and Figs. 1 and 2, below) showed, however, that RST89 and RST91 are not clones.

Fig. 1 shows the tree derived from 16S rRNA alignments by the neighbor-joining method, but similar results were obtained with the MP and ML methods (data not shown). All *R. sphaerocarpa* strains isolated in our previous work had similar 16S rRNA gene sequences [2], and the three included in the phylogenetic tree (RST89^T, RST88bis and RST91) showed >99.6% identity. The *R. sphaerocarpa* strains cluster with other *Bradyrhizobium* species,

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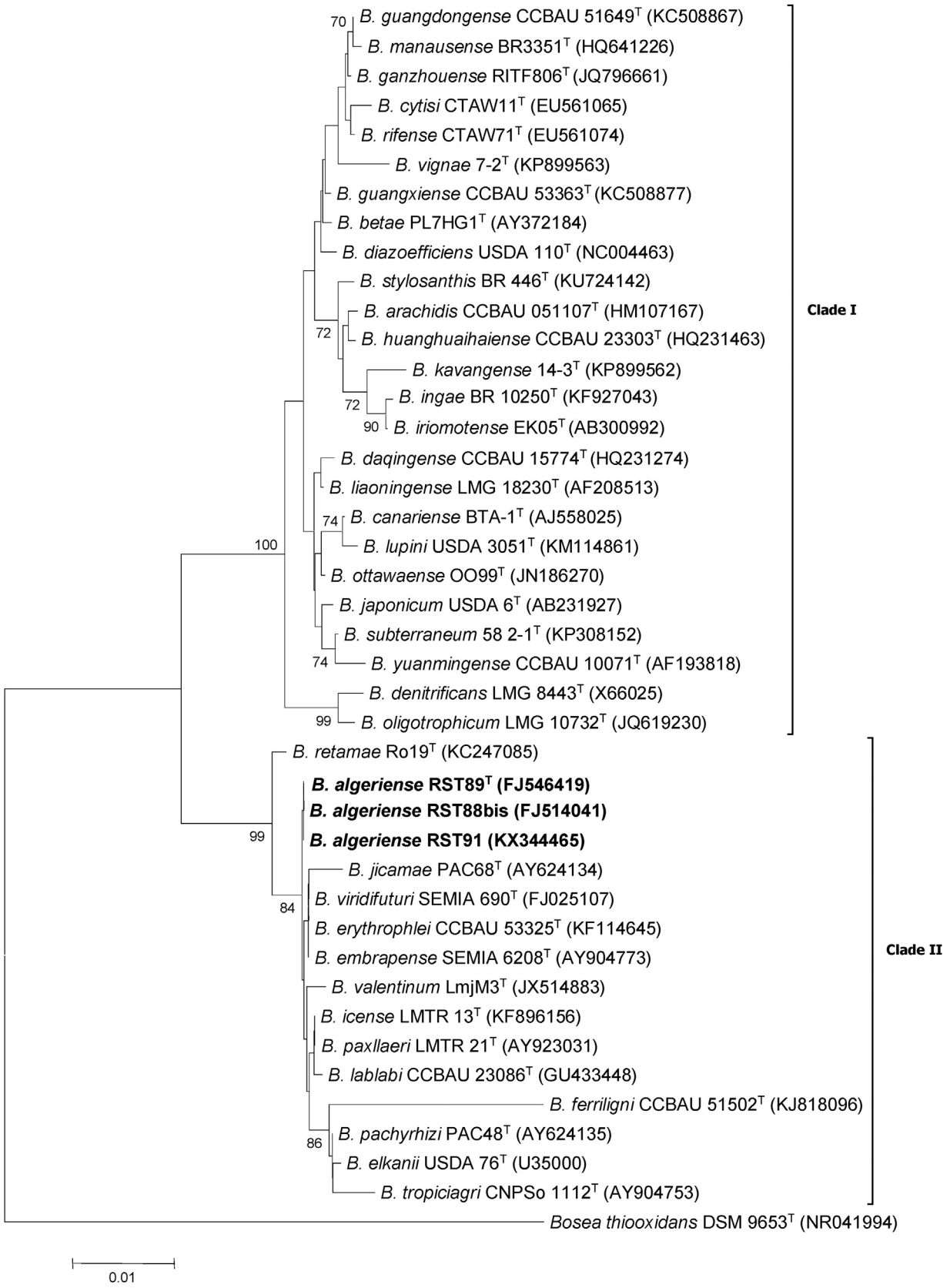

Fig. 1. Phylogenetic tree based on the 16S rRNA sequences (1233 nt) showing the relationships of the strains of *Bradyrhizobium algeriense* sp. nov. and related species. The tree was constructed by the neighbor joining method. Bootstrap values greater than 70% are indicated at nodes. Bar represents 1% substitution. Sequence accession numbers are shown in parenthesis after strain names. The sequence of *Bosea thiooxidans* DSM9653^T was used as an outgroup.

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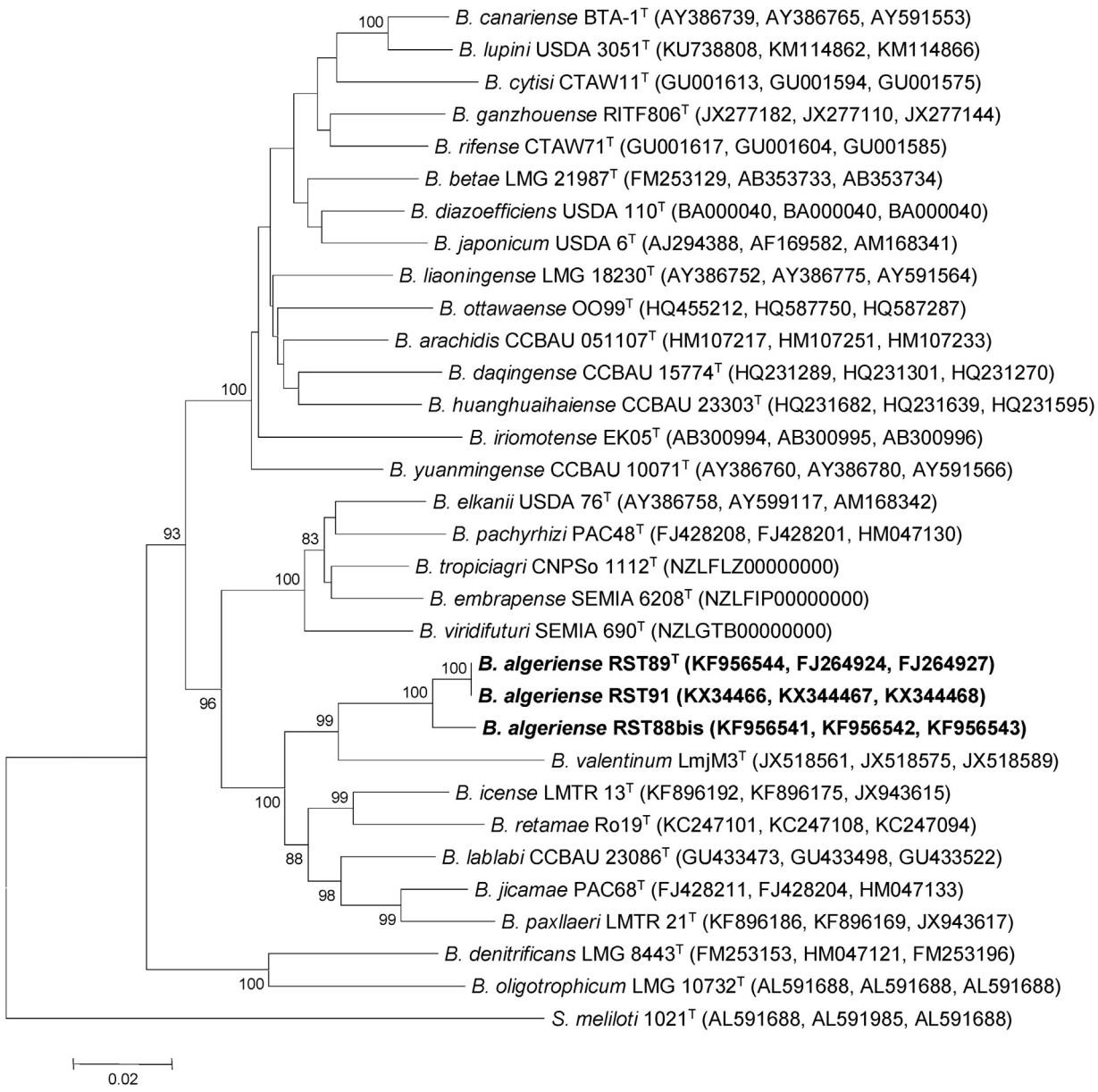

Fig. 2. Neighbor-joining tree based on multiple alignment of partial (1287 nt) concatenated sequences of *atpD*, *glnII* and *recA* genes of *Bradyrhizobium algeriense* sp. nov. (strains RST89^T, RST91 and RST88bis), and its closely related species within the genus *Bradyrhizobium* (see Supplementary Fig. S2 for alignment). The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bootstrap values greater than 70% are indicated at nodes. Bar, 2% substitution. Sequence accession numbers of *atpD*, *glnII* and *recA* genes are shown in parenthesis after strain names. The corresponding sequences from *Sinorhizobium meliloti* 1021 were used as outgroup.

mainly *Bradyrhizobium elkanii* USDA 76^T, *B. lablabi* CCBAU 23086^T, *Bradyrhizobium valentinum* LmjM3^T, *Bradyrhizobium icense* LMTR 13^T, and *Bradyrhizobium paxllaeri* LMTR 21^T, which were their closest relatives, with about 99.5% identity, all within Clade II, a distinct clade in the *Bradyrhizobium* genus [24,33] that also includes *Bradyrhizobium tropiciagri* CNPSo 1112^T and *Bradyrhizobium ferriligni* CCBAU 51502^T, with 98.9 and 97.8% identities, respectively. Within the *Bradyrhizobium* genus the two distinct clades were clearly separated by 16S rRNA gene analysis, as it has been previously reported [24,33]. Type strains within Clade I exhibited identities between 96.6 and 97.5% when their 16S rRNA gene sequences were compared with that of RST89^T (Fig. 1; data not shown). This clade includes *Bradyrhizobium japonicum*, which is the type species of the genus. Due to the high sequence identities observed among 16S rRNAs from some species of Clades I and II, analyses of other genes were required in order to complement DNA-DNA hybridization results in taxonomic studies at the species level [40].

The *atpD*, *glnII* and *recA* loci have been proposed for species delineation within *Bradyrhizobium* [24]. Multilocus sequence analyses of concatenated *atpD*, *glnII* and *recA* sequences gave similar results irrespective of the tree inference method, and the NJ tree is shown in Fig. 2. *R. sphaerocarpa* isolates grouped in a unique branch (100% bootstrap), and showed high identity among them (>98.4%). The *R. sphaerocarpa* strain cluster was closer to *B. valentinum* than to any other *Bradyrhizobium* species, but clearly separated from them. Particularly, identity values for the pairwise comparison of *atpD-glnII-recA* concatenated genes between strain RST89^T and the type strains from the closest species *B. lablabi*, *B. valentinum*, *B. jicamae*, *B. icense*, *B. retamae* and *B. paxllaeri*, were 93.9, 93.5, 93.3, 92.9, 92.3 and 92.1%, respectively. These results suggest that *R. sphaerocarpa* strains RST89^T, RST91 and RST88bis belong to a new *Bradyrhizobium* species different to other bradyrhizobia.

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Table 2
Differential characteristics between *Bradyrhizobium algeriense* strains isolated from *Retama sphaerocarpa* and related species in this genus.[a]

Characteristic/strains	1	2	3	4	5	6	7	8	9
Generation time (h) (YM broth, pH 7)	>20	>20	>20	10–12 [4]	6–7 [28]	>20	ND	11–12 [9]	>6 [8]
Growth at:									
1.0% NaCl	–	–	–	–	+	–	–	+	+
pH 4.0	+	+	+	+	+[b]	+	–	–	+
pH 10.0	–	–	–	+	–	+	–	+	–
37 °C	–	–	–	+	–	–	–	+	+
Resistance to (μg/mL^{-1}):									
Ampicillin (50)	+	+	+	+	+	+	+	–	+[b]
Streptomycin (10)	–	–	–	–	–	–	–	–	–
Erythromycin (50)	–	–	–	+	+[b]	–	+	+	+
Gentamycin (30)	–	–	–	–	–	–	–	–	W
Spectinomycin (50)	–	–	–	–	–	–	–	–	–
Kanamycin (50)	–	–	–	–	–	–	–	–	–
Rifampicin (5)	–	–	–	+	+	–	–	–	+
Utilization of C sources:									
D-Glucose	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	– [28]	+	w	+	+
D-Galactose	+	+	+	+	+	+	+	+	+
D-Mannose	–	–	–	+[b]	– [28]	+	– [14]	–	+
Citrate	–	–	–	+	–	–	–	–	+
Malonate	–	–	–	+	–	–	+	–	+
Fructose	+	+	+	+[b]	+	–	– [14]	+	+
Utilization of N sources:									
Cysteine	–	–	–	– [4]	–	–	–	–	+
Histidine	+	+	+	+	+	–	–	–	+

+: positive, –: negative, w: weak or variable. ND: not determined. Data taken from previous work are indicated by reference numbers.

[a] Species/Strains: **1**, *B. algeriense* RST89^T; **2**, *B. algeriense* RST91; **3**, *B. algeriense* RST88bis; **4**, *B. lablabi* CCBAU 23086^T; **5**, *B. jicamae* PAC68^T; **6**, *B. valentinum* LmjM3^T; **7**, *B. retamae* Ro19^T; **8**, *B. paxllaeri* LMTR 21^T; **9**, *B. diazoefficiens* USDA 110^T.

[b] Our own data are not consistent with those reported previously.

We used Average Nucleotide Identity (ANI) of partial genomic sequences as a measure of genome relatedness and as a substitute for DNA–DNA hybridizations [29,5,6,8–10]. The calculated ANIm values for pairwise genomic comparisons between *R. sphaerocarpa* strains and type strains from the closest species (*B. valentinum* LmjM3^T, *B. lablabi* 23086^T, *B. jicamae* PAC68^T, *B. retamae* Ro19^T) were always <90%, and even lower (< 85%) for *B. elkanii* USDA 76^T/*B. japonicum* USDA 6^T and the *Bradyrhizobium* photosynthetic bacteria (Table 1).

Phenotypic characterization of *R. sphaerocarpa* strains was performed using properties previously shown to be useful for *Bradyrhizobium* species differentiation [4,28,43,44]. Type strains of relevant species within the *Bradyrhizobium* genus were included as reference. The three *R. sphaerocarpa* strains behaved as a homogeneous group, and their differential characteristics are listed in Table 2. Further phenotypic characterization was carried out by comparing whole-cell MALDI-TOF mass spectra of related strains. The similarity dendrogram resulting from this comparison showed that *R. sphaerocarpa* strains RTS89^T and RST91 form a distinct group, separated from type strains within the *Bradyrhizobium* genus (Fig. 3). Finally, the cellular fatty acid composition of strain RST89^T was compared with its closest *Bradyrhizobium* species type strains (*B. retamae* Ro19^T, *B. jicamae* PAC68^T, *B. lablabi* CCBAU 23086^T, *B. pachyrhizi* PAC48^T, and *B. elkanii* USDA 76^T) (Table 3). All the tested strains had the following common fatty acids: 16:0, 18:0, 18:1 ω7c 11-methyl, and Summed Feature 8 (18:1 ω7c/18:1 ω6c), but their percentage varied. Summed Feature 8 and 16:0 were the two most dominant fatty acids in all the tested strains, which is consistent with previous reports for the genus *Bradyrhizobium* [39]. Despite the overall similarities, a differential pattern consisting of nine fatty acids was detected for strain RST89^T (Table 3).

Symbiotic genes are usually found in mobile elements (symbiotic islands in the case of *Bradyrhizobium* species [19]) and their diversity usually reflects differences in plant host range rather than in taxonomical placement. However, their analysis provides valuable information for symbiotic specificity and legume host range of rhizobia species [22,36]. In the case of the symbiotic *nodC* gene (Supplementary Fig. S3), the *B. algeriense* strains are grouped together with *B. valentinum* LmjM3^T, *B. retamae* Ro19^T, and *B. icense* LMTR 13^T. All these strains share a common ancestor with *B. lablabi* CCBAU 23086^T and *B. paxllaeri* LMTR 21^T isolated in China from *Lablab purpureus* nodules and in Peru from *Phaseolus lunatus*, respectively. All *B. algeriense nodC* sequences (RST89^T, RST91 and RST88bis) shared a 99.0% similarity and appeared to be part of the recently described symbiovar retamae (Supplementary Fig. S3) [14]. This phylogeny clearly differs from that obtained for ANI and

Table 3
Fatty acid patterns of *B. algeriense* RST89^T and related strains.[†]

Fatty acid*	1	2	3	4	5	6
9:0	0.56	–	0.97	0.31	–	–
10:0	–	–	–	–	0.68	–
12:0	–	1.08	2.11	1.58	4.64	2.04
14:0	0.76	2.90	2.45	1.37	–	2.55
16:0	19.01	16.20	13.39	12.39	19.62	12.60
16:0 ω5c	–	1.18	–	–	–	–
16:1 ω5c	–	–	–	1.24	–	–
17:0 cyclo	1.04	1.24	–	–	–	–
17:0 anteiso	–	–	–	–	1.62	–
17:1 ω8c	–	–	–	–	0.92	–
17:1 ω6c	–	–	–	–	0.95	–
17:0	–	–	–	–	0.55	–
18:0	1.67	1.48	2.86	1.88	3.29	2.40
18:0 iso	–	–	1.39	–	0.95	–
18:1 ω7c 11-methyl	10.89	13.92	2.79	0.96	2.12	3.36
18:1 ω9c	–	–	1.18	0.70	2.82	1.09
19:0 cyclo ω8c	1.55	9.10	–	11.87	–	–
20:0 ω6,9c	–	–	–	0.74	–	–
Summed Feature 3	1.20	–	1.32	–	1.23	1.32
Summed Feature 5	–	0.58	0.60	–	2.45	–
Summed Feature 6	–	–	–	–	0.42	–
Summed Feature 8	63.30	52.33	70.94	66.96	57.74	74.64

[†]Species/Strains: 1, *B. algeriense* RST89^T; 2, *B. retamae* Ro19^T; 3, *B. jicamae* PAC68^T; 4, *B. pachyrhizi* PAC48^T; 5, *B. lablabi* CCBAU 23086^T; 6, *B. elkanii* USDA 76^T; 7. (all data were obtained in this study) *. Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed Feature 3, 16:1 ω7c/16:1 ω6c; Summed Feature 5, 18:2ω6, 9c/18:0 ante; Summed Feature 6, 19:1 ω11c/19:1 ω9c; Summed Feature 8, 18:1 ω7c/18:1 ω6c.

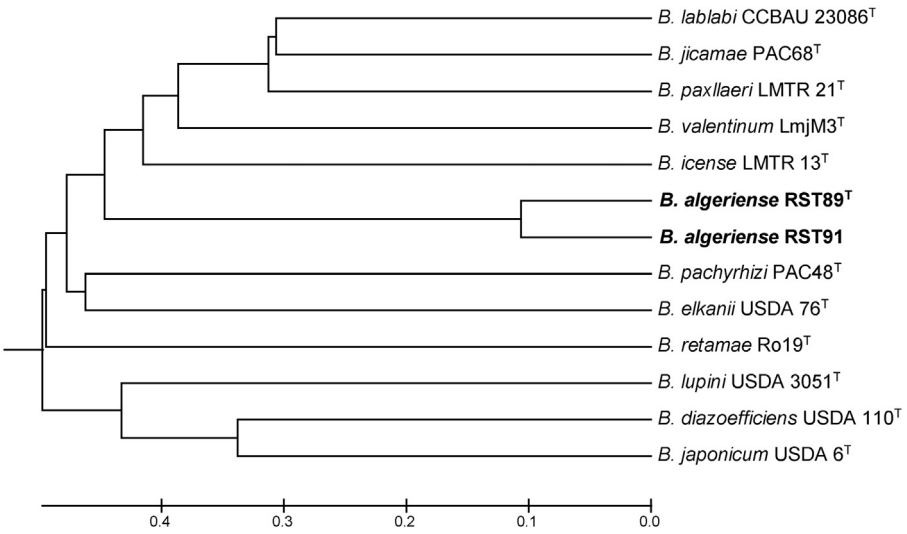

Fig. 3. Whole-cell MALDI-TOF mass spectrometric differentiation of *Bradyrhizobium algeriense* strains. A dendrogram was constructed by applying average linkage clustering and Pearson's distance correlation from a similarity matrix of identical mass peaks, computed from whole-cell mass spectra of all *Bradyrhizobium* strains analyzed. The dendrogram was generated by considering the average value of two independent determinations for each strain. The horizontal bar indicates Euclidean distance.

Table 4
Plant host range of *B. algeriense* strains.[a]

Plant hosts	RST89T	RST88 bis
R. raetam	+/+	+/+
R. sphaerocarpa	+/+	+/+
Lupinus micranthus	+/+	+/+
L. albus	+/+	+/+
L. angustifolius	–	–
Genista numidica	+/+	+/+
Vigna unguiculata	+/–	+/–
Glycine max	–	–

[a] +/+, effective (red) nodules; +/–, ineffective (white) nodules; –/–, no nodules.

housekeeping markers (Figs. 1 and 2, Table 1), and suggests that, similarly to the situation found with other rhizobia [25], symbiotic genes from the symbiovar retamae have been horizontally transferred among distinct species including *B. algeriense*. The structural conservation of nodulation genes correlated well with the symbiotic plant host range of these *R. sphaerocarpa* strains. As shown in Table 4, all three strains were able to establish effective symbioses with other shrubs from the tribe *Genisteae* with overlapping geographical distribution, such as *R. raetam*, *Lupinus micranthus*, *L. albus* and *Genista numidica*, but not with *Lupinus angustifolius*. No symbiosis could be established with soybean (*Glycine max*), whereas an ineffective symbiosis (white nodules) was established with cowpea (*Vigna unguiculata*).

Based on the above phenotypic and genotypic characteristics we propose to classify the *R. sphaerocarpa* strains described in this study into a new species named *B. algeriense* sp. nov., with strain RST89 as the type strain (RST89^T).

Description of *B. algeriense* sp. nov.

B. algeriense (al.ge.ri.en'se. N.L. neut. adj. *algeriense*, pertaining to Algeria, the source of the plant from which the type strain was isolated) was isolated from nodules collected on roots of Mediterranean shrubby legume *R. sphaerocarpa* growing in several ecological–climatic areas of Northeastern Algeria. The isolates are Gram-negative rods as other species of the genus, and grow extremely slowly on YMA plates. Colonies are non-mucoid, creamy in color and with 1.5–2 mm size after 10 days on YMA. Generation time was between 19 and 23 h in YM broth. Optimal growth temperature is between 28 °C and 30 °C. No growth was observed at 37 °C. The optimum pH for growth was 7.0 to 8.0. No growth was detected in the presence of 1% NaCl. The strains use mannitol, D-glucose, D-galactose, sorbose and D-fructose as carbon sources. Strains do not grow on cellobiose, dextrin, sucrose, L-rhamnose, D-mannose, malonate and citrate as sole carbon source. They can assimilate histidine, but not cysteine, as sole nitrogen source. Nine fatty acids were detected, and both Summed Feature 8 (18:1 ω7c/18:1 ω6c) and 16:0 were the predominant fatty acids in RST89^T. All strains are resistant to tetracycline (5 μg mL^{-1}), chloramphenicol (20 μg mL^{-1}), and ampicillin (50 μg mL^{-1}). They do not grow in the presence of spectinomycin (50 μg mL^{-1}), kanamycin (50 μg mL^{-1}), gentamycin (30 μg mL^{-1}), streptomycin (100 μg mL^{-1}), or rifampicin (5 μg mL^{-1}). The genome sequences of *B. algeriense* RST91 and *B. algeriense* RST89^T (Genbank Bioprojects PRJNA438074 and PRJNA438070, respectively) were determined. *B. algeriense* is genomically divergent from other species of the *Bradyrhizobium* genus, sharing less than 90% Average Nucleotide Identity with their closest relatives, *B. lablabi* CCBAU 23086^T, *B. valentinum* LmjM3^T, *B. icense* LMTR 13^T, *B. jicamae* PAC68^T and *B. paxllaeri* LMTR 21^T. They establish diazotrophic root nodule symbioses with *R. sphaerocarpa*, *R. raetam*, *L. micranthus*, *L. albus* and *G. numidica*, but not with *L. angustifolius* or *G. max*. The type strain, RST89^T (= LMG 27618^T = CECT 8363^T) was isolated from a root nodule of *R. sphaerocarpa* grown in Toudja (Algeria), and its genome has a G + C mole % of 62.12.

The Digital Protologue Taxonumber for *B. algeriense* is TA00370.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2018.03.004.

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