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Bénédicte Lafay, Elina Coquery, Philippe M Oger. Bradyrhizobium commune sp. nov., isolated from nodules of a wide range of native legumes across the Australian continent. *International Journal of Systematic and Evolutionary Microbiology*, 2023, 73 (7), pp.005971. 10.1099/ijsem.0.005971 . hal-04191550

HAL Id: hal-04191550

<https://hal.science/hal-04191550>

Submitted on 30 Aug 2023

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International Journal of Systematic and Evolutionary Microbiology

***Bradyrhizobium commune* sp. nov., isolated from nodules of a wide range of native legumes across the Australian continent**

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ABSTRACT

Bradyrhizobia are particularly abundant in Australia, where they nodulate native legumes growing in the acidic and seasonally dry soils that predominate in these environments. They are essential to Australian ecosystems by helping legumes to compensate for nutrient deficiencies and the low fertility of Australian soils. During a survey of Australian native rhizobial communities in 1994–1995, several *Bradyrhizobium* genospecies were identified, among which genospecies B appeared to be present in various edaphic and climatic conditions and associate with a large range of leguminous hosts across the whole continent. We took advantage of the recent sequencing of the genome of strain BDV5040^T, representative of *Bradyrhizobium* genospecies B, to re-evaluate the taxonomic status of this lineage. We further characterized strain BDV5040^T based on morpho-physiological traits and determined its phylogenetic relationships with the type strains of all currently described *Bradyrhizobium* species using both small subunit (SSU) rRNA gene and complete genome sequences. The digital DNA–DNA hybridization relatedness with any type strain was less than 35% and both SSU rRNA gene and genome phylogenies confirmed the initial observation that this strain does not belong to any formerly described species within the genus *Bradyrhizobium*. All data thus support the description of the novel species *Bradyrhizobium commune* sp. nov. for which the type strain is BDV5040^T (=CFBP 9110^T=LMG 32898^T), isolated from a nodule of *Bossiaea ensata* in Ben Boyd National Park in New South Wales, Australia.

Keywords

Australia, *Bradyrhizobium*, genome phylogeny, native legume host, SSU rRNA gene

Author Notes

GenBank/EMBL/DDBJ/PIR accession numbers: Z94812 (SSU rRNA gene), AJ920043 (nodA), CP061379 (chromosome), GCA_015624505 (genome assembly).

Abbreviations

ANI, average nucleotide identity; BNF, biological nitrogen fixation; dDDH, digital DNA–DNA hybridization; GBDP, Genome blast Distance Phylogeny; GTDB, Genome Taxonomy Database; LB, Luria–Bertani; LPSN, List of Prokaryotic names with Standing in Nomenclature; NF, nodulation factor; SSU, small subunit; YMA, yeast–mannitol agar.

Biological nitrogen fixation (BNF) is the largest input pathway by which newly fixed nitrogen (N) enters the biosphere [1]. It is performed exclusively by certain bacteria and archaea (diazotrophic prokaryotes) which convert atmospheric dinitrogen (N₂) into bio-available forms (NH₃) via the enzyme complex nitrogenase that both diazotrophs and plants use. The largest natural source of N in the terrestrial biosphere is expected to result from the mutualistic interactions between legumes (Fabaceae) and their nitrogen-fixing partners, soil bacteria collectively referred to as rhizobia [2]. Fabaceae is the third largest angiosperm family [3], comprising about 770 genera and 23 000 species [4], 70% of which can form N-fixing symbioses with rhizobia [5]. Legumes are most remarkable for their wide evolutionary diversification and cosmopolitan distribution, and many are of considerable agricultural or ecological importance, generally reflecting their ability to develop symbiotic associations with rhizobia. As part of this symbiosis, specialized organs, called nodules, are elicited on the root (or occasionally the stem) of the legume where BNF takes place. Inside the nodule, the 'rhizobia' differentiate into bacteroids that perform nitrogen fixation fueled by plant-provided carbon substrate at nanomolar oxygen levels.

The rhizobia belong to hundreds of species restricted to about 20 genera within the *Hyphomicrobiales* (Alphaproteobacteria) and the *Burkholderiales* (Betaproteobacteria) [6]. Among these, *Bradyrhizobium* (*Hyphomicrobiales: Nitrobacteraceae*) is of particular interest. Bradyrhizobia are comparatively slow growing as opposed to species of *Rhizobium*. They were first characterized from soybean (*Glycine max*), the most important grain legume in the world with a 2020/2021 production of about 360 million tonnes [7] and have since been isolated from numerous legume species across different continents and climatic zones. They are the most widely used rhizobia in agriculture because of their ability to symbiosis with legumes of high agronomic importance. Their use therefore leads to a decrease on the dependence of nitrogen fertilisers for the promotion of legume crop growth and contribute to the nitrogen enrichment of terrestrial ecosystems, thus playing a very important role in the preservation of the environment. Bradyrhizobia are the most ubiquitous and abundant bacteria in soils [8] where they may live as nonsymbiotic saprophytes [9]. They nodulate the widest range of legume genera [10] and are the most frequently isolated rhizobia [5]. They constitute the dominant, possibly exclusive, rhizobial symbionts of legumes in the most basal lineages within the Caesalpinoideae [11], *i.e.* not considering the mimosoid clade that was recently included in that sub-family [12]. *Bradyrhizobium* is also the sole genus found naturally in symbiotic association with *Parasponia* (Cannabaceae), the only known non-leguminous plants that can establish a nitrogen fixing rhizobial mutualism [13, 14]. Remarkably, whereas the classical path to nodule formation relies on nodulation factors (NFs) in the majority of known rhizobial lineages, some *Bradyrhizobium* have evolved alternative NF-independent nodulation strategies [15], involving the type three secretion system (T3SS) [16] and a as yet uncharacterized non-NF/non-T3SS mechanism identified in symbioses involving *Aeschynomene* and photosynthetic bradyrhizobia [17]. *Bradyrhizobium* is therefore believed to be the most ancestral rhizobial symbiont of legumes, from which all other rhizobial lineages are expected to have evolved [10, 18].

Bradyrhizobia are particularly dominant in Australia, where they nodulate native legumes growing in the acidic and seasonally dry soils that predominate in these environments [19–23]. They are essential to Australian ecosystems by helping legumes, which constitute a significant part of the vascular flora (about 10% of the estimated 18 000 native plant species), to circumvent nutrient deficiencies and the low fertility of Australian soils. A number of new lineages was characterized among Australian bradyrhizobia isolated from nodules of indigenous legumes based on SSU rRNA sequence analyses, at least some of which were expected to constitute novel species [19–22].

We here describe a new *Bradyrhizobium* species corresponding to Australian *Bradyrhizobium* genospecies B [19], with BDV5040^T for which the complete genome sequence was recently obtained [24] as type strain. *Bradyrhizobium* genospecies B is widespread throughout the Australian continent in different climatic and edaphic conditions and exhibits a broad host range encompassing both the Papilionoideae and Caesalpinioideae (including the mimosoid clade) subfamilies [19–22]. We thus propose naming this novel species *Bradyrhizobium commune* sp. nov.

Isolation and ecology

Strain BDV5040^T was isolated in 1995 from a root nodule of *Bossiaea ensata* (Fabaceae: Papilionoideae: Bossiadeae) collected in Ben Boyd National Park, New South Wales, Australia (37° 129' S, 149° 579' E), during a survey of rhizobia associated to native shrubby legumes in south-eastern Australia [19]. It is a representative of *Bradyrhizobium* genospecies B which was isolated from a diversity of Australian native hosts belonging to the main Fabaceae lineages growing in a range of environmental conditions [19–22]. It induces effective nitrogen-fixing nodules on its original host [19]. Genospecies B was initially represented by 141 strains characterized in Australia using SSU rRNA gene analyses [19–22]. However, all but one strains were lost about fifteen years ago due to the malfunctioning of the storage facility where they were kept. Strain BDV5040^T is currently the only available genospecies B representative.

The strain was short-term cultured on modified yeast extract–mannitol agar (YMA) medium at 25 °C [25] and, for long-term preservation, was stored in yeast extract–mannitol broth supplemented with 30% glycerol (v/v) at –80 °C, as well as lyophilized.

The strain is deposited at the French Collection for Plant-associated Bacteria [Centre International de Ressources Microbiennes, (CIRM) – Collection Française des Bactéries associées aux Plantes (CFBP), INRAE] and at the Belgian Coordinated Collections of Microorganisms (BCCM/LMG).

SSU rRNA gene phylogeny

Numerous *Bradyrhizobium* species have been described since the initial characterization of strain BDV5040^T 25 years ago [19]. We thus assessed anew the phylogenetic position of strain BDV5040^T within the genus *Bradyrhizobium* based on SSU rRNA gene sequence. Strain BDV5040^T complete SSU rRNA gene (1493 base pairs) was compared to those of the type strains of the 76 current *Bradyrhizobium* species as listed in the List of Prokaryotic names with Standing in Nomenclature (LPSN) database [26] (accessed on 31 January 2022). The SSU rRNA gene sequences were obtained from the National Center for Biotechnology Information (NCBI) Genbank database and analysed using SeaView version 5.0.4 [27]. Sequences were aligned using ClustalO [28] and a maximum likelihood phylogeny was inferred using PhyML version 3.1 [29] with the general time reversible model of nucleotide evolution with substitution rates varying over sites according to the invariable sites plus gamma (I+g) distribution as recommended by Abadi *et al.* [30]. Internal branch supports were evaluated using the approximate likelihood ratio test based on a Shimodaira-Hasegawa-like procedure (n=1,000) [29]. The resulting phylogeny confirmed that strain BDV5040^T does not group with any formerly described species and represents a novel lineage within the *Bradyrhizobium japonicum* clade (Fig. 1).

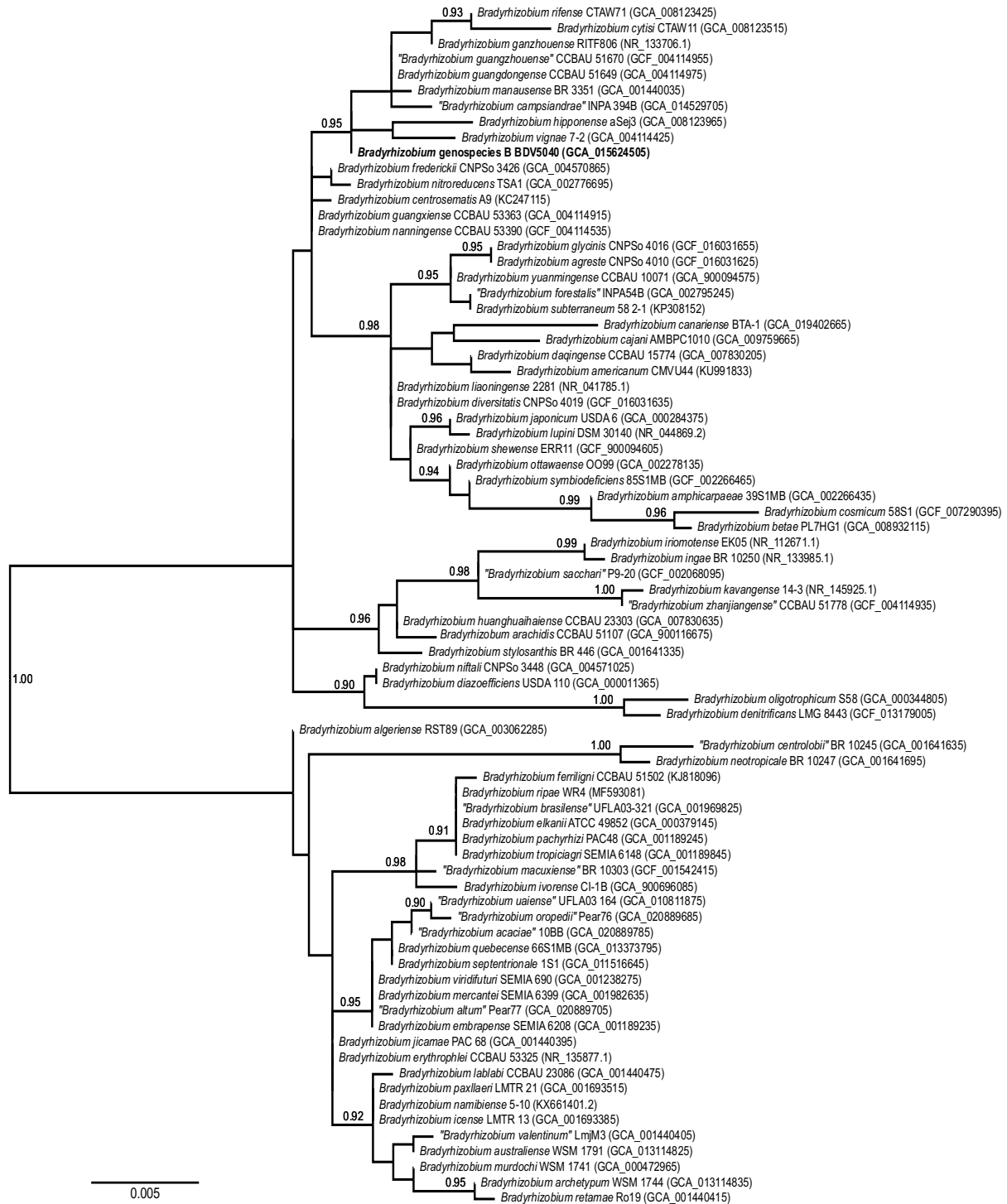


Fig. 1. SU rRNA gene maximum-likelihood phylogeny of *Bradyrhizobium* genospecies B BDV5040^T among all available *Bradyrhizobium* type strains. The numbers above branches are aLRT support values (only values above 0.90 are shown).

Genome phylogeny

We further investigated the relationships of strain BDV5040^T to known species of *Bradyrhizobium* using whole genome sequence comparison. The complete genome sequence of strain BDV5040^T has

been reported elsewhere [24]. The genome consists of a 7.62 Mb chromosome with an average G+C content of 63.92 mol%. The genome-based taxonomic position of BDV5040^T was assessed using the Type (strain) Genome Server (TYGS) at Leibniz Institute DSMZ [31, 32]. In connection with the LPSN database, the TYGS determines the closest type strain genomes, calculates intergenomic distances using the Genome blast Distance Phylogeny approach (GBDP) and digital DNA–DNA hybridization (dDDH) values with confidence intervals, and infers a minimum-evolution tree with branch support [32]. Additionally, average nucleotide identity (ANI) values were computed using the FastANI Calculator [33] on the Genome Taxonomy Database (GTDB) website [34]. These analyses (performed on 8 February 2022) confirmed that strain BDV5040^T constitutes a separate species within the genus *Bradyrhizobium* (Fig. 2; Table 1).

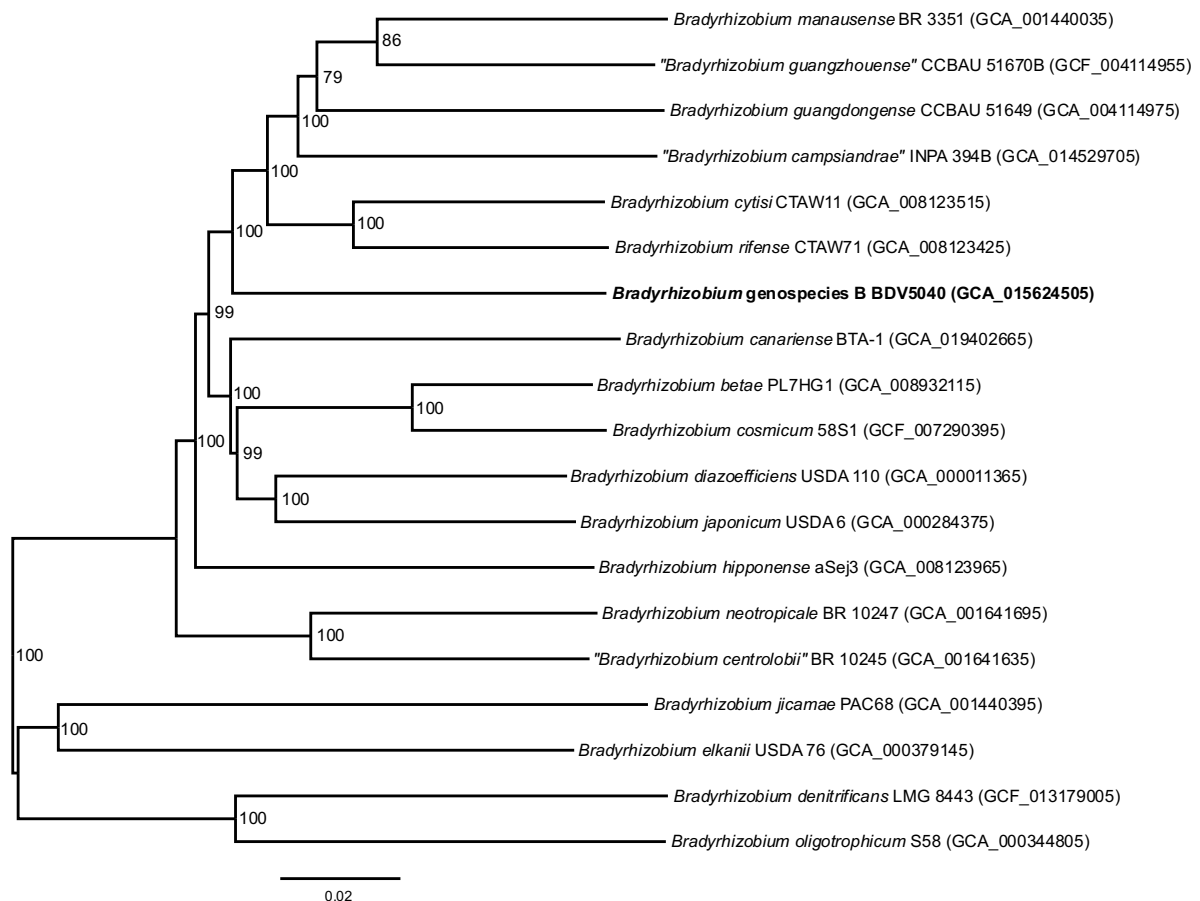


Fig. 2. Genome phylogeny of *Bradyrhizobium* genospecies B BDV5040^T with most closely related *Bradyrhizobium* type strains and *Bradyrhizobium* major lineage representatives. The tree was inferred with FastME from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5 (derived from d4 distances). The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications (only values above 60 are shown).

Table 1. Digital DNA–DNA hybridization (dDDH) values with confidence intervals (CIs) and average nucleotide identity (ANI) values between *Bradyrhizobium* genospecies B BDV5040^T and *Bradyrhizobium* type strains with genome data. Genome sequence accession numbers and major characteristics are provided. dDDH values were calculated using TYGS. ANI were computed using the GTDB FastANI calculator.

<i>Bradyrhizobium</i> type strain*	Genome assembly accession ^b	Genome length (bp)	Genome %G+C	Protein number	dDDH [†] (%)	CI (%)	ANI (%)
' <i>B. acaciae</i> ' 10BB	GCA_020889785	8 467 363	63.69	7728	23.8	21.5 - 26.3	82.84
<i>B. agreste</i> CNPSo 4010	GCF_016031625	7 871 244	63.81	7273	31.9	29.5 - 34.4	87.45
<i>B. algeriense</i> RST89	GCA_003062285	8 824 342	62.14	9374	23.4	21.1 - 25.8	81.65
' <i>B. altum</i> ' Pear77	GCA_020889705	9 749 879	63.32	8843	24.1	21.8 - 26.6	82.86
<i>B. amphicarpaceae</i> 39S1MB	GCA_002266435	7 044 517	64.66	6441	32.1	29.7 - 34.6	87.80
<i>B. arachidis</i> CCBAU 51107	GCA_900116675	9 792 874	63.63	9312	33.4	31.0 - 35.9	88.27
<i>B. archetypum</i> WSM 1744	GCA_013114835	7 015 311	62.26	6298	22.5	20.2 - 25.0	81.69
<i>B. australiense</i> WSM 1791	GCA_013114825	7 435 178	61.88	6654	22.4	20.1 - 24.8	81.43
<i>B. betae</i> PL7HG1	GCA_008932115	7 419 402	64.36	6780	33.4	31.0 - 35.9	88.32
' <i>B. brasiliense</i> ' UFLA03-321	GCA_001969825	8 595 048	63.93	7632	23.9	21.6 - 26.4	83.00
<i>B. cajani</i> AMBPC1010	GCA_009759665	8 372 827	63.96	7348	33.5	31.0 - 36.0	88.13
' <i>B. campsiandrae</i> ' INPA 394B	GCA_014529705	9 051 172	63.65	8467	32.6	30.2 - 35.1	87.97
<i>B. canariense</i> BTA-1	GCA_019402665	8 555 725	63.16	7737	32.3	29.8 - 34.8	87.66
' <i>B. centrolobii</i> ' BR 10245	GCA_001641635	10 106 545	63.22	8821	31.8	29.4 - 34.3	87.40
<i>B. cosmicum</i> 58S1	GCF_007290395	7 304 136	64.33	6924	33.2	30.8 - 35.7	88.07
<i>B. cytisi</i> CTAW11	GCA_008123515	8 849 532	63.17	7900	34.7	32.3 - 37.2	88.74
<i>B. daqingense</i> CCBAU 15774	GCA_007830205	7 885 317	63.74	7442	31.9	29.5 - 34.4	87.49
<i>B. denitrificans</i> LMG 8443	GCF_013179005	8 333 290	64.75	7596	22.7	20.4 - 25.2	81.7
<i>B. diazoefficiens</i> USDA 110	GCA_000011365	9 105 828	64.06	8317	34.5	32.1 - 37.0	88.86
<i>B. diversitatis</i> CNPSo 4019	GCF_016031635	8 437 014	63.93	7915	32.4	30.0 - 34.9	87.70
<i>B. elkanii</i> ATCC 49852	GCA_000379145	9 482 180	63.72	8962	24	21.7 - 26.5	83.02
<i>B. embrapense</i> SEMIA 6208	GCA_001189235	8 267 800	63.98	7721	23.7	21.4 - 26.1	82.86
' <i>B. forestalis</i> ' INPA54B	GCA_002795245	8 235 644	63.87	7360	32.8	30.4 - 35.3	87.91
<i>B. frederickii</i> CNPSo 3426	GCA_004570865	8 291 487	63.85	7506	32.4	30.0 - 34.9	87.68
<i>B. glycinis</i> CNPSo 4016	GCF_016031655	7 790 296	63.73	7322	32	29.6 - 34.5	87.56
<i>B. guangdongense</i> CCBAU 51649	GCA_004114975	8 437 991	63.26	7648	32.4	30.0 - 34.9	87.82
<i>B. guangxiense</i> CCBAU 53363	GCA_004114915	8 200 121	63.91	7796	32.1	29.7 - 34.6	87.63
' <i>B. guangzhouense</i> ' CCBAU 51670	GCF_004114955	8 138 177	63.39	7653	32.8	30.4 - 35.3	88.13
<i>B. hipponense</i> aSej3	GCA_008123965	8 813 045	62.86	7834	32.3	29.9 - 34.9	87.51
<i>B. huanghuaihaiense</i> CCBAU 23303	GCA_007830635	9 223 029	63.94	8622	33.7	31.3 - 36.3	88.4
<i>B. icense</i> LMTR 13	GCA_001693385	8 322 773	62.03	7425	22.5	20.3 - 25.0	81.85
<i>B. ivorensis</i> CI-1B	GCA_900696085	9 409 880	64.21	8546	24.1	21.8 - 26.6	83.14
<i>B. japonicum</i> USDA 6	GCA_000284375	9 207 384	63.67	8829	33.9	31.4 - 36.4	88.42
<i>B. jicamae</i> PAC 68	GCA_001440395	8 714 978	62.4	7745	23	20.7 - 25.4	82.03
<i>B. lablabi</i> CCBAU 23086	GCA_001440475	8 797 398	62.63	7781	23	20.7 - 25.4	82.03

<i>Bradyrhizobium</i> type strain*	Genome assembly accession ^b	Genome length (bp)	Genome %G+C	Protein number	dDDH [†] (%)	CI (%)	ANI (%)
<i>B. lupini</i> DSM 30140	GCA_014194605	9 125 394	63.7	8822	33.8	31.4 - 36.4	88.44
' <i>B. macuxiense</i> ' BR 10303	GCF_001542415	8 713 343	63.25	8184	23.5	21.2 - 26.0	82.63
<i>B. manausense</i> BR 3351	GCA_001440035	9 133 688	62.87	7794	32.6	30.2 - 35.1	87.99
<i>B. mercantei</i> SEMIA 6399	GCA_001982635	8 842 806	63.99	8096	23.8	21.5 - 26.3	83.04
<i>B. murdochi</i> WSM1741	GCA_000472965	7 950 819	62.1	7513	22.6	20.3 - 25.1	81.63
<i>B. nanningense</i> CCBAU 53390	GCF_004114535	8 284 532	63.44	7716	32.2	29.8 - 34.7	87.73
<i>B. neotropiale</i> BR 10247	GCA_001641695	8 676 530	63.6	7682	31.5	29.1 - 34.0	87.34
<i>B. niftali</i> CNPSo 3448	GCA_004571025	9 786 055	63.53	8664	34.6	32.2 - 37.2	88.70
<i>B. nitroreducens</i> TSA1	GCA_002776695	8 197 857	64.31	7268	32.6	30.1 - 35.1	87.84
<i>B. oligotrophicum</i> S58	GCA_000344805	8 264 165	65.13	7228	22.7	20.5 - 25.2	81.93
' <i>B. oropedii</i> ' Pear76	GCA_020889685	9 026 748	63.57	8155	23.9	21.6 - 26.4	82.84
<i>B. ottawaense</i> OO99	GCA_002278135	8 677 089	63.82	7774	32.8	30.4 - 35.3	88.00
<i>B. pachyrhizi</i> PAC48	GCA_001189245	8 706 184	63.76	8213	23.9	21.6 - 26.4	82.92
<i>B. paxllaeri</i> LMTR 21	GCA_001693515	8 290 608	62.49	7430	23	20.7 - 25.4	82.06
<i>B. quebecense</i> 66S1MB	GCA_013373795	9 032 145	63.82	7999	23.8	21.5 - 26.3	82.92
<i>B. retamae</i> Ro19	GCA_001440415	8 466 225	61.87	7481	22.6	20.4 - 25.1	81.53
<i>B. rifense</i> CTAW71	GCA_008123425	9 952 701	63.02	8975	34.8	32.3 - 37.3	88.91
' <i>B. sacchari</i> ' P9-20	GCF_002068095	8 685 818	63.84	8163	33.1	30.7 - 35.7	88.11
<i>B. septentrionale</i> 1S1	GCA_011516645	10 598 256	63.49	9227	23.9	21.6 - 26.4	82.96
<i>B. shewense</i> ERR11	GCF_900094605	9 162 066	63.23	8018	32.7	30.3 - 35.3	88.03
<i>B. stylosanthis</i> BR 446	GCA_001641335	8 801 645	64.56	8141	33.4	30.9 - 35.9	88.28
<i>B. symbiodeficiens</i> 85S1MB	GCF_002266465	7 039 503	64.27	6585	32.3	29.9 - 34.8	87.88
<i>B. tropiciagri</i> SEMIA 6148	GCA_001189845	9 767 314	63.49	9348	23.9	21.6 - 26.3	82.89
' <i>B. uaiense</i> ' UFLA03 164	GCA_010811875	9 868 029	63.27	9162	23.7	21.4 - 26.2	82.78
' <i>B. valentinum</i> ' LmjM3	GCA_001440405	8 808 080	61.92	7770	22.9	20.6 - 25.4	81.59
<i>B. vignae</i> 7-2	GCA_004114425	8 169 430	63.22	7297	32.3	29.8 - 34.8	87.50
<i>B. viridifuturi</i> SEMIA 690	GCA_001238275	8 811 922	64.03	8790	23.7	21.4 - 26.2	82.92
<i>B. yuanmingense</i> CCBAU 10071	GCA_900094575	8 201 482	63.77	7776	31.9	29.5 - 34.4	87.39
' <i>B. zhanjiangense</i> ' CCBAU 51778	GCF_004114935	9 342 022	62.94	8857	32.7	30.3 - 35.2	87.79

*Names between quotation marks are not validly published names under the International Code of Nomenclature of Prokaryotes (ICNP).

[†]GBDP formula d4 corresponding to the sum of all identities found in high-scoring segment pairs (HSPs) divided by overall HSP length [37].

Bradyrhizobium genospecies B was initially characterized using restriction fragment length polymorphism and SSU rRNA gene sequencing [19–22], and its relationships to other rhizobial lineages assessed using SSU rRNA gene phylogeny when only a limited number of *Bradyrhizobium* species had been formerly described. *Bradyrhizobium* genospecies B then represented a separate lineage related to *B. japonicum* [19–22]. The reappraisal of the SSU rRNA gene phylogenetic position of strain BDV5040^T using an updated sampling of *Bradyrhizobium* species validates the original proposal that it represents a distinct *Bradyrhizobium* species within the *B. japonicum* supergroup [35,

36]. This is further substantiated by the whole genome phylogeny analysis, as well as the inter-species dDDH (all below 35%) and ANI values between the proposed new species and the type strains for known *Bradyrhizobium* species for which a genome sequence is available. All ANI values are below the threshold of 95% that corresponds to the recommended cut-off point of 70% DNA–DNA hybridization for delineation of bacterial species [33]. They range from 87.3 to 88.9% for the pairwise comparisons with type strains of the *B. Japonicum* supergroup, the highest ANI values being observed with *B. rifense*, *B. cytisi*, *B. niftali* and *B. diazoefficiens*, and ranged 82.6–83.1%, 81.4–82.0% and 81.7–81.9% for the comparisons with type strains of the *B. elkanii* supergroup, the *B. jicamae* supergroup, and the photosynthetic *Bradyrhizobium* supergroup, respectively. Accordingly, dDDH values were highest for species of the *B. Japonicum* supergroup, and *B. rifense*, *B. cytisi*, *B. niftali* and *B. diazoefficiens* within it. However, when considering the phylogenetic data, strain BDV5040^T is not most closely related to any known *Bradyrhizobium* species in particular. Although grouping significantly with multiple type strains of *Bradyrhizobium*, it mainly appears to be intermediate between major lineages of the *B. Japonicum* supergroup.

Physiology and chemotaxonomy

Phenotypic tests were performed at 28 °C and included the ability to grow in modified YMA medium [25] with the addition of 0.0, 0.5, 1.0, 1.5 or 2.0% NaCl, in modified YMA medium at pH 4.0, 5.0, 6.0, 6.5, 6.8, 7.0, 7.5, 8.0, 8.5 or 9.0, and in solid Luria–Bertani (LB) medium. The ability to grow at various temperatures (15, 20, 28, 34, 37 and 40 °C) was also tested. All analyses were conducted in triplicate and evaluated over the course of 10 days of incubation. Growth of strain BDV5040^T is observed at 15–37 °C, pH 6.5–8.0 and only NaCl concentration below 0.5%. Optimal growth was obtained at 28 °C, pH 6.5 and in the absence of NaCl. Antibiotic tolerance was evaluated using the disc-diffusion method on YMA plates with the following antibiotics (concentration per disc): ampicillin (10 µg ml⁻¹), chloramphenicol (30 µg ml⁻¹), erythromycin (15 µg ml⁻¹), gentamycin (30 µg ml⁻¹), kanamycin (30 µg ml⁻¹), nalidixic acid (30 µg ml⁻¹), rifampicine (10 µg ml⁻¹), and streptomycin (10 µg ml⁻¹). Strain BDV5040^T could tolerate kanamycin, rifampicine and streptomycin. Enzymatic activities and carbon (C) source utilization capacities were evaluated using the API 20NE kit (bioMérieux) following the manufacturer's recommendations. YM without mannitol was used as basal medium and bromothymol blue as indicator of acid or alkaline reaction when the use of each C source was evaluated. The main properties are included in the species description.

Description of *Bradyrhizobium commune* sp. nov.

Bradyrhizobium commune (com.mu'ne. L. neut. adj. commune, common, widespread, referring to the widespread distribution of the species across variable environmental conditions and the ability to associate with a diversity of Australian legume hosts).

Cells are Gram-negative, non-spore-forming aerobic rods. Colonies are circular, mucoid, pearl white and convex on YMA medium after 7 days of growth at 28 °C. Optimum growth occurs at a pH of 6.5 and 28 °C, although growth will also occur at pH 6.0–8.0 and 15–37 °C. Strain BDV5040^T does not grow in the presence of NaCl or in LB medium. It is resistant to kanamycin (30 µg ml⁻¹), rifampicine (10 µg ml⁻¹) and streptomycin (10 µg ml⁻¹). The type strain is susceptible to ampicillin (10 µg ml⁻¹), chloramphenicol (30 µg ml⁻¹), erythromycin (15 µg ml⁻¹), gentamycin (30 µg ml⁻¹) and nalidixic acid (30 µg ml⁻¹). Strain BDV5040^T tests negative for nitrate reduction, indole production, glucose fermentation and gelatin hydrolysis. The type strain tests positive for urease, arginine dihydrolase, β-galactosidase and β-glucosidase activities. This strain is capable of assimilating d-glucose as carbon source but has tested negative for the assimilation of d-mannitol, d-mannose, maltose, l-arabinose, N-acetylglucosamine, adipic acid, capric acid, malic acid, phenylacetic acid, trisodium citrate and potassium gluconate. It may induce effective nitrogen-fixing nodules on its original host.

The type strain is BDV5040^T (=CFBP 9110^T=LMG 32898^T), isolated from nodules of *Bossiaea ensata* in Australia. The DNA G+C content of strain BDV5040^T is 62.3 mol%. Molecular accession numbers for type strain are: SSU rDNA (Z94812), *nodA* (AJ920043), chromosome (CP061379), genome assembly (GCA_015624505).

Funding information

This work received no specific grant from any funding agency

Author contributions

Conceived designed and performed the experiments: B.L., E.C., O.G. Analysed the data: B.L., O.G. Drafted the manuscript: B.L., O.G. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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