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***Bradyrhizobium hardenbergiae* sp. nov., isolated from *Hardenbergia violacea* in Australia, represents a novel basal lineage of the *B. elkanii* supergroup**

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ABSTRACT

Bradyrhizobia are widespread across the Australian continent, where they are essential to Australian ecosystems by helping legumes to compensate nutrient deficiencies and low fertility of Australian soils. Among the *Bradyrhizobium* genospecies identified during a survey of Australian native rhizobia communities in 1994-1995, genospecies L appeared to be only distantly related to any *Bradyrhizobium* lineages known at the time. We take advantage of the recent sequencing of the genome of strain BDV5419, the original strain corresponding to *Bradyrhizobium* genospecies L, to re-assess this lineage taxonomic status. We characterized further strain BDV5419 based on morpho-physiological traits and determined its phylogenetic relationships with the type strains of the 88 currently known *Bradyrhizobium* species based on sequence comparisons of SSU rRNA genes and complete genomes. The digital DNA–DNA hybridization relatedness with any type strain was less than 33% and both SSU rRNA gene and genome phylogenies confirmed that this strain does not belong to any formerly described species within the *Bradyrhizobium* genus. Whereas its position within the lineage encompassing the *B. elkanii* and *B. jicamae* supergroups is unresolved in the SSU rDNA phylogeny, strain BDV5419 appears to be one of most basal lineages of the *B. elkanii* supergroup in the genome comparison. All data thus support the description of the novel species *Bradyrhizobium hardenbergiae* sp. nov. which type strain is BDV5419^T (= CFBP 9111^T = LMG 32897^T), isolated from a nodule of *Hardenbergia violacea* in Black Mountain Nature Reserve, in Canberra, ACT, Australia.

Keywords

Bradyrhizobium, Australia, native legume host, SSU rDNA, genome phylogeny

Abbreviations

ACT: Australian Capital Territory

ANI: Average Nucleotide Identity

ICNP: International Code of Nomenclature of Prokaryotes

dDDH: digital DNA-DNA Hybridization

GBDP: Genome BLAST Distance Phylogeny

LB: Luria-Bertani

LPSN: List of Prokaryotic names with Standing in Nomenclature

OGRI: Overall Genome Relatedness Index

YMA: yeast extract–mannitol agar

Bradyrhizobium bacteria (*Hyphomicrobiales: Nitrobacteraceae*) are the most ubiquitous and abundant bacteria in soils (1). Whilst they may live as saprophytes (2), they are foremost known as nodule-inducing nitrogen-fixing bacteria capable of associating symbiotically with Fabaceae (3). They are the most frequently isolated rhizobia (4) and nodulate the widest range of legume genera (5), among which the most basal lineages within the Caesalpinoideae (6). In addition, *Bradyrhizobium* is the only genus found in the wild in symbiotic association with *Parasponia* (Cannabaceae), the sole currently known non-leguminous plants that can establish a nitrogen fixing rhizobial mutualism (7,8). *Bradyrhizobium* is also remarkable among rhizobia for having evolved alternative Nod-factor independent nodulation strategies (9). The *Bradyrhizobium* genus has thus been proposed to be the most ancestral symbiont of legumes, from which other rhizobia-legume symbioses evolved (5,10). Importantly, *Bradyrhizobium* bacteria interact symbiotically with legumes of high agronomic importance, among which grain legume, and are the most widely used rhizobia in agriculture. They thus limit the use of nitrogen fertilizers for the promotion of legume crop growth, and more generally, contribute to the nitrogen enrichment of the terrestrial ecosystem, hence playing a very important role in the preservation of the environment.

The *Bradyrhizobium* genus harbouring a single species was created in 1982 to account for their slow growth comparatively to other rhizobia (11) and only three species had been formerly described up until the years 2000. *Bradyrhizobium* has long been considered a 'taxonomically difficult' group of organisms due to their highly conserved SSU rRNA gene sequence and to the poor correlation between the groupings formed on the basis of genotypic and phenotypic traits. The emergence of new molecular and bioinformatic methods in the last 20 years as well as the extensive use of whole genome sequence data is now providing a clearer picture of the diversity of this genus. To this date, *Bradyrhizobium* comprises eighty-eight species organized into three major lineages (3) and/or four of the seven supergroups/superclades defined by genome phylogeny (12,13), with intermingled symbiotic and non-symbiotic taxa.

In Australia, *Bradyrhizobium* bacteria are particularly abundant and prevail in Australian acidic and seasonally dry soils (14-18), where they help legumes that constitute about 10% of the estimated 18,000 native plant species, to circumvent nutrient deficiencies and low fertility of Australian soils. Among novel lineages identified in a survey of Australian bradyrhizobia isolated from nodules of indigenous legumes based on SSU rRNA gene sequence analyses (14), *Bradyrhizobium* genospecies L could not unambiguously be affiliated to either of *Bradyrhizobium* major lineages despite its SSU rRNA gene exhibiting the *B. elkanii* lineage signature (14).

We here describe a new *Bradyrhizobium* species corresponding to Australian *Bradyrhizobium* genospecies L (14), with BDV5419 for which the complete genome sequence was recently obtained (19) as type strain. *Bradyrhizobium* genospecies L was isolated only once, from *Hardenbergia violacea*. We thus propose naming this novel species *Bradyrhizobium hardenbergiae* sp. nov.

Isolation and Ecology

Strain BDV5419 was isolated in 1995 from a root nodule of *Hardenbergia violaceae* (Fabaceae: Papilionoideae: Phaeseoleae) collected in Black Mountain Nature Reserve, in Canberra, Australian Capital Territory, Australia (35°16'1.20"S, 149°05'60.00"E), during a survey of rhizobia associated to native shrubby legumes in south-eastern Australia (14). It is a representative of *Bradyrhizobium* genospecies L which was isolated in a single occasion (14). It induces effective nitrogen-fixing nodules on its original host (14).

The strain was short-term cultured on modified yeast extract–mannitol agar (YMA) medium at 25°C (20) 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).

16S rRNA gene phylogenies

The first characterization of strain BDV5419 about twenty-five years ago suggested that it represents a novel lineage loosely related to the *B. elkanii* lineage based on SSU rRNA gene sequence phylogeny (14). Many *Bradyrhizobium* species have since been described, which calls for the phylogenetic position of strain BDV5419 within the *Bradyrhizobium* genus to be reassessed. Strain BDV5419 SSU rRNA gene was compared to those of the 88 current *Bradyrhizobium* type species as listed in the List of Prokaryotic names with Standing in Nomenclature (LPSN) database (21) (accessed on March 10th 2024). The SSU rRNA gene sequences were obtained from NCBI Genbank/RefSeq database and analyzed using SeaView version 5.0.4 (22). Sequences were aligned using ClustalO and a maximum likelihood phylogeny was inferred using PhyML version 3.1 (23) with the general time reversible (GTR) 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.* (24). Internal branch supports were evaluated using the approximate likelihood ratio test based on a Shimodaira-Hasegawa-like procedure ($n=1,000$) (23). The resulting phylogeny confirmed that strain BDV5419 does not group with any formerly described species and represents a novel lineage most basal to the *B. elkanii* lineage (3) that encompasses both the *B. elkanii* and *B. jicamae* supergroups (12, 13) (Figure 1). Including type strains of other *Nitrobacteraceae* type species did not affect the grouping of strain BDV5419 within the *B. elkanii* lineage (Data not shown).

Genome Features

We further investigated the relationships of strain BDV5419 to known species of *Bradyrhizobium* using whole genome sequence comparison. The complete sequence of strain BDV5419 genome has been reported elsewhere (19). It consists in a 7.40 Mb chromosome with an average G+C content of 64.6 % and encodes 6,879 protein-coding genes, 48 tRNAs, one copy each of the 5S, 16S and 23S rRNA genes, and 94 pseudogenes. Average Nucleotide Identities (ANI) between strain BDV5419 and the 77 *Bradyrhizobium* species with whole genome sequence data (computed using GTDB FastANI Calculator (25) on March 3rd 2024) showed *Bradyrhizobium* genospecies L to be distinct from any known *Bradyrhizobium* species and more closely related to members of the *B. elkanii* supergroup (Table 1). The genome-based taxonomic status of strain BDV5419 was further evaluated using the Type (strain) Genome Server (TYGS) at Leibniz Institute DSMZ (26,27). In connection to 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 infer a minimum evolution tree with branch support (27). These analyses (performed on March 15th 2024) confirmed that strain BDV5419 constitutes a separate species within the *Bradyrhizobium* genus, most basal with *B. ivorensis*, *B. semiaridum* and “*B. macuxiense*” to the *B. elkanii* supergroup (Figure 2; Table 1).

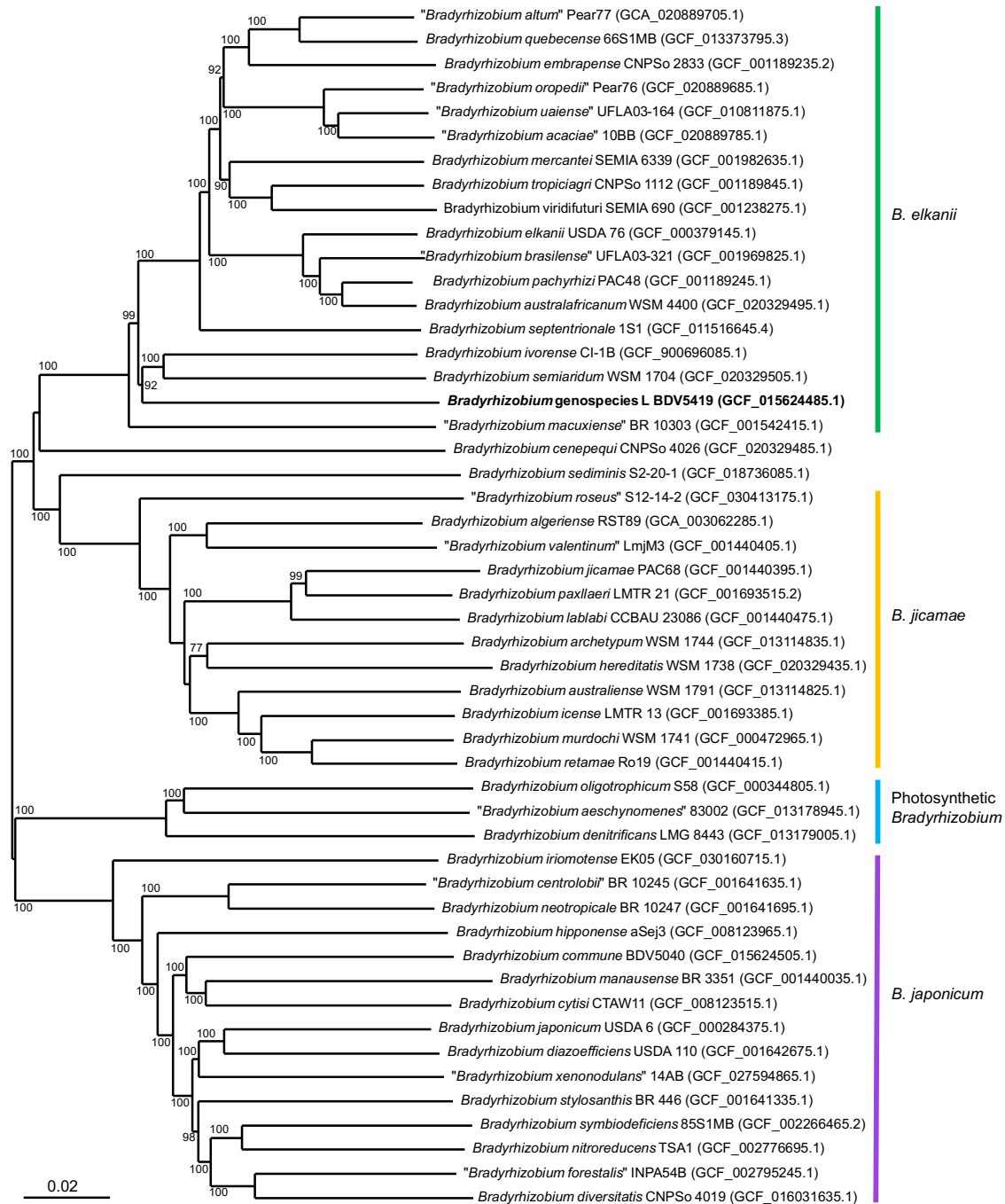


Figure 2. Genome phylogeny of *Bradyrhizobium* genospecies L BDV5419 with all available type strains of *B. elkanii*, *B. jicamae* and Photosynthetic *Bradyrhizobium* supergroups and representatives of the species diversity of the *B. japonicum* supergroup. Supergroup colouring is according to Avontuur *et al.* (12). 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.

Table 1. Digital DNA-DNA hybridization (dDDH) values with confidence intervals (CI) and Average Nucleotide Identities (ANI) between *Bradyrhizobium* genospecies L BDV5419 and *Bradyrhizobium* type strains with genome data. Genome sequence accession numbers and major characteristics are provided. dDDH values were calculated using TYGS (26, 27). ANIs were computed using GTDB FastANI Calculator (25).

<i>Bradyrhizobium</i> type strain ^a	Genome assembly accession	Genome length (bp)	Genome %G+C	Protein number	dDDH ^b (%)	CI (%)	ANI (%)
" <i>B. acaciae</i> " 10BB	GCF_020889785.1	8 471 967	63,69	8102	31.70	[29.3 - 34.2]	87.68
" <i>B. aeschynomenes</i> " 83002	GCF_013178945.1	7 522 254	65,42	6743	23.20	[20.9 - 25.6]	82.11
<i>B. agreste</i> CNPSo 4010	GCF_016031625.1	7 877 331	63,81	7292	24.00	[21.7 - 26.5]	83.10
<i>B. algeriense</i> RST89	GCA_003062285.1	8 824 342	62,14	9374	24.90	[22.6 - 27.4]	82.92
" <i>B. altum</i> " Pear77	GCA_020889705.1	9 758 985	63,32	9656	31.90	[29.5 - 34.4]	87.71
<i>B. amphicarpaceae</i> 39S1MB	GCF_002266435.2	7 044 517	64,66	6536	23.80	[21.5 - 26.2]	83.14
<i>B. arachidis</i> CCBAU 51107	GCF_015291705.1	9 865 973	63,62	9313	24.60	[22.3 - 27.1]	83.56
<i>B. archetypum</i> WSM 1744	GCF_013114835.1	7 017 218	62,26	6656	24.10	[21.8 - 26.6]	82.73
<i>B. australafricanum</i> WSM 4400	GCF_020329495.1	9 684 669	63,52	9241	32.10	[29.7 - 34.7]	87.93
<i>B. australiense</i> WSM 1791	GCF_013114825.1	7 436 256	61,88	7200	23.90	[21.6 - 26.3]	82.56
<i>B. barranii</i> 144S4	GCF_017565645.3	11 399 526	63,13	11285	24.10	[21.8 - 26.5]	83.05
<i>B. betae</i> PL7HG1	GCF_008932115.1	7 419 402	64,36	7103	23.90	[21.6 - 26.4]	83.19
" <i>B. brasiliense</i> " UFLA03-321	GCF_001969825.1	8 595 048	63,93	7961	32.20	[29.8 - 34.7]	87.98
<i>B. cajani</i> AMBPC1010	GCF_009759665.1	8 381 257	63,96	7672	24.20	[21.9 - 26.6]	83.24
" <i>B. campsiandrae</i> " UFLA 01-1174	GCF_014529705.1	9 084 813	63,63	8764	23.70	[21.4 - 26.2]	82.93
<i>B. canariense</i> BTA-1	GCF_019402665.1	8 561 472	63,16	8182	24.00	[21.7 - 26.5]	83.07
<i>B. cenepequi</i> CNPSo 4026	GCF_020329485.1	8 472 857	62,31	8181	24.90	[22.5 - 27.3]	83.36
" <i>B. centrolobii</i> " BR 10245	GCF_001641635.1	10 111 113	63,22	9545	24.00	[21.7 - 26.5]	83.10
<i>B. commune</i> BDV5040	GCF_015624505.1	7 622 528	63,92	7107	23.80	[21.5 - 26.2]	83.15
<i>B. cosmicum</i> 58S1	GCF_007290395.1	7 304 136	64,33	6925	23.90	[21.5 - 26.3]	83.10
<i>B. cytisi</i> CTAW11	GCF_008123515.1	8 854 489	63,17	8449	24.00	[21.7 - 26.4]	83.16
<i>B. daqingense</i> CCBAU 15774	GCF_021044685.1	7 998 085	63,72	7566	23.80	[21.5 - 26.2]	82.82
<i>B. denitrificans</i> LMG 8443	GCF_013179005.1	8 333 290	64,75	7596	23.00	[20.7 - 25.4]	81.81
<i>B. diazoefficiens</i> USDA 110	GCF_001642675.1	9 106 064	64,06	8493	24.20	[21.9 - 26.7]	83.38
<i>B. diversitatis</i> CNPSo 4019	GCF_016031635.1	8 450 368	63,93	8050	24.20	[21.9 - 26.7]	83.04
<i>B. elkani</i> USDA 76	GCF_000379145.1	9 482 467	63,72	8973	32.20	[29.8 - 34.7]	87.99
<i>B. embrapense</i> CNPSo 2833	GCF_001189235.2	8 267 832	63,98	7736	31.60	[29.2 - 34.1]	87.68
" <i>B. forestalis</i> " INPA54B	GCF_002795245.1	8 252 029	63,86	7606	24.10	[21.8 - 26.6]	83.07
<i>B. frederickii</i> CNPSo 3426	GCF_004570865.1	8 294 086	63,85	7853	24.00	[21.7 - 26.5]	83.26
<i>B. glycinis</i> CNPSo 4016	GCF_016031655.1	7 794 411	63,73	7393	24.00	[21.7 - 26.4]	82.89
<i>B. guangdongense</i> CCBAU 51649	GCF_004114975.1	8 437 991	63,26	8042	24.00	[21.7 - 26.5]	82.95
<i>B. guangxiense</i> CCBAU 53363	GCF_004114915.1	8 200 121	63,91	7796	23.80	[21.5 - 26.3]	83.05
" <i>B. guangzhouense</i> " CCBAU 51670	GCF_004114955.1	8 138 177	63,39	7654	23.50	[21.2 - 25.9]	82.84
<i>B. hereditatis</i> WSM 1738	GCF_020329435.1	7 871 253	61,99	7462	24.10	[21.8 - 26.6]	82.54
<i>B. hipponense</i> aSej3	GCF_008123965.1	8 826 946	62,86	8456	23.70	[21.4 - 26.1]	82.84
<i>B. huanghuaihaiense</i> CCBAU 23303	GCF_007830635.1	9 226 856	63,94	8715	24.10	[21.8 - 26.5]	83.37
<i>B. icense</i> LMTR 13	GCF_001693385.1	8 322 773	62,03	7965	24.20	[21.9 - 26.7]	82.84
<i>B. iriomotense</i> EK05	GCF_030160715.1	9 789 577	62,83	9109	24.00	[21.7 - 26.5]	82.95

Bradyrhizobium type strain^a	Genome assembly accession	Genome length (bp)	Genome %G+C	Protein number	dDDH^b (%)	CI (%)	ANI (%)
<i>B. ivorense</i> CI-1B	GCF_900696085.1	9 412 302	64,21	8590	32.80	[30.4 - 35.4]	88.30
<i>B. japonicum</i> USDA 6	GCF_000284375.1	9 207 384	63,67	8701	24.10	[21.8 - 26.5]	83.12
<i>B. jicamae</i> PAC68	GCF_001440395.1	8 738 846	62,4	8497	24.50	[22.2 - 27.0]	83.23
<i>B. lablabi</i> CCBAU 23086	GCF_001440475.1	8 817 291	62,63	8553	24.50	[22.1 - 26.9]	83.13
<i>B. liaoningense</i> 2281	GCF_030160735.1	7 899 289	63,91	8011	24.10	[21.8 - 26.6]	83.20
" <i>B. macuxiense</i> " BR 10303	GCF_001542415.1	8 722 143	63,25	8202	30.90	[28.5 - 33.4]	87.16
<i>B. manausense</i> BR 3351	GCF_001440035.1	9 145 311	62,87	8735	23.70	[21.4 - 26.2]	82.89
<i>B. mercantei</i> SEMIA 6399	GCF_001982635.1	8 842 857	63,99	8142	31.80	[29.4 - 34.3]	88.10
<i>B. murdochi</i> WSM 1741	GCF_000472965.1	7 952 271	62,09	7570	24.10	[21.8 - 26.6]	82.74
<i>B. nanningense</i> CCBAU 53390	GCF_004114535.1	8 290 088	63,44	7750	23.90	[21.6 - 26.3]	83.07
<i>B. neotropicale</i> BR 10247	GCF_001641695.1	8 679 329	63,6	8152	24.00	[21.7 - 26.5]	83.04
<i>B. niftali</i> CNPSo 3448	GCF_004571025.1	9 787 683	63,53	9214	24.20	[21.9 - 26.7]	83.38
<i>B. nitroreducens</i> TSA1	GCF_002776695.1	8 199 267	64,31	7569	24.20	[21.9 - 26.7]	83.37
<i>B. oligotrophicum</i> S58	GCF_000344805.1	8 264 165	65,13	7164	23.20	[20.9 - 25.6]	82.17
" <i>B. oropedii</i> " Pear76	GCF_020889685.1	9 043 029	63,57	8709	31.70	[29.3 - 34.2]	87.67
<i>B. ottawaense</i> OO99	GCF_002278135.2	8 606 328	63,83	8012	24.20	[21.8 - 26.6]	83.25
<i>B. pachyrhizi</i> PAC48	GCF_001189245.1	8 711 973	63,76	8253	31.90	[29.5 - 34.4]	87.82
<i>B. paxllaeri</i> LMTR 21	GCF_001693515.2	8 377 719	62,51	7901	24.40	[22.1 - 26.9]	83.28
" <i>B. prioritasperba</i> " BL16A	GCF_032397745.1	9 083 454	63,05	8395	21.60	[19.4 - 24.0]	80.02
<i>B. quebecense</i> 66S1MB	GCF_013373795.3	9 172 345	63,78	8778	31.80	[29.4 - 34.3]	87.91
<i>B. retamae</i> Ro19	GCF_001440415.1	8 466 225	61,87	8344	23.90	[21.6 - 26.4]	82.63
<i>B. rifense</i> CTAW71	GCF_008123425.1	9 952 932	63,02	9508	23.80	[21.5 - 26.3]	83.00
" <i>B. roseus</i> " S12-14-2	GCF_030413175.1	7 319 411	63,33	6905	24.20	[21.9 - 26.6]	82.91
" <i>B. sacchari</i> " BR 10280	GCF_002068095.1	8 698 798	63,84	8158	23.90	[21.6 - 26.4]	83.13
<i>B. sediminis</i> S2-20-1	GCF_018736085.1	5 554 208	63,58	5289	24.00	[21.7 - 26.5]	82.72
<i>B. semiaridum</i> WSM 1704	GCF_020329505.1	6 712 655	65,1	6301	32.40	[29.9 - 34.9]	88.15
<i>B. septentrionale</i> 1S1	GCF_011516645.4	10 410 654	63,51	9996	32.20	[29.8 - 34.7]	88.02
<i>B. shewense</i> ERR11	GCF_900094605.1	9 163 226	63,23	8630	23.90	[21.6 - 26.3]	83.21
<i>B. stylosanthis</i> BR 446	GCF_001641335.1	8 801 717	64,56	8138	24.20	[21.9 - 26.6]	83.41
<i>B. symbiodeficiens</i> 85S1MB	GCF_002266465.2	7 039 503	64,27	6586	23.80	[21.5 - 26.2]	83.06
<i>B. tropiciagri</i> CNPSo 1112	GCF_001189845.1	9 767 314	63,49	9391	31.80	[29.4 - 34.3]	87.90
" <i>B. uaiense</i> " UFLA03-164	GCF_010811875.1	9 972 761	63,26	10057	31.80	[29.4 - 34.3]	87.59
" <i>B. valentinum</i> " LmjM3	GCF_001440405.1	8 845 258	61,92	8740	24.40	[22.1 - 26.9]	82.90
<i>B. vignae</i> 7-2	GCF_004114425.1	8 175 609	63,21	7762	23.90	[21.6 - 26.4]	82.90
<i>B. viridifuturi</i> SEMIA 690	GCF_001238275.1	8 811 922	64,03	8844	31.60	[29.2 - 34.1]	87.78
" <i>B. xenonodulans</i> " 14AB	GCF_027594865.1	8 276 659	63,99	7720	24.00	[21.7 - 26.5]	83.32
<i>B. yuanmingense</i> CCBAU 10071	GCF_900094575.1	8 201 522	63,77	7854	23.90	[21.6 - 26.4]	82.87
" <i>B. zhanjiangense</i> " CCBAU 51778	GCF_004114935.1	9 342 022	62,94	8858	24.00	[21.7 - 26.4]	82.98
<i>B. zhengyangense</i> WYCCWR 13023	GCF_022012485.1	9 004 494	62,87	8692	23.60	[21.3 - 26.0]	82.69

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

^b GBDP formula d4 corresponding to the sum of all identities found in High Scoring segment Pairs (HSPs) divided by overall HSP length (29)

The initial characterization of *Bradyrhizobium* genospecies L relied on SSU rRNA gene restriction fragment length polymorphisms and sequencing followed by SSU rDNA phylogeny to assess its relationships to then known rhizobial lineages (14). *Bradyrhizobium* genospecies L appeared to be related to, although distant from, *B. elkanii*. The reevaluation of the SSU rRNA gene phylogenetic position of strain BDV5419 among an up-to-date sampling of *Bradyrhizobium* species confirms that it represents a distinct *Bradyrhizobium* species, more closely related to the *B. elkanii* lineage (3) than to either the *B. japonicum* or the Photosynthetic *Bradyrhizobium* lineages. However, the SSU rRNA gene phylogeny does not resolve further the relationships of *Bradyrhizobium* genospecies L with members of the *B. elkanii* and *B. jicamae* supergroups that were later individualized using whole genome phylogenetic analyses (12, 13). Overall genomic relatedness indices (OGRI) dDDH and ANI between strain BDV5419 and the 77 *Bradyrhizobium* type strains for which a genome sequence is currently available confirmed the clear delineation of strain BDV5419, all pairwise comparisons being well below the inter-species 70% (dDDH) and 95-96% (ANI) threshold values (28). In contrast to the SSU rRNA gene sequence data, highest OGRI values unambiguously identified the species of the *B. elkanii* supergroup as the closest relatives of BDV5419 (dDDH values ranging from 30.9 to 32.8% and ANI values from 87.16 to 88.3%). Indeed, OGRIs between strain BDV5419 and species of the *B. jicamae* supergroups (23.9-24.9% for dDDH and 82.54-83.28% for ANI) were in the similar, intermediary, value range than that observed with species of the *B. japonicum* supergroup (23.5-24.6% for dDDH and 82.69-83.56% for ANI). Strain BDV5419 was more highly divergent from species of the Photosynthetic supergroup (23.0-23.2% for dDDH and 81.81-82.17% for ANI), and most from *B. prioritasuperba*. Interestingly, OGRIs for this species were in the range of dDDH and ANI values observed between strain BDV5419 and type species of other *Nitrobacteraceae* (Table 2).

Table 2. Digital DNA-DNA hybridization (dDDH) values with confidence intervals (CI) and Average Nucleotide Identities (ANI) between *Bradyrhizobium* genospecies L BDV5419 and type strains of *Nitrobacteraceae* type species with genome data. Genome sequence accession numbers and major characteristics are provided. dDDH values were calculated using TYGS (26, 27). ANIs were computed using GTDB FastANI Calculator (25).

<i>Nitrobacteraceae</i> type species type strain	Genome assembly accession	Genome length (bp)	Genome %G+C	Protein number	dDDH ^a (%)	CI (%)	ANI (%)
<i>Afipia felis</i> B-91-007352	GCF_900445155.1	4 195 257	60.72	4049	20.90	[18.7 - 23.4]	79.47
<i>Nitrobacter winogradskyi</i> Nb-255	GCF_000012725.1	3 402 093	62.05	3262	22.10	[19.8 - 24.5]	79.61
<i>Pseudolabrys taiwanensis</i> CC-BB4	GCF_003367395.1	5 593 741	64.39	5286	19.90	[17.7 - 22.3]	78.37
<i>Pseudorhodoplanes sinuspersici</i> RIPI 110	GCF_002119765.1	5 971 615	60.32	5795	19.60	[17.4 - 22]	77.79
<i>Rhodoplanes roseus</i> 941	GCF_003258865.1	6 879 536	69.35	6828	19.90	[17.7 - 22.3]	78.00
<i>Rhodopseudomonas palustris</i> 2.1.6	GCF_034479375.1	5 305 909	65.98	4736	22.60	[20.3 - 25.1]	81.67
<i>Tardiphaga robiniae</i> LMG 26467	GCF_013359755.1	6 318 076	61.51	5935	21.40	[19.2 - 23.8]	80.30
<i>Undibacter mobilis</i> GY_H	GCF_003367195.1	4 098 220	63.28	3910	19.90	[17.7 - 22.3]	78.30
<i>Variibacter gotjawalensis</i> GJW-30	GCF_002355335.1	4 586 237	62.23	4441	19.90	[17.7 - 22.3]	77.51

^a GBDP formula d4 corresponding to the sum of all identities found in High Scoring segment Pairs (HSPs) divided by overall HSP length (29)

Among species of the *B. elkanii* supergroup, closest relatives to strain BDV5419 were

B. ivorensis, *B. semiaridum*, *B. septentrionale*, *B. brasilense* and *B. elkanii* according to the dDDH values and *B. ivorensis*, *B. semiaridum*, *B. mercantei*, and *B. septentrionale* according to the ANI values. Accordingly, the genome-based phylogeny identifies strain BDV5419 as a novel species of the *B. elkanii* supergroup. In turn, alike the SSU rRNA gene phylogeny, it places it among the basal and most divergent lineages of this group with strain BDV5419 being most closely related to *B. ivorensis* and *B. semiaridum* although separated from them by long branches.

SSU rRNA gene phylogeny and OGRIs whilst providing valuable indication of bacterial inter-species relatedness failed to establish unambiguously the position of strain BDV5419 within the *Bradyrhizobium* genus. The complex evolutionary signal linking strain BDV5419 to the other *Bradyrhizobium* species could only be recovered using whole genome sequence-based phylogeny. This supports the recent proposition to center the taxonomic description of bacterial taxa on genome phylogeny using whole genome sequence data (28).

Physiology and Chemotaxonomy

Phenotypic tests were performed at 28 °C and included ability to grow in modified YMA medium (20) with the addition of 0.0%, 0.5%, 1%, 1.5% or 2% 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 temperature (15°C, 20°C, 28°C, 34°C, 37°C 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 BDV5419 is observed at 15–34°C, pH from 6.5–8.5 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 BDV5419 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.

Protologue

Bradyrhizobium hardenbergiae ((har.den.ber'gi.ae. N.L. gen. n. adj. *hardenbergiae*, of *Hardenbergia violaceae*, from which the type strain was isolated).

Cells are Gram-negative, non-spore-forming aerobic rods. Colonies are circular, mucoid, opaque white and convex on YMA medium after 7 days of growth at 28 °C. Optimum growth occurs at pH 6.5 and 28 °C, although growth will also occur at pH 6.0–8.5, and 15–34°C. Strain BDV5419 does not grow in the presence of NaCl or in LB medium. It is resistant to kanamycin (30 µg/ml), rifampicine (10 µg/ml) and streptomycin (10 µg/ml). The type strain is susceptible to ampicillin (10 µg/ml), chloramphenicol (30 µg/ml), erythromycin (15 µg/ml), gentamycin (30 µg/ml) and nalidixic acid (30 µg/ml). Strain BDV5419 tests negative for

indole production, glucose fermentation and gelatin hydrolysis. The type strain tests positive for nitrate reduction, urease, arginine dihydrolase, β -galactosidase and β -glucosidase activities. This strain is capable of assimilating D-glucose, D-maltose, D-mannose, L-arabinose, malic acid, phenylacetic acid and trisodium citrate as carbon source as carbon source but tested negative for assimilation of D-mannitol, N-acetylglucosamine, adipic acid, capric acid and potassium gluconate is negative. It may induce effective nitrogen-fixing nodules on its original host.

The type strain is BDV5419^T (=CFBP 9111^T = LMG 32897^T), isolated from nodules of *Hardenbergia violacea* in Australia. The DNA G+C content of strain BDV5419^T is 64.6 mol%. Molecular accession numbers for type strain are: SSU rDNA gene (Z94821), chromosome (CP061378), genome assembly (GCA_015624485).

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