

Supplementary Materials

Title: *Bradyrhizobium ontarionense* sp. nov. isolated from *Aeschynomene indica* (Indian jointvetch) harbours photosynthesis, nitrogen fixation and nitrous oxide (N₂O) reductase genes.

Authors: Eden S. P. Bromfield* and Sylvie Cloutier

Affiliation: Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, K1A 0C6, Canada.

***Correspondence:** Eden S. P. Bromfield; Email: eden.bromfield@agr.gc.ca

[illegible]

Strain	<i>atpD</i>	<i>glnII</i>	<i>gyrB</i>	<i>recA</i>	<i>rpoB</i>	<i>16S rRNA</i>	<i>nifHDK</i>	<i>nosZ</i>	<i>pufML</i>
<i>B. oligotrophicum</i> S58 ^T	AP012603	AP012603	AP012603	AP012603	AP012603	AP012603	AP012603	AP012603	AP012603
<i>‘B. oropedii’</i> Pear76 ^T	JACMYO01	JACMYO01	JACMYO01	JACMYO01	JACMYO01	JACMYO01	JACMYO01	NA	NA
<i>B. ottawaense</i> OO99 ^T	CP029425	CP029425	CP029425	CP029425	CP029425	CP029425	CP029425	CP029425	NA
<i>B. pachyrhizi</i> PAC48 ^T	LFIQ01	LFIQ01	LFIQ01	LFIQ01	LFIQ01	LFIQ01	LFIQ01	NA	NA
<i>B. paxllaeri</i> LMTR 21 ^T	CP042968	CP042968	CP042968	CP042968	CP042968	CP042968	CP042968	NA	NA
<i>B. quebecense</i> 66S1MB ^T	CP088022	CP088022	CP088022	CP088022	CP088022	CP088022	CP088022	NA	NA
<i>B. retamae</i> Ro19 ^T	LLYA01	LLYA01	LLYA01	LLYA01	LLYA01	LLYA01	LLYA01	NA	NA
<i>B. rifense</i> CTAW71 ^T	VSSS01	VSSS01	VSSS01	VSSS01	VSSS01	VSSS01	VSSS01	NA	NA
<i>B. ripae</i> WR4 ^T	NA	-	-	-	-	MF593082	NA	NA	NA
<i>‘B. sacchari’</i> BR 10280 ^T	LWIG01	LWIG01	LWIG01	LWIG01	LWIG01	LWIG01	LWIG01	NA	NA
<i>‘B. sediminis’</i> S2-20-1 ^T	CP076134	CP076134	CP076134	CP076134	CP076134	CP076134	CP076134	NA	NA
<i>B. semiaridum</i> CNPSo ^T	JAGKJJ01	JAGKJJ01	JAGKJJ01	JAGKJJ01	JAGKJJ01	JAGKJJ01	NA	NA	NA
<i>B. septentrionalis</i> 1S1 ^T	CP088285	CP088285	CP088285	CP088285	CP088285	CP088285	CP088285	NA	NA
<i>B. shewense</i> ERR11 ^T	FMAI01	FMAI01	FMAI01	FMAI01	FMAI01	FMAI01	FMAI01	FMAI01	NA
<i>B. stylosanthis</i> BR 446 ^T	LVEM01	LVEM01	LVEM01	LVEM01	LVEM01	LVEM01	LVEM01	LVEM01	NA
<i>B. subterraneum</i> 58 2-1 ^T	-	-	-	-	-	KP308152	NA	NA	NA
<i>B. symbiodeficiens</i> 85S1MB ^T	CP029427	CP029427	CP029427	CP029427	CP029427	CP029427	NA	NA	NA
<i>B. tropiciagri</i> CNPSo 1112 ^T	LFLZ01	LFLZ01	LFLZ01	LFLZ01	LFLZ01	LFLZ01	LFLZ01	NA	NA
<i>‘B. uaiense’</i> UFLA03-164 ^T	VKHP01	VKHP01	VKHP01	VKHP01	VKHP01	VKHP01	VKHP01	NA	NA
<i>‘B. valentinum’</i> LmjM3 ^T	LLXX01	LLXX01	LLXX01	LLXX01	LLXX01	LLXX01	LLXX01	NA	NA
<i>B. vignae</i> 7-2 ^T	RDQF01	RDQF01	RDQF01	RDQF01	RDQF01	RDQF01	RDQF01	NA	NA
<i>B. viridifuturi</i> SEMIA 690 ^T	LGTB01	LGTB01	LGTB01	LGTB01	LGTB01	LGTB01	LGTB01	NA	NA
<i>‘B. xenonodulans’</i> 14AB	CP089391	CP089391	CP089391	CP089391	CP089391	CP089391	CP089391	CP089391	NA
<i>B. yuanmingense</i> CCBAU 10071 ^T	FMAE01	FMAE01	FMAE01	FMAE01	FMAE01	FMAE01	FMAE01	NA	NA
<i>‘B. zhanjiangense’</i> CCBAU 51778 ^T	CP022221	CP022221	CP022221	CP022221	CP022221	CP022221	CP022221	NA	NA
<i>‘B. zhengyangense’</i> WYCCWR13023 ^T	JAKLTY01	JAKLTY01	JAKLTY01	JAKLTY01	JAKLTY01	JAKLTY01	JAKLTY01	JAKLTY01	NA
<i>Bradyrhizobium</i> sp. BTAi1	CP000494	CP000494	CP000494	CP000494	CP000494	CP000494	CP000494	CP000494	CP000494
<i>Bradyrhizobium</i> sp. ORS278	CU234118	CU234118	CU234118	CU234118	CU234118	CU234118	CU234118	NA	CU234118
<i>Bradyrhizobium</i> sp. ORS285	LT859959	LT859959	LT859959	LT859959	LT859959	LT859959	LT859959	LT859959	LT859959
<i>Bradyrhizobium</i> sp. ORS375	NA	NA	NA	NA	NA	NA	NA	NA	CAFI01
<i>Bradyrhizobium</i> sp. PSBB068	NA	NA	NA	NA	NA	NA	NA	NA	CP069300
<i>Bradyrhizobium</i> sp. SSBR45G	NA	NA	NA	NA	NA	NA	NA	NA	BSCT01
<i>Rhodopseudomonas palustris</i> HAa2	NA	NA	NA	NA	NA	NA	NA	NA	CP000250

NA, Not applicable, or, sequence not available in public databases.

- , Sequence not used in phylogenetic analyses either because sequence too short or not available in database

Table S2 Occurrence of the *nosZ* gene in genome sequences of species type strains of the genus *Bradyrhizobium*.

Species and type strain	Genome sequence accession no.	Symbiosis with legume host
<i>nosZ</i> gene present:		
<i>B. ontarionense</i> sp. nov. A19 ^T	CP088156	Yes
' <i>B. aescynomenes</i> ' 83002 ^T	JABFDM	Yes
<i>B. betae</i> PL7HG1 ^T	CP044543	No
<i>B. cenepequi</i> CNPSo 4026 ^T	JAGKJI	Yes
' <i>B. centrolonii</i> ' BR10245 ^T	LUUB	Yes
<i>B. cosmicum</i> 58S1 ^T	CP041656	No
<i>B. diazoefficiens</i> USDA 110 ^T	CP011360	Yes
' <i>B. forestalis</i> ' INPA54B ^T	PGVG	Yes
<i>B. frederickii</i> CNPSo 3426 ^T	SPQS	Yes
<i>B. guangdongense</i> CCBAU 51649 ^T	CP030051-CP030052 (plasmid)	Yes
<i>B. guangxiense</i> CCBAU 53363 ^T	CP022219-CP022220 (plasmid)	Yes
<i>B. lablabi</i> CCBAU 23086 ^T	LLYB	Yes
<i>B. nanningense</i> CCBAU 53390 ^T	LBJC	Yes
<i>B. neotropale</i> BR10247 ^T	LSEF	Yes
<i>B. nitroreducens</i> TSA1 ^T	LFJC	No
<i>B. oligotrophicum</i> S58 ^T	AP012603	Yes
<i>B. ottawaense</i> OO99 ^T	CP029425	Yes
<i>B. shewense</i> ERR11 ^T	FMAI	Yes
<i>B. stylosanthi</i> BR446 ^T	LVEM	Yes
' <i>B. xenonodulans</i> ' 14AB	CP089391	Yes
<i>B. zhengyangense</i> WYCCWR 13023 ^T	JAKLTY	Yes
<i>nosZ</i> gene absent:		
' <i>B. acaciae</i> ' 10BB ^T	JACMYK	Yes
<i>B. agreste</i> CNPSo 4010 ^T	JACCHP	Yes
<i>B. algeriense</i> RST89 ^T	PYCM	Yes
' <i>B. altum</i> ' Pear77 ^T	JACMYP	Yes
<i>B. amphicarpaceae</i> 39S1MB ^T	CP029426	No
<i>B. arachidis</i> CCBAU 051107 ^T	FPBQ	Yes
<i>B. archetypum</i> WSM 1744 ^T	JA AVLW	Yes
<i>B. australiense</i> WSM 1791 ^T	JA AVLX	Yes
<i>B. australafricanum</i> WSM 4400 ^T	JAGKJL	Yes
<i>B. barranii</i> 144S4 ^T	CP086136-CP086139	Yes
' <i>B. brasiliense</i> ' UFLA03-321 ^T	MPVQ00000000	Yes
<i>B. cajani</i> AMBPC1010 ^T	WQNE	Yes
' <i>B. campsiandrae</i> ' UFLA 01-1174 ^T	JA ANIH	No
<i>B. canariense</i> BTA-1 ^T	VSST	Yes
<i>B. commune</i> BDV5040 ^T	CP061379	Yes
<i>B. cytisi</i> CTAW11 ^T	VSSR	Yes
<i>B. daqingense</i> CCBAU 15774 ^T	CP0882014	Yes
<i>B. diversitatis</i> CNPSo 4019 ^T	JACEGD	Yes
<i>B. elkanii</i> USDA 76 ^T	ARAG	Yes
<i>B. embrapense</i> CNPSo 2833 ^T	LFIP	Yes
<i>B. glycinis</i> CNPSo 4016 ^T	JACCHQ	Yes
' <i>B. guangzhouense</i> ' CCBAU 51670 ^T	CP030053-CP030054	Yes
<i>B. hereditatis</i> WSM 1738 ^T	JAGKJK	Yes
<i>B. hipponense</i> aSej3 ^T	VSTH	Yes
<i>B. huanghuaihaiense</i> CCBAU 23303 ^T	VLLA	Yes
<i>B. icense</i> HAMB1 3584 ^T	CP016428	Yes
<i>B. ivorensis</i> CI-1B ^T	CAADFC	Yes
<i>B. japonicum</i> USDA6 ^T	AP012206	Yes
<i>B. jicamae</i> PAC68 ^T	NZ_LLXZ	Yes
' <i>B. macuxiense</i> ' BR10303 ^T	LNCU	Yes
<i>B. manausense</i> BR3351 ^T	LJYG	Yes
<i>B. mercantei</i> SEMIA 6399 ^T	MKFI	No
<i>B. murdochi</i> WSM 1741 ^T	AXAU	Yes
<i>B. nifitali</i> CNPSo 3448 ^T	SPQT	Yes
' <i>B. oropedii</i> ' Pear76 ^T	JACMYO	Yes
<i>B. pachyrhizi</i> PAC48 ^T	LFIQ	Yes
<i>B. paxllaeri</i> LMTR21 ^T	CP042968	Yes
<i>B. quebecense</i> 66S1MB ^T	CP088022-CP088025	Yes
<i>B. retamae</i> Ro19 ^T	LLYA	Yes
<i>B. rifense</i> CTAW71 ^T	VSSS	Yes
' <i>B. sacchari</i> ' BR10280 ^T	LWIG	Yes
' <i>B. sediminis</i> ' S2-20-1 ^T	CP076134	No
<i>B. septentrionale</i> 1S1 ^T	CP088284-CP088287	Yes
<i>B. semiaridum</i> WSM 1704 ^T	JAGKJJ	No
<i>B. symbiodeficiens</i> 85S1MB ^T	CP029427	No
<i>B. tropiciagri</i> CNPSo 1112 ^T	LFLLZ	Yes
' <i>B. uaiense</i> ' UFLA03-164 ^T	VKHP	Yes
' <i>B. valentinum</i> ' LmjM3 ^T	LLXX	Yes
<i>B. vignae</i> 7-2 ^T	RDQF	Yes
<i>B. viridifuturi</i> SEMIA 690 ^T	LGTB	Yes
<i>B. yuanmingense</i> CCBAU 10071 ^T	FMAE	Yes
' <i>B. zhanjiangense</i> ' CCBAU 51778 ^T	CP022221	Yes

Table S3 Growth characteristics of **1.** *B. ontarionense* sp. nov. A19^T, **2.** *B. oligotrophicum* S58^T, **3.** '*B. aeschynomenes*' 83002^T, **4.** *B. denitrificans* IFAM 1005^T, and, **5.** *B. japonicum* USDA6^T.

Characteristic	1	2	3	4	5
Growth on YEM agar medium*					
10 °C	-	-	±	-	±
37 °C	-	+	+	+	-
pH 5	+	+	+	+	+
pH 10	-	-	+	±	+
0.5% NaCl	-	-	±	-	±
1% NaCl	-	-	±	-	±
2% NaCl	-	-	-	-	-
Acid production †	7.5	ND	ND	ND	ND

* Positive, +; weak, ± ; negative, – ; not determined, ND. Values are based on three replicates.

† Acid production on YEM agar medium determined as described by Bromfield et al., (2010).

Table S4 Fatty acid profiles of *Bradyrhizobium* strains: **1.** *B. ontarionense* sp. nov., A19^T, **2.** *B. oligotrophicum* S58^T, **3.** '*B. aescynomenes*' 83002^T, **4.** *B. denitrificans* IFAM 1005^T, and, **5.** *B. japonicum* USDA6^T.

Fatty Acid	1	2	3	4	5 [†]
12:0	0.7	0.5		0.4	
14:0	0.3	0.3	0.3	0.1	
16:0	16.9	16.0	15.2	15.6	13.1
16:1 ω5c					3.6
16:1 ω11c	0.3			0.1	
17:1 ω8c		0.5	0.5		
17:1 ω6c		0.3	0.5	0.1	
17:0		0.6	0.6	0.3	
18:0	1.1	0.7	0.6	1.1	0.8
18:1 ω5c	0.2	0.2	0.2	0.2	
18:1ω7c 11-methyl	8.0	10.9	11.6	8.7	6.7
18:3 ω6c (6,9,12)	0.2	0.1		0.1	
19:0 iso		0.2			
19:0 cyclo ω8c			0.8	1.8	
20:0		0.5			
20:0 iso			1.3	3.2	
20:1 ω7c		0.8		0.2	
Summed feature 3 [†]	1.9	2.6	2.5	0.4	1.1
Summed feature 7 [†]		0.2		0.1	
Summed feature 8 [†]	70.4	65.7	65.8	67.4	74.8

* Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 2, 12:0 aldehyde/?; Summed feature 3, 16:1 ω6c/16:1 ω7c; Summed feature 5, 18:0 ante/18:2 ω6,9c; Summed feature 8, 18:1 ω6c/18:1 ω7c.

† Data from: Yu X, Cloutier S, Tambong J, Bromfield ESP. *Int J Syst Evol Microbiol* 2014; 64: 3202–3207.

Table S5 Phenotypic characteristics of **1.** *B. ontarionense* sp. nov., A19^T, **2.** *B. oligotrophicum* S58^T, **3.** *B. denitrificans* IFAM 1005^T, **4.** *B. aeschynomenes* 83002^T, **5.** *B. japonicum* USDA 6^T.

Characteristic	1	2	3	4	5 ^a	Characteristic	1	2	3	4	5 ^a
C-source utilization (Biolog)^b											
Dextrin	-	-	-	-	-	Glycyl-L-Proline	-	-	-	-	-
D-Maltose	-	-	-	-	-	L-Alanine	-	-	-	-	-
D-Trehalose	-	-	-	-	-	L-Arginine	-	-	-	-	-
D-Cellobiose	-	-	-	-	-	L-Aspartic Acid	-	-	-	+	-
Gentiobiose	-	-	-	-	-	L-Glutamic Acid	-	-	-	-	-
Sucrose	-	-	-	-	-	L-Histidine	-	-	-	-	-
D-Turanose	-	-	-	-	-	L-Pyroglutamic Acid	-	-	-	+	-
Stachyose	-	-	-	-	-	L-Serine	-	-	-	-	-
D-Raffinose	-	-	-	-	-	Pectin	-	±	-	-	-
α-D-Lactose	-	-	-	-	-	D-Galacturonic Acid	-	+	+	+	+
D-Melibiose	-	-	-	-	-	L-Galactonic Acid Lactone	-	+	+	+	-
β-Methyl-DGlucoside	-	-	-	-	-	D-Gluconic Acid	±	+	±	-	-
D-Salicin	-	-	-	-	-	D-Glucuronic Acid	-	+	+	+	+
N-Acetyl-DGlucosamine	-	-	-	-	-	Glucuronamide	-	+	+	+	+
N-Acetyl-β-DMannosamine	-	-	-	-	-	Mucic Acid	-	±	±	-	+
N-Acetyl-DGalactosamine	-	-	-	-	-	Quinic Acid	+	+	±	+	±
N-Acetyl Neuraminic Acid	-	-	-	-	-	D-Saccharic Acid	-	+	±	+	+
α-D-Glucose	±	-	-	+	-	p-HydroxyPhenylacetic Acid	-	±	-	-	-
D-Mannose	-	-	-	-	-	Methyl Pyruvate	-	±	±	+	+
D-Fructose	-	-	-	±	-	D-Lactic Acid Methyl Ester	-	±	-	-	-
D-Galactose	±	±	-	±	-	L-Lactic Acid	-	±	-	-	+
3-Methyl Glucose	-	-	-	-	-	Citric Acid	-	-	-	+	-
D-Fucose	+	+	±	+	+	α-Keto-Glutaric Acid	-	-	-	±	-
L-Fucose	-	±	±	-	-	D-Malic Acid	+	+	-	+	+
L-Rhamnose	-	-	-	-	-	L-Malic Acid	±	+	-	+	+
Inosine	-	-	-	-	-	Bromo-Succinic Acid	-	-	-	±	-
D-Sorbitol	±	+	±	±	-	Tween 40	+	+	±	±	-
D-Mannitol	±	+	+	±	-	γ-Amino-Butyric Acid	-	±	-	-	-
D-Arabitol	±	+	+	+	-	α-HydroxyButyric Acid	-	±	-	-	-
myo-Inositol	-	±	±	-	-	β-Hydroxy-D,LButyric Acid	±	+	±	+	+
Glycerol	±	+	+	+	+	α-Keto-Butyric Acid	-	-	-	-	-
D-Glucose- 6-PO4	-	±	±	-	-	Acetoacetic Acid	±	+	+	-	-
D-Fructose- 6-PO4	-	±	+	-	-	Propionic Acid	+	+	+	-	-
D-Aspartic Acid	-	±	-	-	-	Acetic Acid	+	+	+	-	-
Gelatin	-	±	-	-	-	Formic Acid	+	+	v	+	+
Chemical Sensitivity (Biolog)^b											
1% Sodium Lactate	±	±	-	-	+	Vancomycin	-	±	±	+	+
Fusidic Acid	-	-	-	-	-	Tetrazolium Violet	±	+	+	+	+
D-Serine	-	-	-	-	-	Tetrazolium Blue	±	+	+	+	+
Troleandomycin	±	+	-	-	-	Nalidixic Acid	+	±	-	±	+
Rifamycin SV	±	+	±	+	+	Lithium Chloride	-	-	-	-	-
Minocycline	±	+	±	+	+	Potassium Tellurite	-	-	-	-	-
Lincomycin	+	-	+	+	+	Aztreonam	+	±	+	+	+
Guanidine HCl	-	-	-	-	-	Sodium Butyrate	-	+	-	-	-
Niaproof 4	-	-	-	-	-	Sodium Bromate	-	-	-	-	-

^a Data from Bromfield ESP, Cloutier S and Nguyen HDT. *Int J Syst Evol Microbiol* 2020; 70: 442-449.

^b BIOLOG GEN III MicroPlates (120 hours incubation at 28 °C): +, Positive; ±, weak; -, negative; v, variable. ND, not determined. Values are based on three replicates.

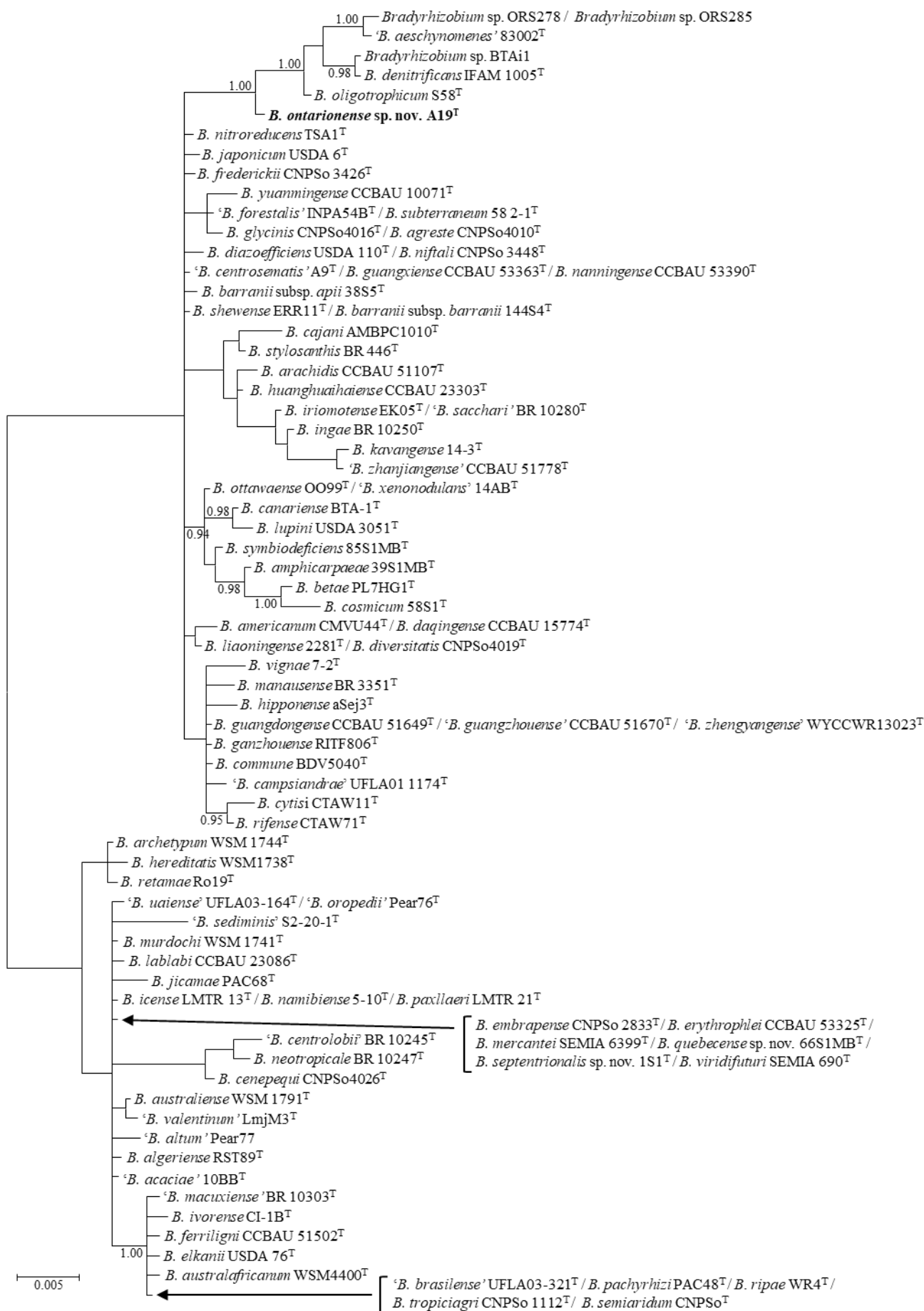


Figure S1. Bayesian tree of 16S rRNA gene sequences of *Bradyrhizobium ontarionense* sp. nov. strain A19^T and reference strains. Best fit substitution model: HKY + G + I. Posterior probabilities ≥ 0.9 are shown. Scale, expected number of substitutions per site. To include all species of *Bradyrhizobium* in the tree, alignment lengths of sequences were trimmed to 1300 bp.

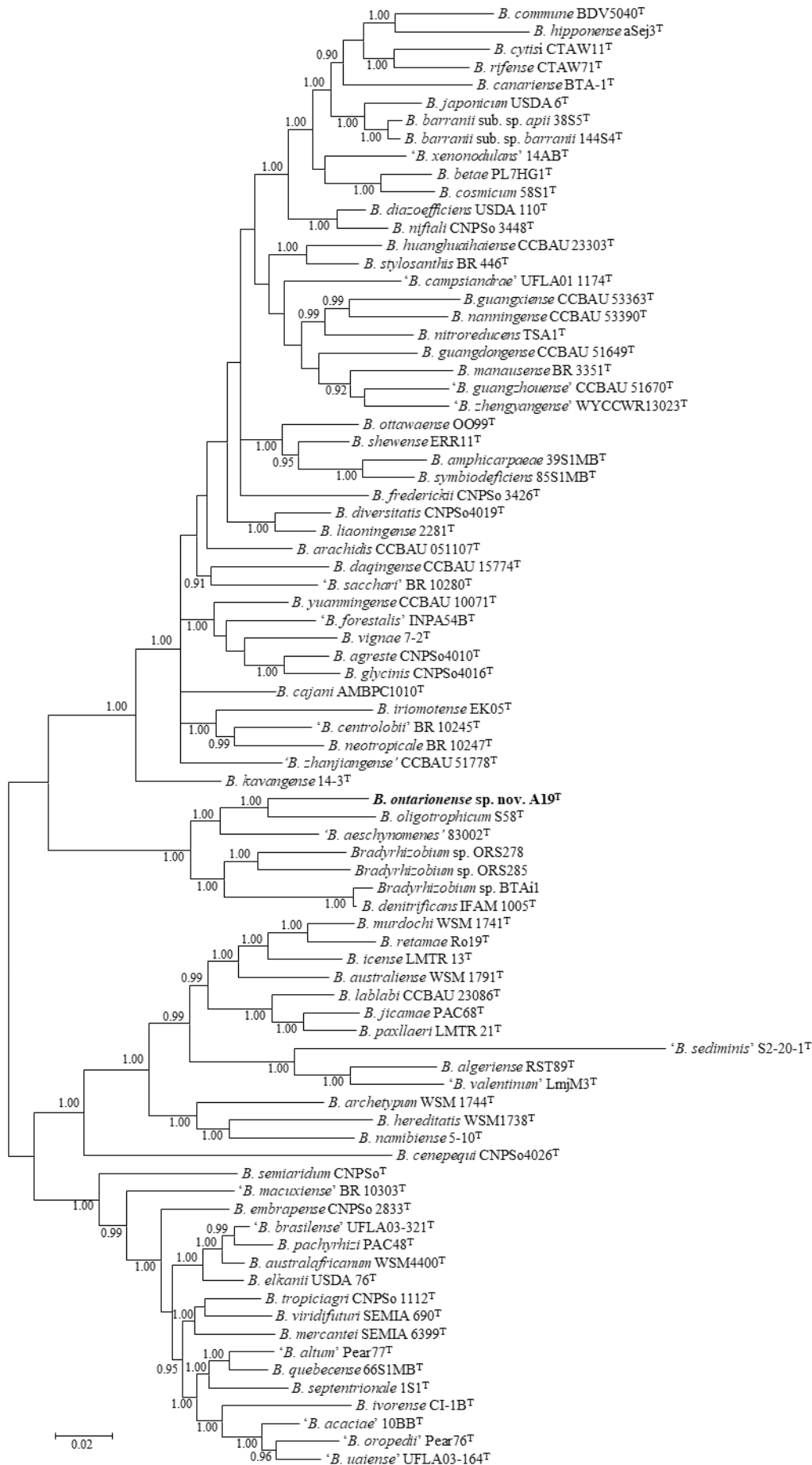
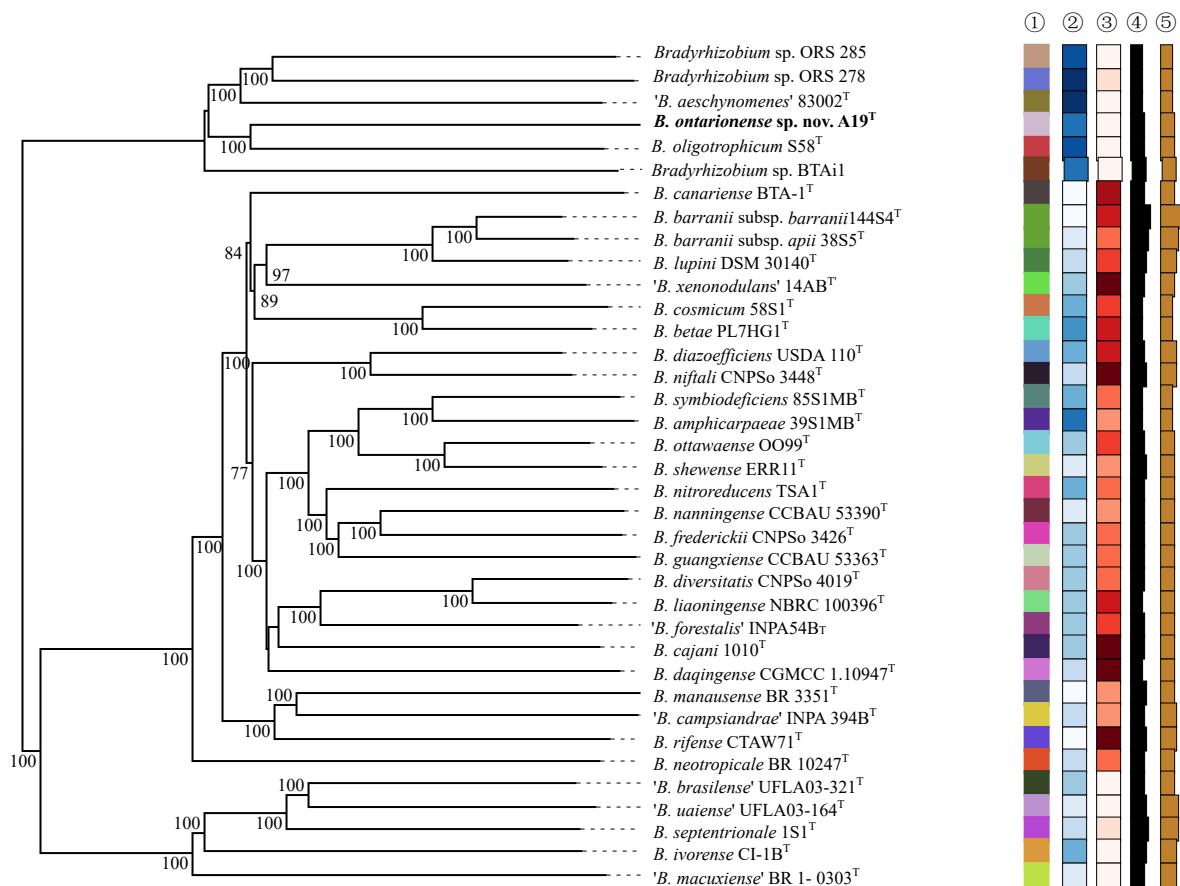
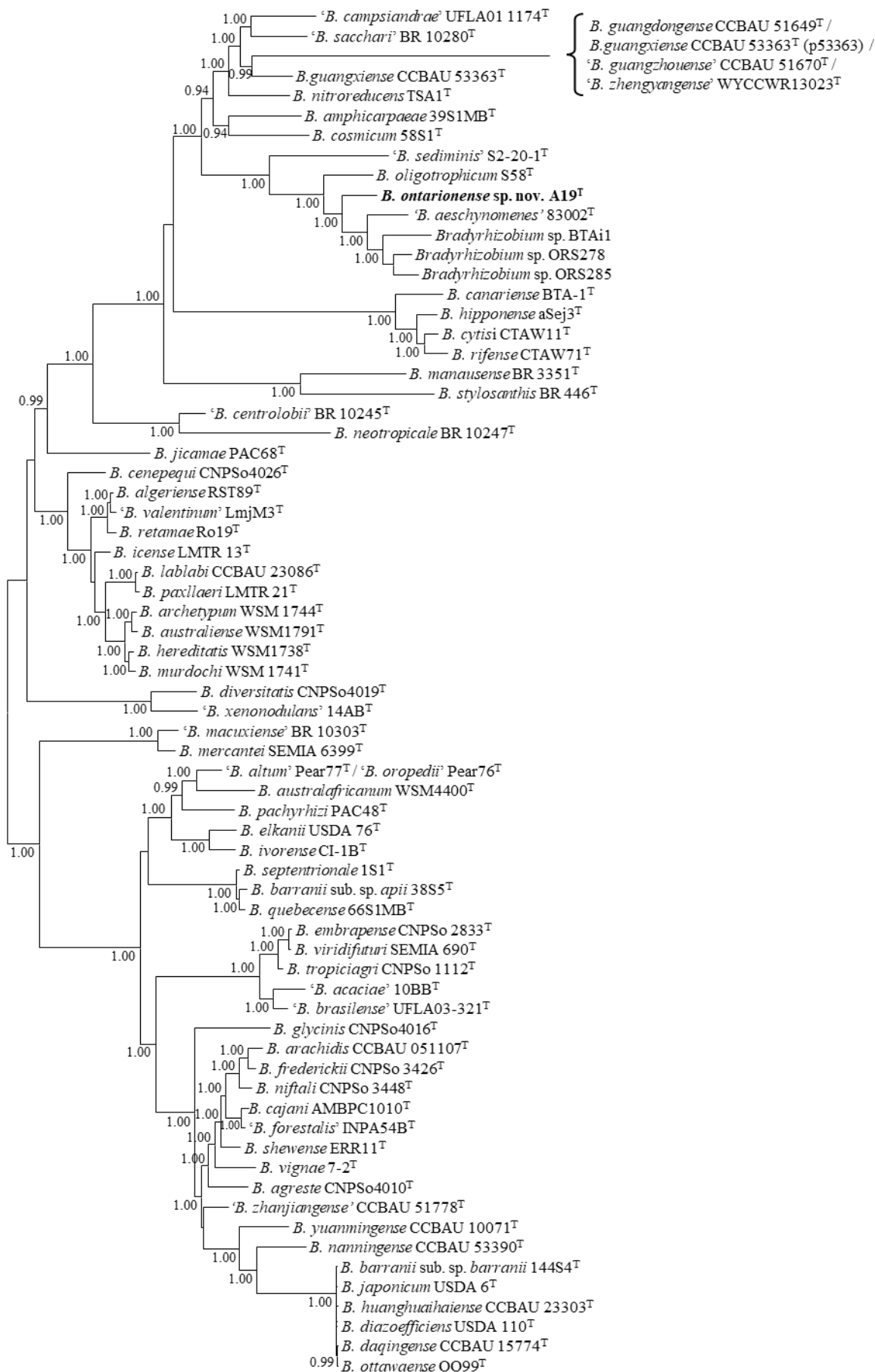


Figure S2 Bayesian phylogenetic tree (GTR+G+I substitution model) of *atpD-glnII-recA-gyrB-rpoB* concatenated partial housekeeping gene sequences (2679 bp alignment length) for *Bradyrhizobium ontarionense* sp. nov. strain A19^T and reference taxa of the genus *Bradyrhizobium*. Posterior probabilities ≥ 0.9 are shown. Bar, expected substitutions per site.



① dDDH species assignment	② G+C %	③ Delta values	④ Genome Size	⑤ Number of proteins
 <i>B. ontarionense</i> sp. nov.	 Min. (62.9)	 Min. (0.12)	 Min. (7.0Mb)	 Min. (6,441)
	 Max. (65.5)	 Max. (0.28)	 Max. (11.4 Mb)	 Max. (10,154)

Figure S3 Phylogenomic tree based on TYGS implementation showing *Bradyrhizobium ontarionense* sp. nov. A19^T and most closely related reference taxa of the genus *Bradyrhizobium*. The tree was inferred with FastME 2.1.6.1 (Ciufo et al. 2018) from Genome Blast Distance Phylogeny (GBDP) distances calculated from genome sequences. Branch lengths are scaled according to GBDP distance formula d5. The numbers above branches represent GBDP pseudo-bootstrap support values ($\geq 70\%$) from 100 replications, with an average branch support of 94%. The tree is rooted at the mid-point. Leaf labels are annotated by affiliation to species (1), genomic G+C content (2), delta values (3), overall genome sequence length (4) and number of proteins (5). Delta statistics permit assessment of accuracy in terms of tree-likeness; the lower the delta value, the greater the accuracy (Holland et al. 2002).



0.05

Figure S4. Bayesian tree of concatenated *nifHDK* gene sequences (3479bp) of *Bradyrhizobium ontarionense* sp. nov. strain A19^T and reference taxa of the genus *Bradyrhizobium*. Best fit substitution model, GTR+G+I. Posterior probabilities ≥ 0.9 are shown. Bar, expected substitutions per site.

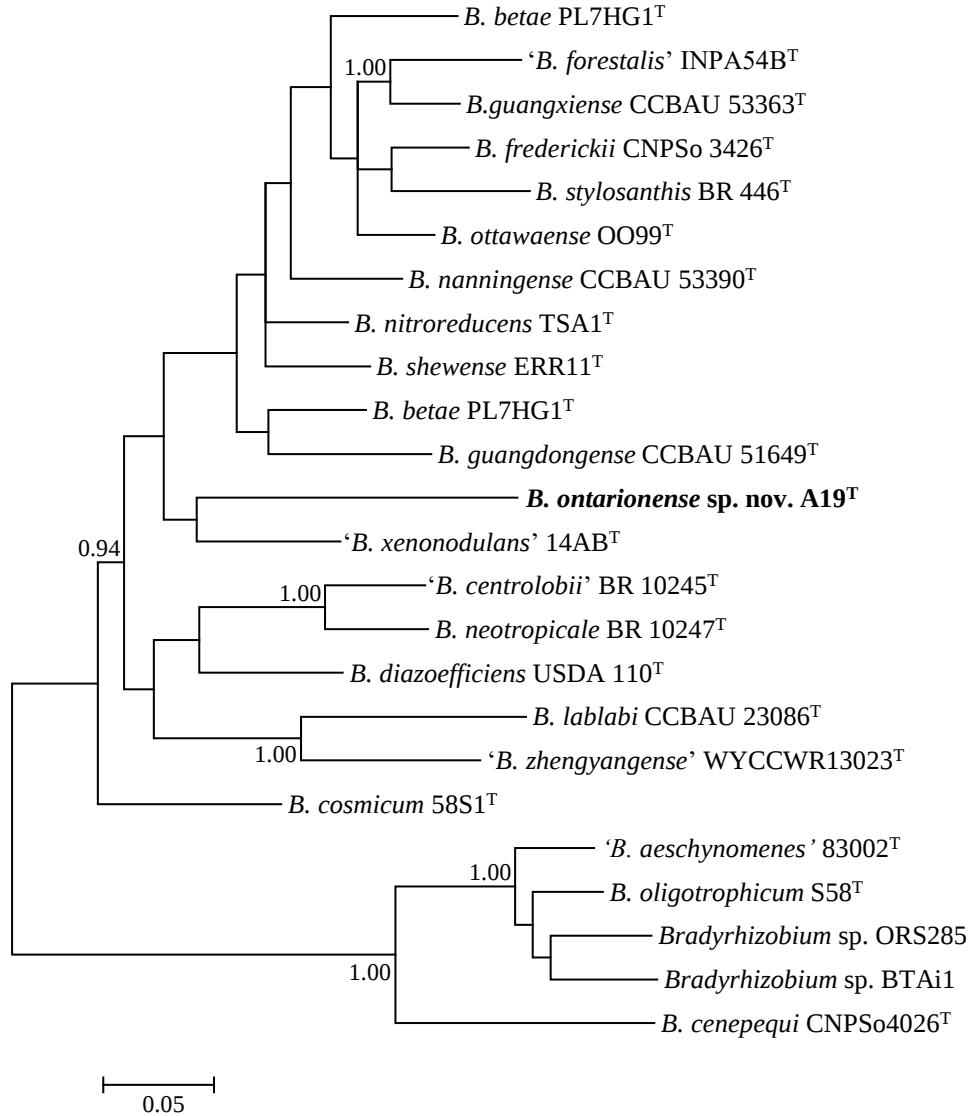


Figure S5 Bayesian phylogenetic tree (GTR+G+I substitution model) of nitrous oxide reductase (*nosZ*) gene sequences (1748 bp) for *Bradyrhizobium ontarionense* sp. nov. strain A19^T and reference taxa consisting of type strains of described species of the genus *Bradyrhizobium*. Posterior probabilities ≥ 0.90 are indicated. Bar represents expected substitutions per site.

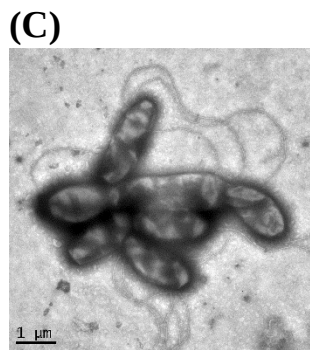
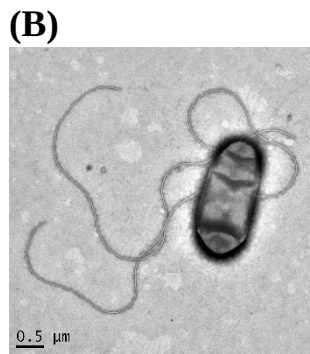
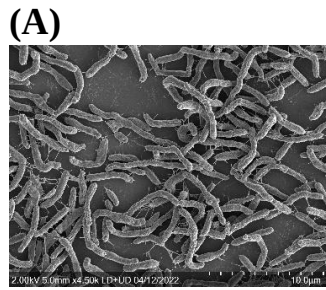


Figure S6 Scanning (A) and transmission (B and C) electron microscope images showing morphological features of cells of *Bradyrhizobium ontarionense* sp. nov A19^T. Scale bars (μm) are indicated in the images.