

Supplementary material

## Diversity and specificity of rhizobia from nodules of the neotropical tree legumes *Inga* and *Lysiloma* (Caesalpinioideae-mimosoid clade)

Diana Hernández-Oaxaca<sup>1</sup>, Karen Claro<sup>1</sup>, Marco A. Rogel<sup>1</sup>, Mónica Rosenblueth<sup>1</sup>, Jorge A. Velasco Trejo<sup>2</sup>, Enrique Alarcón-Gutiérrez<sup>2</sup>, José Antonio García-Pérez<sup>3</sup>, Julio Martínez-Romero<sup>1</sup>, Leif Eriksson<sup>1</sup>, Euan K. James<sup>4</sup> and Esperanza Martínez-Romero<sup>1,\*</sup>

<sup>1</sup> Centro de Ciencias Genómicas UNAM Cuernavaca, Morelos, México

<sup>2</sup> Instituto de Biotecnología y Ecología Aplicada, Universidad Veracruzana Xalapa, Veracruz, México

<sup>3</sup> Facultad de Biología, Universidad Veracruzana, Xalapa, Veracruz, México

<sup>4</sup> The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK 2

\* Correspondence: [emartine@ccg.unam.mx](mailto:emartine@ccg.unam.mx)

The scripts and outputs of the generated bioinformatic analyzes are available in the repository: <https://github.com/DianaOaxaca/Bradyrhizobium-analysis>

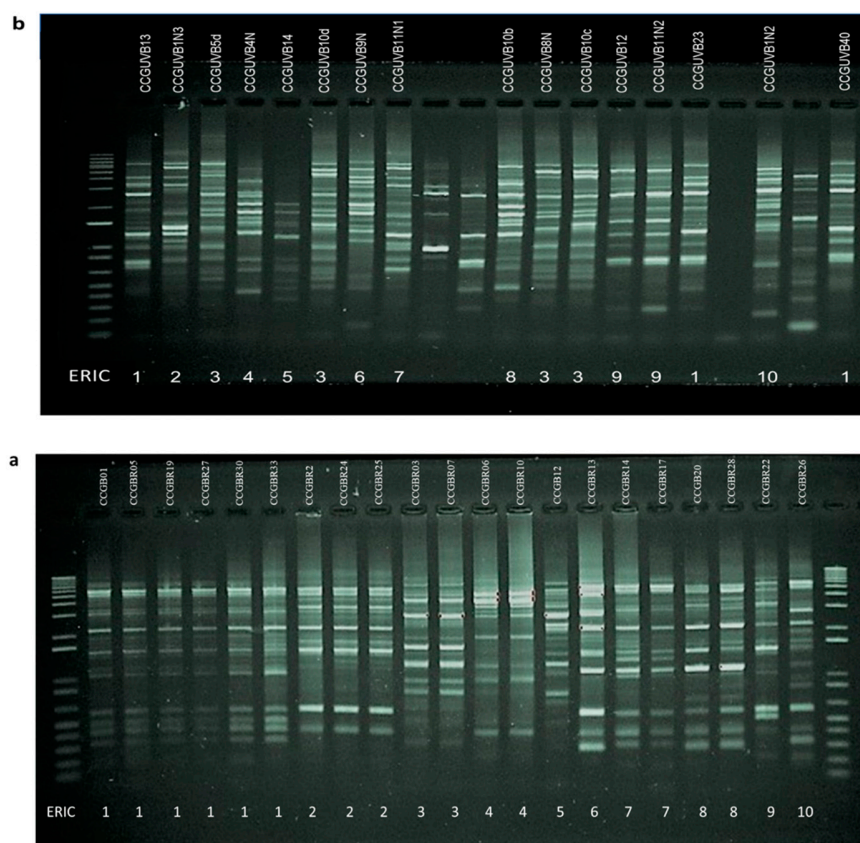


Figure S1. ERIC-PCR profiles. a) *Lysiloma* sp. isolated strains, b) *Inga vera* isolated strains.

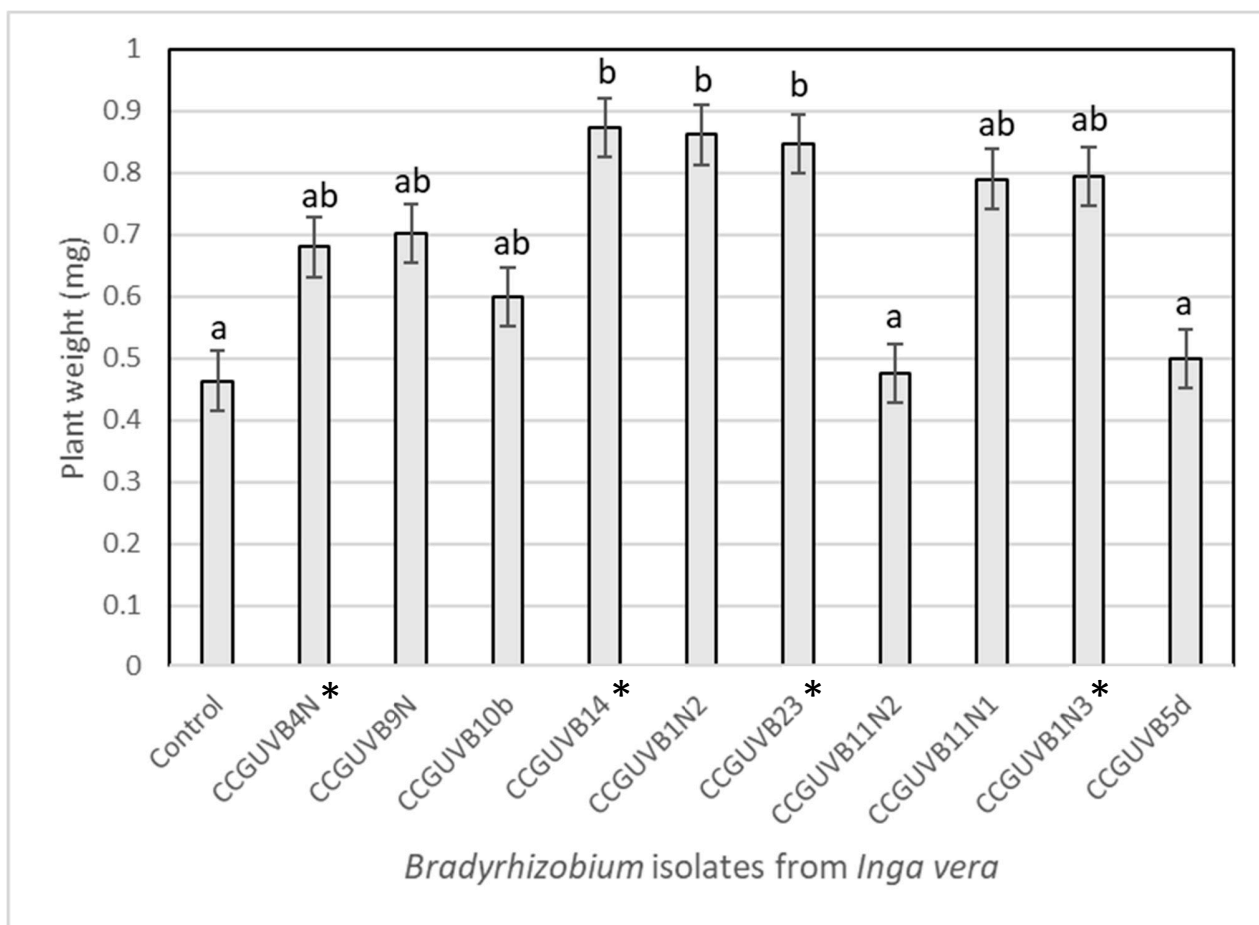


Figure S2. Fresh weight of the above ground portion of the plant of *Inga vera* inoculated with the different *Bradyrhizobium* isolates. The standard deviation is shown. Different letters correspond to significant different values according to Tukey test ( $p < 0.5$ ). The control was without inoculation. \* strains selected for sequencing.

Table S1. Accession numbers of representative genomes of *Bradyrhizobium* species used in the phylogenomic analysis.

Species name	Assembly accession number	Species name	Assembly accession number
<i>B. agreste</i> CNPSO 4010T	GCF_016031625.1	<i>B. stylosanthis</i> BR446T	GCF_001641335.1
<i>B. algeriense</i> RST89T	GCF_003062285.1	<i>B. symbiodeficiens</i> 85S1MBT	GCF_002266465.2
<i>B. amphicarpaceae</i> 39S1MBT	GCF_002266435.2	<i>B. tropiciagri</i> SEMIA 6148T	GCF_001189845.1
<i>B. arachidis</i> CCBAU 051107T	GCF_015291705.1	<i>B. uaiense</i> UFLA 03164T	GCF_010811875.1
<i>B. archetypum</i> WSM1744T	GCF_013114835.1	<i>B. valentinum</i> LmjM3T	GCF_001440405.1
<i>B. australiense</i> WSM1791T	GCF_013114825.1	<i>B. vignae</i> LMG 28791T	GCF_900324035.1
<i>B. betae</i> PL7HG1T	GCF_008932115.1	<i>B. viridifuturi</i> SEMIA 690T	GCF_001238275.1
<i>B. brasiliense</i> UFLA 03321T	GCF_002776695.1	<i>B. yuanmingense</i> CCBAU 10071T	GCF_900094575.1
<i>B. cajani</i> AMBPC 1010T	GCF_009759665.1	<i>B. zhanjiangense</i> CCBAU 51778T	GCF_004114935.1
<i>B. campsiandrae</i> INPA 01394BT	GCF_014529705.1	<i>B. japonicum</i> E109	GCF_000807315.1
<i>B. centrolobii</i> BR10245T	GCF_001641635.1	<i>B. japonicum</i> Is-34	GCF_000773865.1
<i>B. cosmicum</i> 58S1T	GCF_007290395.1	<i>B. japonicum</i> J5	GCF_001887695.1
<i>B. cytisi</i> CTAW11T	GCF_008123515.1	<i>B. japonicum</i> CCBAU 15354	GCF_000261545.1
<i>B. daqingense</i> CGMCC 1 10947T	GCF_007830205.1	<i>B. japonicum</i> CCBAU 15517	GCF_000261565.1
<i>B. diazoefficiens</i> USDA 110T	GCF_000011365.1	<i>B. japonicum</i> CCBAU 83623	GCF_000261625.1
<i>B. diversitatis</i> CNPSO 4019T	GCF_016031635.1	<i>B. japonicum</i> CCBAU 15618	GCF_000261585.1
<i>B. elkanii</i> USDA 76T	GCF_000379145.1	<i>B. japonicum</i> CCBAU 25435	GCF_000261605.1
<i>B. embrapense</i> SEMIA 6208T	GCF_001189235.2	<i>B. japonicum</i> FNI	GCF_001038185.1
<i>B. forestalis</i> INPA 54BT	GCF_002795245.1	<i>B. japonicum</i> NBRC 14783	GCF_006539645.1
<i>B. frederickii</i> CNPSO 3426T	GCF_004570865.1	<i>B. japonicum</i> SEMIA 5079	GCF_000661935.1
<i>B. glycinis</i> CNPSO 4016T	GCF_016031655.1	<i>B. japonicum</i> SZCCT0280	GCF_018130365.1
<i>B. guangdongense</i> CCBAU 51649T	GCF_004114975.1	<i>B. japonicum</i> SZCCT0395	GCF_018130735.1
<i>B. guangxiense</i> CCBAU 53363T	GCF_004114915.1	<i>B. japonicum</i> SZCCT0402	GCF_018130795.1
<i>B. guangzhouense</i> CCBAU 51670T	GCF_004114955.1	<i>B. japonicum</i> UBMA197	GCF_002108935.1
<i>B. hipponense</i> aSej3T	GCF_008123965.1	<i>B. japonicum</i> USDA 123	GCF_000482525.1
<i>B. huanghuaihaiense</i> CGMCC 1.10948T	GCF_007830635.1	<i>B. japonicum</i> USDA 38	GCF_000472745.1
<i>B. icense</i> LMTR 13T	GCF_001693385.1	<i>B. liaoningense</i> SZCCT0293	GCF_018130525.1
<i>B. ivorensensis</i> CI1BT	GCF_900696085.1	<i>B. liaoningense</i> SZCCT0399	GCF_018130785.1
<i>B. japonicum</i> USDA 6T	GCF_000284375.1	<i>Bsp</i> MOS001	GCF_004634465.1
<i>B. jicamae</i> PAC68T	GCF_001440395.1	<i>B. quebecense</i> 66S1MBT	GCF_017493175.3
<i>B. lablabi</i> CCBAU 23086T	GCF_001440475.1	<i>B. septentrionale</i> 1S1T	GCF_011516645.4
<i>B. macuxiense</i> BR10303T	GCF_001542415.1	<i>Nitrobacter</i> sp. 62-13	GCA_001896955.1
<i>B. manausense</i> BR3351T	GCF_001440035.1	<i>N. sp.</i> 62-23	GCA_001897285.1
<i>B. mercantei</i> SEMIA 6399T	GCF_001982635.1	<i>N. sp.</i> Nb-311A	GCF_000152905.1
<i>B. murdochi</i> WSM1741T	GCF_000472965.1	<i>N. hamburgensis</i> X14 T	GCF_000013885.1

Bnanningense CCBAU 53390T	GCF_004114535.1	N.vulgaris Ab1	GCF_002028545.1
BneotropicaleBR10247T	GCF_001641695.1	N.winogradskyi NBRC14297	GCF_006539545.1
B. niftali CNPSo 3448T	GCF_004571025.1	N.winogradskyi Nb-255T	GCF_000012725.1
B. nitroreducensTSA1T	GCF_002776695.1	14-inga	SAMN28667582*
B. oligotrophicumS58T	GCF_000344805.1	1N3-inga	SAMN28667583*
B ottawaense OO99T	GCF_002278135.2	23-inga	SAMN28667584*
B. pachyrhizi PAC48T	GCF_001189245.1	4N-inga	SAMN28667585*
B. paxllaeri LMTR21T	GCF_001693515.2	B12-lysi	SAMN28667586*
B. retamaeRo19T	GCF_001440415.1	B20-lysi	SAMN28667587*
B. rifense CTAW71T	GCF_008123425.1	B51278-lysidiv	SAMN28667588*
B. sacchari p9-20T	GCF_002068095.1	B51279-lysidivRis	SAMN28667589*
B. shewense ERR11T	GCF_900094605.1	BCCG4001-lysi	SAMN28667590*

The last **T** indicates it is a type strain.

\* BioSample accession numbers of assemblies generated in this work.

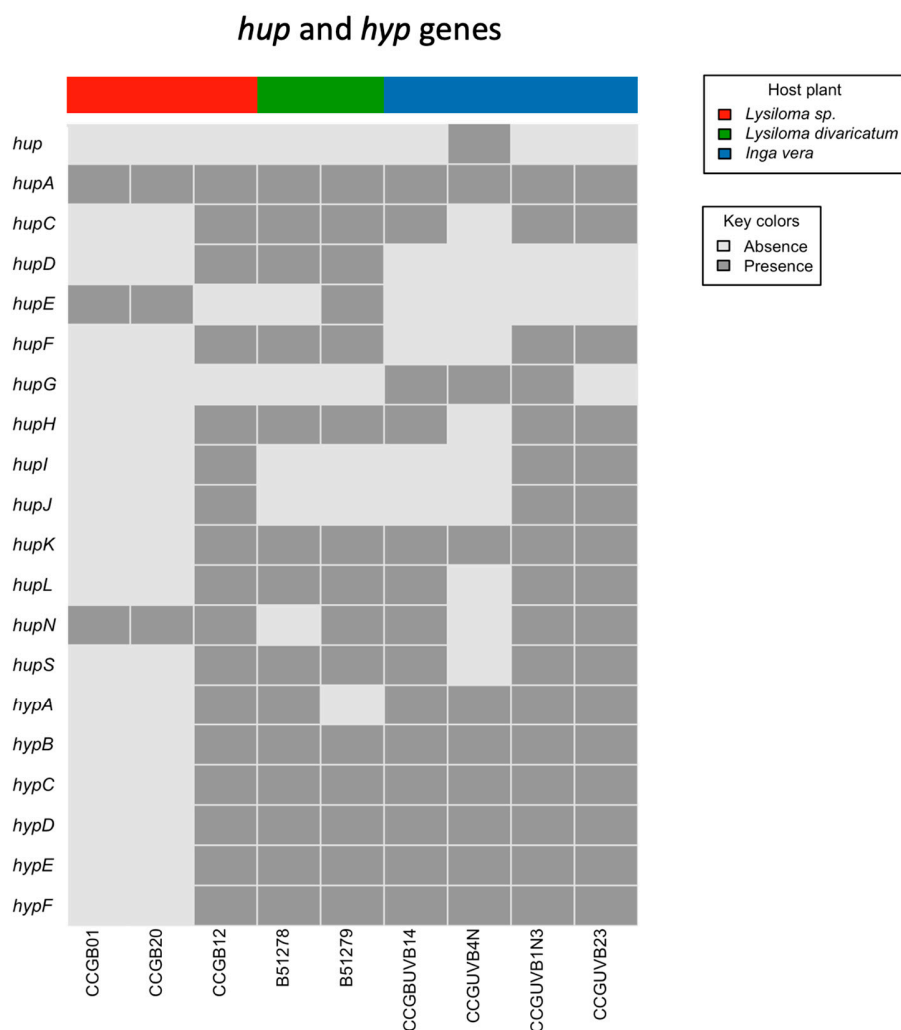
Table S2. Accession numbers of representative genomes of *Bradyrhizobium* species used to perform ANIm analysis.

Specie name	Assembly accession number	Specie name	Assembly accession number
<i>Bradyrhizobium_agreste</i>	GCF_016031625.1_ASM1603162v1	<i>Bradyrhizobium_manausense</i>	GCF_001440035.1_ABySSBR3351v1 R
<i>Bradyrhizobium_algeriense</i>	GCF_003062295.1_ASM306229v1 R	<i>Bradyrhizobium_mercantei</i>	GCF_001982635.1_ASM198263v1 R
<i>Bradyrhizobium_amphicarpaceae</i>	GCF_002266435.2_ASM226643v2 R	<i>Bradyrhizobium_nanningense</i>	GCF_004114535.1_ASM411453v1 R
<i>Bradyrhizobium_arachidis</i>	GCF_015291705.1_ASM1529170v1 R	<i>Bradyrhizobium_neotropicale</i>	GCF_001641695.1_ASM164169v1 R
<i>Bradyrhizobium_betae</i>	GCF_008932115.1_ASM893211v1 R	<i>Bneotropicale_MPMI1</i>	GCF_017614905.1_ASM1761490v1
<i>Bradyrhizobium_brasilense</i>	GCF_012689435.1_ASM1268943v1 R	<i>Bradyrhizobium_nitroreducens</i>	GCF_002776695.1_ASM277669v1 R
<i>Bradyrhizobium_brasilense</i>	GCF_900101405.1_IMG-taxon_2654588101	<i>Bradyrhizobium_oligotrophicum</i>	GCF_000344805.1_ASM34480v1 R
<i>Bradyrhizobium_cajani</i>	GCF_009759665.1_ASM975966v1 R	<i>Bradyrhizobium_ottawaense</i>	GCF_002278135.2_ASM227813v2 R
<i>Bradyrhizobium_campsiandrae</i>	GCF_014530645.1_ASM1453064v1 R	<i>Bradyrhizobium_pachyrhizi</i>	GCF_009759865.1_ASM975986v1 R
<i>Bradyrhizobium_canariense</i>	GCF_002108775.1_ASM210877v1 R	<i>Bradyrhizobium_paxllaeri</i>	GCF_001693515.2_ASM169351v2 R
<i>Bradyrhizobium_centrolobii</i>	GCF_001641635.1_ASM164163v1 R	<i>Bradyrhizobium_retamae</i>	GCF_001440415.1_ASM144041v1 R
<i>Bradyrhizobium_cosmicum</i>	GCF_000284275.1_ASM28427v1 R	<i>Bradyrhizobium_rifense</i>	GCF_008123425.1_ASM812342v1 R
<i>Bradyrhizobium_cytisi</i>	GCF_008123515.1_ASM812351v1 R	<i>Bradyrhizobium_sacchari</i>	GCF_007828095.1_ASM782809v1 R
<i>Bradyrhizobium_daqingense</i>	GCF_007830205.1_ASM783020v1 R	<i>Bradyrhizobium_shewense</i>	GCF_900094605.1_IMG-taxon R
<i>Bradyrhizobium_daqingense</i>	<u>GCF_021044685.1_ASM2104468v1/</u>	<i>Bradyrhizobium_stylosanthis</i>	GCF_001641335.1_ASM164133v1 R
<i>Bradyrhizobium_diaoefficiens</i>	GCF_001642675.1_ASM164267v1 R	<i>Bradyrhizobium_symbiodeficiens</i>	GCF_011604665.1_ASM1160466v1 R
<i>Bradyrhizobium_diversitatis</i>	GCF_016031635.1_ASM1603163v1 R	<i>Bradyrhizobium_tropiciagri</i>	GCF_001189845.1_ASM118984v1 R
<i>Bradyrhizobium_elkanii</i>	GCF_000379145.1_ASM37914v1 R	<i>Bradyrhizobium_macuxiense</i>	GCF_001542415.1_ASM154241v1 R
<i>Bradyrhizobium_elkanii</i> USDA76	GCF_000379145.1	<i>Bradyrhizobium_uaiense</i>	GCF_010811875.1_ASM1081187v1 R
<i>Bradyrhizobium_embrapense</i>	GCF_001189235.2_ASM118923v2 R	<i>Bradyrhizobium_valentinum</i>	GCF_001440385.1_ASM144038v1 R
<i>Bradyrhizobium_erythrophlei</i>	GCF_900142985.1_IMG-taxon R	<i>Bradyrhizobium_vignae</i>	GCF_900324035.1_BRAD3257 R
<i>Bradyrhizobium_forestalis</i>	GCF_002795245.1_ASM279524v1 R	<i>Bradyrhizobium_viridifuturi</i>	GCF_001238275.1_ASM123827v1 R
<i>Bradyrhizobium_glycinis</i>	GCF_016031655.1_ASM1603165v1 Lv	<i>Bradyrhizobium_yuanmingense</i>	GCF_001439885.1_ABySSBR3267 R
<i>Bradyrhizobium_guangdongense</i>	GCF_004114975.1_ASM411497v1 R	<i>Bradyrhizobium_zhanjiangense</i>	GCF_004114935.1_ASM411493v1 R
<i>Bradyrhizobium_guangxiense</i>	GCF_004114915.1_ASM411491v1 R	<i>Bradyrhizobium_zhanjiangense</i>	GCF_004114465.1
<i>Bradyrhizobium_guangzhouense</i>	GCF_004114955.1_ASM411495v1 R	CCGUVB14	SAMN27738322*
<i>Bradyrhizobium_huanghuaihaiense</i>	GCF_007830635.1_ASM783063v1 R	CCGUVB1N3	SAMN27738323*
<i>Bradyrhizobium_icense</i>	GCF_001693385.1_ASM169338v1 R	CCGUVB23	SAMN27738324*
<i>Bradyrhizobium_ivorense</i>	GCF_900696085.1_ERZ807199 R	CCGUVB4N	SAMN27738325*

Bradyrhizobium_japonicum	GCF_000284375.1_ASM28437v1 R	CCGB12	SAMN27738326*
Bradyrhizobium_jicamae	GCF_001440395.1_ASM144039v1 R	CCGB20	SAMN27738327*
Bradyrhizobium_lablabi	GCF_001440475.1_ASM144047v1 Lv	B51278	SAMN27738328*
Bradyrhizobium_liaoningense	GCF_001595995.1_ASM159599v1 R	B51279	SAMN27738329*
Bradyrhizobium liaoningense SZCCT0400	GCF_018130825.1_ASM1813082v1	CCGB01	SAMN27738330*
Bradyrhizobium_lupini	GCF_014194605.1_ASM1419460v1 Lv		

The last **R** indicates if it is a representative genome and **Lv** indicates it is the last version.

\* BioSample accession number of assemblies generated in this work.

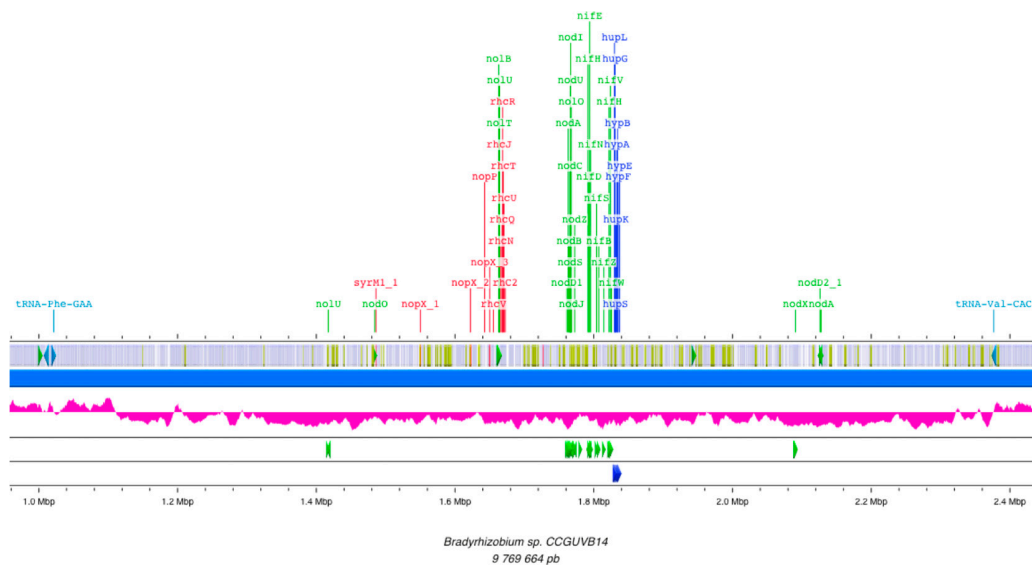


**Figure S3.** Comparative analysis of *hup* and *hyp* genes present in the *Lysiloma* spp. And *I. vera* *Bradyrhizobium* isolates. Colors within the map indicate the presence (light gray) and absence (dark gray) of *hup* and *hyp* genes in each. The top sidebar indicates the legume host: red for *Lysiloma* sp., green for *L. divaricatum* and blue for *I. vera*.





**Figure S4.** *Bradyrhizobium* effect on the growth of *Inga vera* plant. On the left side, plant inoculated with a mixture of CCGB01, CCGB12 and CCGB20; on the right side, plant inoculated with *Bradyrhizobium* CCGUVB23.



**Figure S5.** Zoom view of the putative symbiotic island of CCGUVB14. Bottom-up. The numbers represent the coordinates of the location of the island within the chromosome. The stripe with strong blue markings indicates the *hup* and *hyp* genes. The stripe with green marks indicates the *nod*, *nol*, *noe* and *nif* genes predicted with EggNogg-Mapper. The strip with levels in pink shows the GC content of the chromosomal region. The blue stripe represents the genomic sequence and the outermost stripe indicates the coding sequences (CDS) in light purple, repeat regions in yellow, and green marks *nod* and *nol* genes predicted with prokka, whereas genes involved in the nodulation regulation and protein systems secretion type are marked in red, predicted by EggNogg-Mapper, RASTtk and Prokka. The island region is flanked by t-RNAs (light blue).