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Molecular Characterization of Novel Bradyrhizobium Strains Isolated from Native Legumes of Meghalaya

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Abstract

Meghalaya part of the Indo-Myanmar biodiversity hotspot, harbors diverse native legumes, yet their association with nitrogen-fixing microsymbionts remains poorly understood. Approximately, 100-200 legumes species occur in North-East India, 60% of which are native. Species such as *Desmodium polycarpum* and *Smithia ciliata* are ecologically and medicinally important but their nodulation status is scarely studied. Globally, *Bradyrhizobium*, a slow-growing genus first isolated from *Glycine max*, is known for its metabolic versatility, including chemolithotrophy and photosynthetic gene clusters. More than 36 species have been reported from different agro-ecosystems across Asia and South America, yet information from the state of Meghalaya, India is lacking. This knowledge gap limits understanding of rhizobium-legume interactions in a region with high ecological and evolutionary significance. This study aims to elucidate the diversity and effectiveness of microsymbionts associated with nodulation and nitrogen fixation, highlighting their ecological significance.

1. Introduction

Meghalaya, with a total area of 22,429 km square is located between latitudes 25º00 and 26º10 north and 89º45 and 92º47 east. Its northern, eastern and western borders are shared with Bangladesh and Assam, respectively (Reddy and Baiantimon, 2011). The area is distinguished by a variety of agro-climatic and physical-circumstances and the region is a significant component of the Indo-Myanmar biodiversity hotspot (Myers et al., 2000). There are about 100- 120 legume

species documented from North-East India, out of which 60% are native to the area (Balakrishnan, 1981; Haridasan and Rao, 1987). The genus *Desmodium polycarpum* is one of the legumes that belong to the subfamily *Papillionoideae* (Fabaceae) widely distributes in the tropical and subtropical regions of the world (Tonuitti et al., 2017; Andrews et al., 2017) and the eastern Himalayan region of India (Meghalaya). This plant is known to have medicinal properties for treatment of dysentery, liver diseases, ulcers, eye diseases, etc (Gu et al., 2007). It is also found in Columbia (Delamuta et al., 2012), Argentina (Tonuitti et al., 2017), China (Xu et al., 2016). *Smithia ciiata* is an annual and diffused herb (Bargali et al., 2016) belonging to the subfamily *Papillionoideae* (Fabaceae). It grows on the sloppy hills during rainy seasons, native to Himalayas, tropical and subtropical Asia and is commonly known as fringed Smithia. In India, it is mainly found in Assam, Meghalaya and Mizoram (Manandhar et al., 2009; Panday et al., 2016).

Reports on the nodulation status in crop legumes such as *Glycine max* (Appunu et al., 2008; Appunu et al., 2009) and *Vigna* (Appunu et al., 2009) species by native *Bradyrhizobium* strains have been recorded from agricultural fields of central and southern part of India where the soil is mostly neutral to alkaline and also some parts of Maharashtra (Deshmukh et al., 2013). *Bradyrhizobium* strain (Kanika et al., 2010) has been isolated from pigeon pea (*Cajanus cajan*) growing under arid conditions in Rajasthan and North Western India plains, respectively.

This genus was first isolated from *Glycine max* and described by Jordan (1982) and it include all the slow growing bacteria with a generation time of 10-12 hr. (Andrews et al., 2017) described that the *Bradyrhizobium* species have been reported from China (Yao et al., 2014), Brazil (Fonseca et al., 2012), India (Ojha et al., 2017, Rathi et al., 2018, Chouhan et al., 2024, Bissa et al., 2024). Shamsheldin et al., (2017) reported that there are about more than 36 species that belong to this genus. *Bradyrhizobium* strains are alkaline-producing group, slow growing bacteria, gram negative, rod-shaped with a single polar or subpolar flagellum (Somasegaran and Hoben, 1985; Sessitsch et al., 2002). Some of the *Bradyrhizobium* species could grow as chemolithotrophs in the presence of hydrogen, carbon dioxide and low content of oxygen due to the presence of the enzyme hydrogenase (Berrada et al., 2014). Interestingly, photosynthetic gene clusters have also been studied in *B. japonicum* and *B. elkanii* based on comparative genomics by Avontuur et al., 2023. The recently reported novel species *B. ontarionense* A19^{T} from root nodules of *Aeschynomene indica* has also been reported to cluster with the photosynthetic clade (Bromfield and Cloutier, 2024).

The native legumes of Meghalaya have been neglected despite being a hub of a mega biodiversity center, and there is very little knowledge regarding their likely link with microsymbionts. Despite their spatial and evolutionary significance, such relationships between rhizobium-legume in the North-East region have mostly been ignored and have never been explored for in-depth investigation. Exploring the relationship between the diversity of Meghalaya's native legumes above ground and below ground microsymbionts will be intriguing. Such research is anticipated to help in understanding the role of geographical factors influence rhizobia selection and shed light on evolutionary relationships between the rhizobia gathered from different ecological zones of Meghalaya. The present study can provide insight on the types of microsymbionts associated with nodulation and nitrogen fixation and its diversity and effectiveness contrasted with the process of nitrogen fixing.

2. Material and methods

2.1. Study area and plant collection

D. polycarpum and *S. ciliata* were surveyed and collected from different areas of Meghalaya (Mawryngkneng, Nongstoiñ, Ialong, Jowai, Ummulong, Umroi, Marngar, Umbang, Sohiong, Mairang, Mawthadraishan, Lum Rapleng, Laitlyngkot), Fig 1. The plant specimens were identified with aid of the Regional Centre of the Botanical Survey of India (BSI), Shillong and their accession number were obtained as previously reported (Kharbyngar et al., 2025). The plants were collected and excavated during the period between April-October.

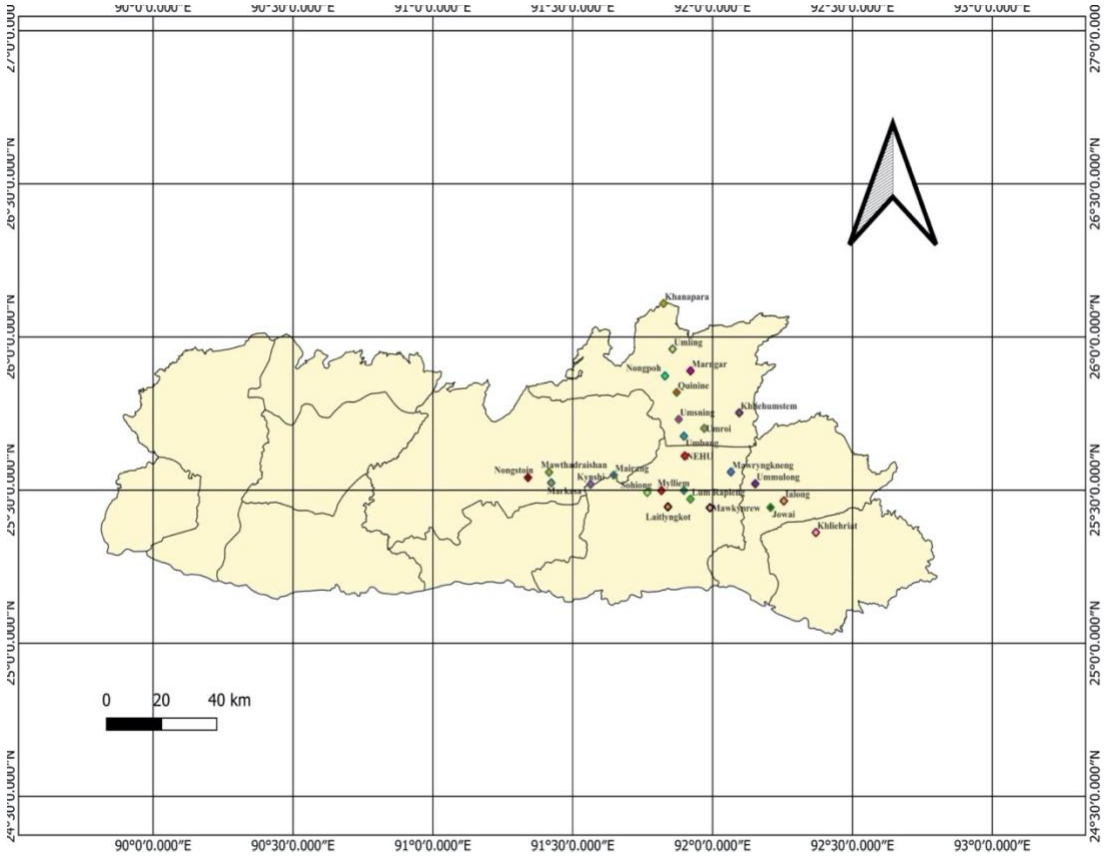

Fig 1. Map showing the location of the collection sites (QGIS 3.28.3 Firenze)

2.2. Isolation and purification of root nodulating bacteria

Fresh and healthy root nodules were separated from the legumes and further sterilized with 90% (w/v) ethanol for 1 minute followed by 0.1% (w/v) fungicide (Bavistin^R) for 5 minutes and 0.1% (w/v) $HgCl_2$ for 3-6 minutes and further washed with autoclaved distilled water in intervals. The sterilized nodules were then taken on to a sterilized watch glass and crushed using a sterile scapel. The root exudates obtained were streaked on Congo red-Yeast Extract Mannitol Agar medium (CR-YEMA) with pH 7 (Vincent 1970) and Somasegaran and Hoben (1994) with minor modifications and incubated at 28ºC in BOD incubator for 4-7 days. The rhizobial colonies were obtained and purified through quadrant streaking and preserved on agar slants/stabs kept at low

temperature (4˚C) for short-term storage or in YEM broth containing 20% (w/v) glycerol at -80˚C for long term storage.

2.3. Genomic DNA isolation

The RNB isolates were cultured on tryptone yeast (TY) agar and broth media and the activated cultures were used for DNA isolation (Howieson and Dilworth, 2016). DNA extraction of the bacterial culture was done by using Phenol Chloroform method described by Cheng and Jiang (2006) with minor modifications. In this method 1ml sterile saline solution was added to a sterile 1.5 ml tube and a loop full of activated culture. The tube was centrifuged at 8,000 rpm for 2 min at room temperature. Supernatant was discarded and pellet was washed with 400 µL of STE (Sodium Chloride Tris-EDTA). Subsequently, the phenol-chloroform was added for DNA purification. The purified DNA obtained was further used as a template for DNA fingerprinting and amplification of various housekeeping genes and symbiotic genes.

2.4. DNA fingerprinting

The genetic diversity of all the strains was analyzed and grouping was done based on DNA fingerprinting through RAPD (Random Amplified Polymorphic DNA) A single *nif* gene-directed RPO1 primer described earlier by Richardson et al., 1995 was used for the random amplification. A total volume of 20 µL reaction mixture containing 1 µL of DNA template, 2 µL of 10x Taq buffer, 3 µL of 25 mM $MgCl_2$, 1.2 µL of dNTP mix (2.5 mM each), 1 µL of DMSO, 1.2 µL of 50 µM RPO1 primer, 0.35 µL of 3 $U\mu L^{-1}$ Taq DNA polymerase and nuclease free water and were set up with cycling conditions as follows: 5 min 94˚C, 5 x (30 s 94˚C, 1 min 55˚C, 1.5 min 72˚C), 30 x (30 s 94˚C, 25 s 58˚C, 30 s 72˚C), followed by final extension of 7 min at 72˚C thermal cycler (ESCO Swift Max Pro).

2.5. PCR amplification of 16S rRNA gene

PCR amplification of 16S rRNA gene was performed using universal primers 18F and 1492R (Weisburg et al., 1991). PCR reaction was carried at a total volume of 25 µL reaction mixture containing 1.25 µL of diluted DNA template, 2.5 µL of 10x Taq buffer, 2 µL of 25 mM MgCl2, 1.5 µL of dNTP mix (2.5 mM each), 1.25 µL of DMSO, 0.6 µL of forward (18F) and reverse primers (1492R), 0.25 µL of 3 $U\mu L^{-1}$ Taq DNA polymerase and nuclease free water. The PCR cycle conditions were set up as follows: 5 min 95˚C, 5 x (1 min 94˚C, 1 min 53˚C, 1 min 72˚C) followed by final extension of 7 min at 72˚C thermal cycler (ESCO Swift Max Pro).

2.6. PCR amplification of housekeeping genes

For multilocus sequence analysis (MLSA), three conserved protein-coding housekeeping genes (*recA, glnII* and *atpD*) were amplified and sequenced. The primers primer pair TSrecAF and TSrecAR as described by Stepkowski et al., 2005 was used for amplification of 600 bp *recA* gene (which codes for recombination protein) in *Bradyrhizobium.* The amplification of 620 bp *glnII* gene (glutamine synthase II) in *Bradyrhizobium* was carried out using the primer pair TSglnIIF and TSglnIIR as described by Stepkowski et al., 2005. The amplification of 500 bp *atpD* gene (which codes for ATP synthase F1 beta subunit) in *Bradyrhizobium* was carried out using the

primer pair atpD294F and atpD771R as described by Gaunt et al., (2001). The PCR reaction mixture for amplification of protein-coding housekeeping genes was similar to that used in 16S rRNA gene with minor modifications.

2.7. PCR amplification of symbiotic genes

Two symbiotic genes (*nodA* and *nifH*) were amplified and sequenced for the selected strains. To amplify an internal fragment of 550 bp nodA gene (which codes for N-acyltransferase nodulation protein) in Bradyrhizobial strains, the primer pair nodAf.brad and nodAr.brad were effectively used as described by Chaintreuil et al., (2001). PCR reaction was carried at a total volume of 25µL reaction mixture containing 1.25 µL of the DNA template, 2.5 µL 10x Taq buffer, volume of 25 mM $MgCl_2$ depending on the type of gene fragment amplified, 1.5 µL of dNTPs mix (2.5 mM each), 1.25 µL of dimethyl sulfoxide (DMSO), 0.5 µL of forward and reverse primers, 0.25 µL of 3 $U\mu L^{-1}$ Taq DNA polymerase and nuclease free water. The amplification of 750bp *nif*H gene (which codes for nitrogenase Fe protein) in *Rhizobium* strains was carried out using primer pair nifHF and nifHI as described by Laguerre et al., (2001). PCR optimization was performed at a total volume of 25µL reaction mixture, containing 1.25 µL of the DNA template, 2.5 µL 10x Taq buffer, volume of 25 mM $MgCl_2$ depending on the type of gene fragment amplified, 1.5 µL of dNTPs mix (2.5 mM each), 1.25 µL of dimethyl sulfoxide (DMSO), 0.5 µL of forward and reverse primers, 0.25 µL of 3 $U\mu L^{-1}$ Taq DNA polymerase and nuclease free water.

2.8. Sequence and phylogenetic analysis

The PCR products for the symbiotic and housekeeping genes were sequenced using the respective primers and the sequencing was carried through an outsource from Biokart India Pvt. Ltd., Bangalore, India. The sequences acquired were analyzed using bioinformatic tools such a GeneTool lite (version 1.0 Double Twist Inc., Oakland, CA, USA) and deposited in the GenBank database to obtain accession numbers for different genes. To conduct molecular phylogenetic analysis, the multiple sequence alignment tool CLUSTALW was used to align the reference sequences needed for comparison that were retrieved from the NCBI. The Kimura 2-parameter model was used to calculate the pair wise evolutionary distances (Kimura, 1980) and using the neighbour-joining method, the phylogenetic trees were inferred (Saitou and Nei, 1987). Using MEGA 7 software, the phylogenetic trees were constructed, their distance was calculated and the evolutionary analysis was performed with bootstrapped value of 1000 replications (Kumar et al., 2016).

3. Results

3.1. Isolation and purification of root nodulating bacteria

During the field survey of leguminous plants that were investigated, it was observed that the mandate plants such as *Desmodium polycarpum* and *Smithia ciliata* were found mostly in patches during rainy season of the year, from the month of May - October. These wild and native legumes of Meghalaya have not been thoroughly explored for its nodulation and its endosymbiotic bacteria. The legume plant, *S. ciliata* was explored for the first time for its nodulation and its microsymbionts associated with them. The nodulation was seen optimum at the height of 3-10 cm

of top soil where the legumes grow and significant nodulation was observed in all the four plants and the nodules were well distributed. The nodules of *S. ciliata* were found to be of determinate aeschynomenoid type, without lenticels and were always associated with the lateral and adventitious roots. The surface of the nodules of *S. ciliata* was pink in colour which indicates the presence of leghaemoglobin. Similarly, the nodules of *D. polycarpum* were pink in colour but they had lenticels and were of desmodioid type as previously described (Kharbyngar et. al, 2025). A total of 54 rhizobial strains were isolated and purified from the root nodules of these two native legumes and these strains were further carried out for molecular characterization.

3.2. DNA fingerprinting through RAPD (Random Amplified Polymorphic DNA)

A total of twenty-six strains isolated from *D. polycarpum* were grouped into five groups (Group 1 to group 5) and fifteen individual genotypes. Highest number of strains were found in group 1 with three strains, NEHU-DP1, NEHU-DP2, both were isolated from Mawryngkneng and NEHU-DP3 from NEHU whereas group 2, 3, 4 and 5, each consist of two strains. Group 2 include the strains NEHU-DP9 and NEHU11 isolated from Nongstoiñ and Ialong, and NEHU- DP 12 from Jowai and NEHU15 from Ummulong, respectively, constituted group 3. Group 4 consists of NEHU-DP14 and NEHU-DP16 isolated from Khliehumstem and Umbang respectively, and the strains NEHU-DP21 from Marngar and NEHU-DP26 from Umroi formed the group 5 (Table 1).

Table 1. Grouping of NEHU-DP microsymbionts based on RPO1 primer

Sl. No	Groups	Isolate	Morphology	Site	District
1	Group 1	NEHU-DP1	Circular, raised, entire, high EPS, fast growing	Mawryngkneng	East Khasi Hills
		NEHU-DP 2	Circular, convex, entire, high EPS, fast growing	Mawryngkneng	
		NEHU-DP 3	Circular, raised, entire, high EPS, slow growing	NEHU	
2	Group 2	**NEHU-DP 9**	Circular, convex, entire, high EPS, fast growing	Nongstoiñ	West Khasi Hills
		NEHU-DP 11	Circular, raised, entire, high EPS, fast growing	Ialong	Jaiñtia Hills
3	Group 3	NEHU-DP 12	Circular, raised, entire, high EPS, slow growing	Jowai	Jaiñtia Hills
		NEHU-DP 15	Circular, raised, entire, high EPS, slow growing	Ummulong	
4	Group 4	NEHU-DP 14	Circular, raised, entire, high EPS, fast growing	Khliehriat	Jaiñtia Hills
		NEHU-DP 16	Circular, convex, entire, high EPS, fast growing	Marngar	Ri-Bhoi
5	Group 5	NEHU-DP 21	Circular, raised, entire, high EPS, fast growing	Umroi	Ri-Bhoi
		NEHU-DP 26	Circular, raised, entire, high EPS, fast growing	Umroi	
6	Group 6	**NEHU-DP 4**	Circular, convex, entire, high EPS, fast growing	NEHU	East Khasi Hills
7	Group 7	**NEHU-DP 5**	Circular, raised, entire, low EPS, fast growing	Sohiong	
8	Group 8	**NEHU-DP 6**	Circular, convex, entire, high EPS, fast growing	Kynshi	West Khasi Hills
9	Group 9	**NEHU-DP 7**	Circular, raised, entire, high EPS, slow growing	Kynshi	
10	Group 10	**NEHU-DP 8**	Circular, convex, entire, high EPS, slow growing	Mawthadraishan	
11	Group 11	**NEHU-DP 10**	Circular, raised, entire, high EPS, fast growing	Nongstoiñ	
12	Group 12	**NEHU-DP 13**	Circular, convex, entire, high EPS, fast growing	Khliehriat	Jaintia Hills
13	Group 13	**NEHU-DP 17**	Circular, raised, entire, low EPS, fast growing	Umbang	Ri-Bhoi
14	Group 14	**NEHU-DP 18**	Irregular, raised, entire, low EPS, fast growing	Quinine	
15	Group 15	**NEHU-DP 19**	Circular, raised, entire, high EPS, slow growing	Nongpoh	
16	Group 16	**NEHU-DP 20**	Circular, convex, entire, high EPS, fast growing	Nongpoh	
17	Group 17	**NEHU-DP 22**	Circular, raised, entire, high EPS, slow growing	Marngar	
18	Group 18	NEHU-DP 23	Circular, raised, entire, high EPS, slow growing	Umling	
19	Group 19	**NEHU-DP 24**	Circular, raised, entire, high EPS, slow growing	Khanapara	
20	Group 20	**NEHU-DP 25**	Circular, raised, entire, high EPS, slow growing	Khanapara	

A total of twenty-eight strains were isolated from *S. ciliata* and its RAPD profile revealed four groups (Group 1- group 4) and nineteen individual genotypes. The highest number of strains was observed in group 2 which includes NEHU-SC8, NEHU-SC11 and NEHU-SC12 isolated from Sohiong, Mairang and Mawthadraishan respectively whereas group 1, 3 and 4 consist of two strains each. Group 1 comprised of the strain NEHU-SC 2 from Lum Rapleng and NEHU-SC3 from Laitlyngkot and group 3 included the strain NEHU-SC17 and NEHU-SC22 isolated from Nongstoiñ and Umbang, respectively. And lastly, group 4 included the strain NEHU-SC 24 and NEHU-27 isolated from Umling and Khanapara, individually (Table 2).

Table 2. Grouping of NEHU-SC microsymbionts based on RPO1 primer.

Sl. No.	Groups	Isolates	Morphology	Site	District
1	Group 1	**NEHU- SC 2**	Circular, convex, entire, high EPS, fast growing	Laitlyngkot	East Khasi Hills
		NEHU- SC 3	Circular, convex, entire, high EPS, fast growing	Laitlyngkot	
2	Group 2	**NEHU- SC 8**	Circular, raised, entire, high EPS, slow growing	Sohiong	East Khasi Hills
		NEHU- SC 11	Circular, raised, entire, high EPS, fast growing	Mairang	West Khasi Hills
		NEHU- SC 12	Circular, raised, entire, high EPS, slow growing	Mawthadraishan	
3	Group 3	NEHU- SC 17	Circular, convex, entire high EPS, fast growing	Nongstoiñ	West Khasi Hills
		NEHU- SC 22	Circular, raised, entire high EPS, fast growing	Umbang	Ri-Bhoi
4	Group 4	**NEHU- SC 24**	Circular, convex, entire, high EPS, fast growing	Umling	Ri-Bhoi
		NEHU- SC 27	Circular, convex, entire, high EPS, fast growing	Khanapara	
5	Group 5	**NEHU- SC 1**	Circular, raised, entire, high EPS, fast growing	Lum Rapleng	East Khasi Hills
6	Group 6	**NEHU- SC 4**	Circular, convex, entire high EPS, fast growing	Mawkynrew	
7	Group 7	**NEHU- SC 5**	Circular, convex, entire high EPS, fast growing	Mylliem	
8	Group 8	**NEHU- SC 6**	Circular, raised, entire, high EPS, slow growing	NEHU	
9	Group 9	**NEHU- SC 7**	Circular, convex, entire high EPS, fast growing	NEHU	
10	Group 10	**NEHU- SC 9**	Circular, convex, entire high EPS, fast growing	Umphrup	
11	Group 11	**NEHU- SC10**	Circular, convex, entire high EPS, slow growing	Mairang	West Khasi Hills
12	Group 12	**NEHU- SC 13**	Circular, convex, entire high EPS, slow growing	Mawthadraishan	
13	Group 13	**NEHU- SC 14**	Circular, convex, entire high EPS, fast growing	Markasa	
14	Group 14	**NEHU- SC-15**	Circular, convex, entire high EPS, slow growing	Nongstoiñ	
15	Group 15	**NEHU-SC 16**	Circular, convex, entire high EPS, slow growing	Nongstoiñ	West Khasi Hills
16	Group 16	**NEHU-SC 18**	Circular, raised, entire, high EPS, fast growing	Ummulong	Jaiñtia Hills
17	Group 17	**NEHU-SC 19**	Circular, convex, entire high EPS, slow growing	Jowai	
18	Group 18	**NEHU-SC 20**	Circular, raised, entire, high EPS, slow growing	Khliehriat	
19	Group 19	**NEHU-SC 21**	Circular, raised, entire, high EPS, slow growing	Khliehriat	
20	Group 20	**NEHU-SC 23**	Circular, raised, entire, high EPS, slow growing	Umling	Ri-Bhoi
21	Group 21	**NEHU-SC 25**	Circular, raised, entire, high EPS, fast growing	Umling	
22	Group 22	**NEHU-SC 26**	Circular, raised, entire, less EPS, fast growing	Khanapara	
23	Group 23	**NEHU-SC 28**	Circular, raised, entire, high EPS, slow growing	Umroi	

3.3. Phylogenetic analysis of the Bradyrhizobium strains on the basis of 16S rRNA gene phylogeny

Despite the fact that the protein-coding gene is necessary to fully resolve the phylogenetic status of the strains, 16S rRNA is still regarded as a prerequisite for the identification and molecular study of the strains, due to its highly conserved sequence. The strains that were selected for MLSA studies were also considered for their 16S rRNA gene sequencing phylogenetic analysis (Fig 2). The strains NEHU-SC23 isolated from Umling, NEHU-SC28 isolated from Umroi of Ri-Bhoi and NEHU-SC16 isolated from Nongstoiñ (West Khasi Hills), formed a group that clustered close to *B. stylosanthis* BR446T (KU724142). The strain NEHU-SC15 isolated from Nongstoiñ

(West Khasi Hills) formed a lineage divergent from *B. jicamae* PAC68T (NR043036). The strains NEHU-DP7 isolated from Kynshi and NEHU-SC13 isolated from Mawthadraishan both of West Khasi Hills, each formed a novel lineage divergent from *B. rifense* CTAW71T(NR116361).

3.4. Phylogenetic analysis of Bradyrhizobium strains on the basis of protein-coding housekeeping gene

To improve the resolution of the 16S rRNA-based phylogeny, a multi locus sequence analysis (MLSA), using three conserved protein-coding housekeeping genes (*recA, glnII* and *atpD*) was performed on sixteen selected *Bradyrhizobium* strains.

3.5. Phylogenetic analysis of the Bradyrhizobium strains on the basis of recA gene phylogeny

On the basis of *recA*, majority of the clusters were formed by the strains from West Khasi Hills and Ri-Bhoi areas. The clustering of strains was connected to their geographical origin as seen in C2 (T-III), where all the strains (NEHU-DP22, NEHU-DP19 and NEHU-DP24) were isolated from Marngar, Nongpoh and Khanapra of Ri-Bhoi areas. However, significant diversity was also found where the microsymbionts from tropical wet regions and with low elevation of Ri-Bhoi areas and high elevations of East and West Khasi Hills formed a diverse clade and lineages. The cluster C1 formed by the strains (NEHU-DP7 and NEHU-DP23) isolated from Kynshi/West Khasi Hills and Umling/Ri-Bhoi district and C3 (NEHU-DP8 & NEHU- SC12) both were isolated from Mawthadraishan/West Khasi Hills, NEHU-SC8 isolated from Sohiong/East Khasi Hills, and NEHU-SC23 isolated from Umling/Ri-Bhoi district which is of different climatic conditions and thereby suggesting their differentiation in its origin. The single novel lineage L1 (NEHU-SC13) isolated from Mawthadraishan, C1 and C2 shows divergence from *B. sacchari* BR10280T (KX065095). Details are illustrated in Fig 3.

3.6. Phylogenetic analysis of the Bradyrhizobium strains on the basis of atpD gene phylogeny

The strains selected for the MLSA studies were also subjected for sequencing of the gene *atpD* encoding for ATP synthase F1 beta subunit and the Maximum Likelihood phylogenetic tree were also constructed (Fig 4). The two strains NEHU-DP7 and NEHU-SC13 that were isolated from Kynshi and Mawthadraishan respectively (West Khasi Hills), formed a clade divergent from *B. ganzhouense* RITF806T (JX277182). The strains NEHU-SC28 isolated from Umroi (Ri-Bhoi) and NEHU-SC15 isolated from Nongstoiñ (West Khasi Hills), each formed separate lineages divergent from *B. iriomotense* EK05T (AB300994). The strains NEHU-SC20 and NEHU-SC21 both were isolated from Khliehriat (Jaiñtia Hills), clustered together diverging from *B. embrapense* SEMIA 6208T (HQ634875). And the strains NEHU-DP8 isolated from Mawthadraishan, NEHU-SC16 isolated from Nongstoiñ, NEHU-SC12 isolated from Mawthadraishan from West Khasi Hills and NEHU-SC23 isolated from Umling (Ri-Bhoi), clustered together to form a clade divergent from *B. elkani* USDA 76T (AY386758).

3.7. Phylogenetic analysis of the Bradyrhizobium strains on the basis of glnII gene phylogeny

In case of the gene *glnII,* the strains NEHU-DP7 and NEHU-SC13 that were isolated from Kynshi and Mawthadraishan respectively (West Khasi Hills), formed a clade divergent from *B.*

ganzhouense RITF806T (JX277110). Similarly, strains NEHU-SC28 isolated from Umroi (Ri-Bhoi) and NEHU-SC15 isolated from Nongstoiñ (West Khasi Hills) form a clade divergent from *B. iriomotense* EK05T (AB300995). NEHU-SC12 isolated from Mawthadraishan (West Khasi Hills) and NEHU-SC23 isolated from Umling (Ri-Bhoi), clustered together to form a clade divergent from *B. mercantei* SEMA6399T (KX690621) and similarly, the strain NEHU-DP8 isolated from Mawthadraishan and NEHU-SC16 isolated from Nongstoiñ (West Khasi Hills) form a separate lineage divergent from *B. mercantei* SEMA6399T (KX690621). And NEHU- SC20 and NEHU-SC21 isolated from Khliehriat (Jaiñtia Hills) each formed a lineage divergent from *B. pachyrhizi* PAC48T (FJ428201). Details are illustrated in Fig 5.

3.8. Phylogenetic analysis of the Bradyrhizobium strains on the basis of concatenated gene (rrs- glnII-atpD-recA) phylogeny

On the basis of *rrs-glnII-atpD-recA* genes, a concatenated gene phylogeny was constructed (Fig 6) and percentage similarity with the type strains was calculated (Table 3).

Table 3. Percentage sequence similarity of *Bradyrhizobium* strains isolated from *D. polycarpum* and *S. ciliata* based on concatenated gene (*rrs- recA glnII-atpD*) phylogeny.

Type Strains	NEHU-DP7	NEHU-SC13	NEHU-SC16	NEHU-SC23	NEHU-SC28
***B. americanum* CMVU44T (KC247141)**	93.37	93.29	89.89	89.99	92.31
***B. arachidis* CCBAU 051107T (HM107233)**	94.63	94.12	91.22	91.39	94.02
***B. betae* PL7HG1T (FJ970378)**	94.53	94.18	89.99	90.07	94.98
***B. canariense* BTA-1T (AY591553)**	93.73	93.55	89.90	89.90	94.71
***B. centrosemae* A9T (KC247145)**	93.30	92.87	90.76	91.29	92.31
***B. cytisi* CTAW11T (GU001575)**	93.57	93.40	89.72	89.63	93.93
***B. daqingense* CCBAU 15774T (HQ231270)**	92.85	92.76	89.41	89.21	91.59
***B. denitrificans* LMG 8443T (EU665419)**	88.07	87.60	88.26	88.35	88.37
***B. elkanii* USDA76T (AY591568)**	89.93	89.39	94.74	94.65	91.07
***B. embrapense* CNPSo 2833T (HQ634899)**	89.81	89.54	94.64	95.59	90.94
***B. ganzhouense* RITF806T (JX277144)**	95.14	94.80	89.98	89.71	93.99
***B. guangdongense* CCBAU 51649T (KC509269)**	93.76	93.41	90.00	89.44	92.02
***B. guangxiense* CCBAU 53363T (KC509279)**	93.75	93.31	90.03	90.55	92.67
***B. huanghuaihaiense* CCBAU 23303T (HQ231595)**	94.98	94.64	89.92	89.91	93.49
***B. icense* LMTR 13T (JX943615)**	88.82	88.45	90.86	90.78	89.42
***B. iriomotense* EK05T (AB300996)**	93.41	93.06	89.09	88.51	93.14
***B. japonicum* USDA 6T (AM168341)**	93.41	93.24	89.58	90.40	96.29
***B. jicamae* LMG 24556T (HQ587415)**	89.11	89.02	90.51	90.97	89.43
***B. lablabi* CCBAU 23086T (GU433522)**	89.26	89.08	92.53	92.35	89.78
***B. liaoningense* LMG 18230T (FM253180)**	94.63	94.46	90.38	90.19	93.67
***B. lupini* USDA 3051T (KM114866)**	93.91	93.57	90.20	90.02	94.38
***B. namibiense* 5-10T (KM378377)**	89.12	89.02	89.36	89.93	88.35
***B. oligotrophicum* LMG 10732T (JQ619231)**	86.78	86.40	87.51	88.16	87.61
***B. ottawaense* OO99T (HQ587287)**	93.47	93.30	90.49	90.67	93.41
***B. pachyrhizi* PAC48T (HM590777)**	89.91	89.37	94.90	95.16	90.03
***B. paxllaeri* LMTR 21T (JX943617)**	88.17	87.98	90.13	90.59	88.87
***B. retamae* Ro19T (KC247094)**	87.98	87.61	89.96	90.14	89.05
***B. rifense* CTAW71T (GU001585)**	95.23	94.89	89.71	89.91	93.21
***B. sacchari* BR10280T (KX065095)**	93.66	93.31	90.30	90.29	92.22
***B. tropiciagri* CNPSo 1112T (FJ391168)**	90.38	90.02	94.91	94.74	91.48
***B. valentinum* LmjM3T (JX518589)**	88.26	87.79	89.36	89.07	88.36
***B. yuanmingense* CCBAU 10071T (AM168343)**	93.22	93.05	91.78	91.78	93.32

The strains NEHU- DP7 and NEHU-SC13 isolated from Kynshi and Mawthadraishan respectively (West Khasi Hills), clustered together to form a clade divergent from *B. ganzhouense* RITF806T

(JX277144). Similarly, strains NEHU-SC16 and NEHU-SC23 isolated from Nongstoiñ (West Khasi Hills) and Umling (Ri-Bhoi) respectively, clustered together to form a clade divergent from *B. pachyrhizi* PAC48T (HM590777). And the strain NEHU-SC28 isolated from Umroi (Ri-Bhoi) form a lineage divergent from *B. japonicum* USDA 6T (AM168341).

3.9. Phylogenetic analysis of the Bradyrhizobium strains on the basis of concatenated gene (glnII- atpD-recA) phylogeny

The concatenated dendogram, which combines the sequences of protein-coding housekeeping genes, excluding the conserved 16S rRNA gene, was also generated, and a finer resolution of the phylogenetic position of the strains was inferred as a result (Fig 7) and percentage similarity with the type strains was calculated (Table 4). The strains NEHU-DP7 and NEHU-SC13 isolated from Kynshi and Mawthadraishan respectively (West Khasi Hills), clustered together to form a clade divergent B. ganzhouense RITF806T (JX277144). Similarly, the strains NEHU-SC12, NEHU-SC23 and NEHU-SC16 isolated from Mawthadraishan (West Khasi Hills), Umling (Ri-Bhoi), and Nongstoiñ (West Khasi Hills) clustered together to form a clade divergent *B. pachyrhizi* PAC48T (HM590777) and NEHU-SC20 and NEHU-SC21 isolated from Khliehriat (Jaiñtia Hills) clustered together to form a clade divergent from *B. embrapense* CNPSo 2833T (HQ634899). The strain NEHU-SC28 isolated from Umroi (Ri-Bhoi) form a lineage divergent from *B. iriomotense* EK05T (AB300996).

3.9. Phylogenetic analysis of RNB strains on the basis of symbiotic genes

3.9.1. Phylogenetic analysis of the Bradyrhizobium strains on the basis of nodA gene phylogeny

On the basis of nodA gene phylogeny, eleven *Bradyrhizobium* strains separated to form seven nodA types (TI-TVII) with 4 novel lineages and 3 clades (Fig 8). The clade C1(T-I) that comprises of the strains NEHU-SC12, NEHU-SC23 and NEHU-DP8 isolated from Mawthadraishan (West Khasi Hills), Umling (Ri-Bhoi) and Mawthadraishan (West Khasi Hills) respectively, formed a cluster divergent from *B. elkanii* USDA 76T (AM117554) isolated from *G. max*, USA. The strains NEHU-SC20 and NEHU-SC21 isolated from Khliehriat (Jaiñtia Hills) clustered together and formed a clade C2(T-III) divergent from *B. pachyrhizi* LMG 24246T (KC509198) isolated from *Pachyrhizus erosus*, Costa Rica. And the last clade C3(T-VI) that includes the strains NEHU-SC6 and NEHU-SC15 isolated from NEHU (East Khasi Hills) and Nongstoiñ (West Khasi Hills) formed a clade divergent from *B. japonicum* USDA 6T (AM117545) isolated from *G. max*, USA. The lineage L1(T-II) includes the strain NEHU-SC16 isolated from Nongstoiñ (West Khasi Hills) divergent from *B. elkanii* USDA 76T (AM117554) isolated from *G. max*, USA. The strain NEHU-SC28 isolated from Umroi (Ri-Bhoi), formed a single lineage L2 (T-IV) divergent from *B. arachidis* CCBAU 051107T (KC509196) isolated from *Arachis hypogaea*, China. The strain NEHU-SC13 isolated from Mawthadraishan and the strain NEHU-DP7 isolated from Kynshi of West Khasi Hills, each formed a separate single lineage L3(T-V) and L4(T-VII) respectively, divergent from *B. japonicum* USDA 6T (AM117545) isolated from *Glycine max*, USA.

3.9.2. Phylogenetic analysis of the Bradyrhizobium strains on the basis of nifH gene phylogeny

The clade C1(T-I) that comprises of the strains NEHU-SC12, NEHU-SC23 and NEHU-DP8 isolated from Mawthadraishan (West Khasi Hills), Umling (Ri-Bhoi) and Mawthadraishan (West Khasi Hills) respectively, formed a cluster divergent from *B. elkanii* USDA 76T (AB094963) isolated from Japan.

Table 4. Percentage sequence similarity of *Bradyrhizobium* strains isolated from *D. polycarpum* and *S. ciliata* based on concatenated gene (*recA-glnII-atpD*) phylogeny.

	NEHU-DP7	**NEHU-SC1**	**NEHU-SC13**	**NEHU-SC16**	**NEHU-SC20**	**NEHU-SC21**	**NEHU-SC23**	**NEHU-SC28**
***B. americanum* CMVU44T (KC247141)**	93.37	89.90	93.29	89.90	89.34	89.45	89.99	92.31
***B. arachidis* CCBAU 051107T (HM107233)**	94.63	91.30	94.12	91.23	90.73	90.73	91.40	94.02
***B. betae* PL7HG1T (FJ970378)**	94.53	89.98	94.18	90.00	89.48	89.13	90.08	94.98
***B. canariense* BTA-1T (AY591553)**	93.73	89.81	93.55	89.90	90.17	90.08	89.91	94.71
***B. centrosemae* A9T (KC247145)**	93.30	91.30	92.86	90.76	90.62	90.54	91.30	92.31
***B. cytisi* CTAW11T (GU001575)**	93.57	89.54	93.40	89.73	89.68	89.96	89.64	93.93
***B. daqingense* CCBAU 15774T (HQ231270)**	92.85	89.12	92.76	89.42	89.33	89.24	89.22	91.59
***B. denitrificans* LMG 8443T (EU665419)**	88.07	88.17	87.61	88.27	88.04	87.67	88.36	88.38
***B. elkanii* USDA76T (AY591568)**	89.93	94.47	89.39	94.74	95.16	95.09	94.65	91.07
***B. embrapense* CNPSo 2833T (HQ634899)**	89.73	95.33	89.46	94.56	96.62	96.96	95.51	90.85
***B. ganzhouense* RITF806T (JX277144)**	95.14	89.71	94.80	89.98	88.89	89.10	89.72	93.99
***B. guangdongense* CCBAU 51649T (KC509269)**	93.75	89.44	93.41	90.01	89.22	89.42	89.44	92.03
***B. guangxiense* CCBAU 53363T (KC509279)**	93.75	90.65	93.31	90.03	89.99	89.72	90.56	92.67
***B. huanghuaihaiense* CCBAU 23303T (HQ231595)**	94.98	89.82	94.63	89.93	89.02	89.61	89.92	93.49
***B. icense* LMTR 13T (JX943615)**	88.83	90.51	88.46	90.87	91.05	90.95	90.78	89.43
***B. iriomotense* EK05T (AB300996)**	93.50	88.51	93.15	89.19	88.27	88.39	88.61	93.23
***B. japonicum* USDA 6T (AM168341)**	93.41	90.31	93.23	89.58	89.34	89.45	90.40	96.28
***B. jicamae* LMG 24556T (HQ587415)**	89.21	90.78	89.11	90.60	90.38	91.02	91.06	89.53
***B. lablabi* CCBAU 23086T (GU433522)**	89.36	92.17	89.17	92.61	91.96	91.78	92.44	89.87
***B. liaoningense* LMG 18230T (FM253180)**	94.63	90.01	94.46	90.39	91.00	90.54	90.20	93.67
***B. lupini* USDA 3051T (KM114866)**	93.91	89.93	93.57	90.20	90.19	90.11	90.02	94.38
***B. namibiense* 5-10T (KM378377)**	89.22	89.83	89.12	89.45	90.09	90.36	90.02	88.44
***B. oligotrophicum* LMG 10732T (JQ619231)**	86.79	87.98	86.41	87.52	88.46	88.07	88.17	87.62
***B. ottawaense* OO99T (HQ587287)**	93.47	90.58	93.30	90.49	89.73	89.84	90.67	93.41
***B. pachyrhizi* PAC48T (HM590777)**	90.00	95.07	89.46	94.99	96.18	96.53	95.25	90.13
***B. paxllaeri* LMTR 21T (JX943617)**	88.27	90.41	88.08	90.22	90.85	90.84	90.68	88.97
***B. retamae* Ro19T (KC247094)**	87.99	89.86	87.62	89.96	90.32	90.67	90.14	89.06
***B. rifense* CTAW71T (GU001585)**	95.23	89.81	94.89	89.71	89.84	89.85	89.91	93.21
***B. sacchari* BR10280T (KX065095)**	93.66	90.20	93.31	90.31	89.43	89.26	90.30	92.22
***B. tropiciagri* CNPSo 1112T (FJ391168)**	90.38	94.56	90.02	94.91	95.41	96.02	94.74	91.49
***B. valentinum* LmjM3T (JX518589)**	88.27	89.07	87.80	89.37	89.22	89.25	89.07	88.37
***B. yuanmingense* CCBAU 10071T (AM168343)**	93.22	91.88	93.05	91.78	90.20	90.65	91.78	93.32

Table 5. Percentage sequence similarity of *Bradyrhizobium* strains isolated from *D. polycarpum* and *S.ciliata* based on concatenated gene (*nod-nif*H) phylogeny.

Type strains	NEHU-DP7	NEHU-DP8	NEHU-SC6	NEHU-C12	NEHU-C13	NEHU-C16	NEHU-C20	NEHU-C21	NEHU-C23	NEHU-C28
***B. arachidis* CCBAU 051107T (KC509196)**	80.66	82.22	80.03	82.22	79.52	81.79	84.29	84.29	82.22	85.82
***B. brasilense* UFLA 03-321T (MPVQ01000056)**	77.92	82.46	77.27	82.46	77.81	82.64	85.78	85.78	82.46	81.67
***B. canariense* Oc6T (FR720454)**	68.56	73.22	67.28	73.22	66.20	74.14	70.51	70.51	73.22	69.10
***B. centrolobii* BR 10245T (LUUB01000057)**	66.35	70.65	66.09	70.65	65.06	70.44	67.21	67.21	70.65	67.26
***B. cytisi* LMG 25866T (KC509202)**	70.84	75.56	70.06	75.56	68.47	76.01	72.81	72.81	75.56	69.94
***B. daqingense* CCBAU 15774T (KC509194)**	96.81	82.59	97.83	82.59	96.12	81.60	81.27	81.27	82.59	82.29
***B. elkanii* USDA 76T (AM117554)**	80.71	96.13	80.71	96.13	80.25	95.96	88.04	88.04	96.13	83.05
***B. embrapense* SEMIA 6208T (LFIP02000007)**	79.07	82.48	78.43	82.48	78.10	82.66	85.22	85.22	82.48	83.17
***B. forestalis* INPA54BT (PGVG01000037)**	85.78	87.30	85.78	87.30	84.52	86.71	88.63	88.63	87.30	91.04
***B. guangdongense* CCBAU 51649T (KC509176)**	62.49	63.05	62.50	63.05	61.88	63.59	61.27	61.27	63.05	60.13
***B. guangxiense* CCBAU 53363T (KC509186)**	66.24	67.46	65.68	67.46	65.09	67.73	65.48	65.48	67.46	63.96
***B. huanghuaihaiense* CCBAU 23303T (KC509197)**	96.81	82.59	97.83	82.59	96.12	81.60	81.27	81.27	82.59	82.29
***B. icense* LMTR 13T (NZ CP016428)**	74.27	76.56	73.59	76.56	72.49	76.80	76.31	76.31	76.56	73.91
***B. iriomotense* EK05T (AB300999)**	65.63	69.17	65.07	69.17	65.23	69.88	66.29	66.29	69.17	64.54
***B. japonicum* USDA 6T (AM117545)**	96.81	82.59	97.83	82.59	96.12	81.60	81.27	81.27	82.59	82.29
***B. jicamae* LMG 24556T (KC509199)**	73.98	78.06	73.30	78.06	71.73	77.40	77.99	77.99	78.06	74.62
***B. lablabi* CCBAU 23086T (JX518553)**	72.08	76.27	71.38	76.27	71.17	76.95	76.50	76.50	76.27	73.86
***B. manausense* BR 3351T (NZ LJYG01000088)**	68.62	71.27	68.09	71.27	68.25	71.97	69.73	69.73	71.27	67.43
***B. pachyrhizi* LMG 24246T (KC509198)**	81.74	92.39	81.74	92.39	80.88	92.21	89.17	89.17	92.39	84.21
***B. paxllaeri* LMTR 21T (NZ MAXB01000110)**	73.05	76.98	72.36	76.98	71.71	77.65	76.34	76.34	76.98	73.25
***B. retamae* Ro19T (KF806459)**	73.59	76.53	72.85	76.53	71.53	76.77	76.55	76.55	76.53	73.93
***B. rifense* CTAW71T (LM994610)**	70.55	73.81	69.30	73.81	67.69	74.27	71.12	71.12	73.81	68.46
***B. sacchari* BR10280T (LWIG01000060)**	88.17	82.67	87.57	82.67	86.35	82.48	83.11	83.11	82.67	83.97
***B. stylosanthis* BR 446T (NZ LVEM01000003)**	68.28	71.68	67.97	71.68	68.04	71.93	68.22	68.22	71.68	66.56
***B. tropiciagri* SEMIA 6148T (LFLZ01000014)**	81.04	82.99	80.41	82.99	80.11	83.18	85.73	85.73	82.99	83.51
***B. valentinum* LmjM3T (JX518540)**	73.37	77.01	72.68	77.01	71.81	77.24	77.03	77.03	77.01	74.65
***B. viridifuturi* SEMIA 690T (LGTB01000005)**	79.07	82.48	78.43	82.48	78.10	82.66	85.22	85.22	82.48	83.17
***B. yuanmingense* CCBAU 10071T (KC509193)**	89.41	82.93	89.59	82.93	88.03	82.74	83.87	83.87	82.93	85.02

The strains NEHU-SC20 and NEHU-SC21 isolated from Khliehriat (Jaiñtia Hills) clustered together and formed a clade C2(T-III) divergent from *B. kavangense* 14-3T (KM378254) isolated from *Vigna unguiculata*, Namibia. And the last clade C3(T-VI) that includes the strains NEHU-DP7 and NEHU-SC6 isolated from Kynshi (West Khasi Hills) and NEHU (East Khasi Hills) respectively, formed a clade divergent from *B. daqingense* CCBAU 15774T (KF962701) isolated from *Glycine max*, Canada. The lineage L1(T-II) includes the strain NEHU-SC16 isolated from Nongstoiñ (West Khasi Hills) divergent from *B. elkanii* USDA 76T (AB094963) isolated

from Japan. The strain NEHU- SC28 isolated from Umroi (Ri-Bhoi), formed a single lineage L2 (T-IV) divergent from *B. arachidis* CCBAU 051107T (KF962700) isolated from *Arachis hypogaea*, Canada. The strain NEHU-SC13 isolated from Mawthadraishan (West Khasi Hills), formed a single lineage L3(T- V) and divergent from *B. yuanmingense* CCBAU 10071T (EU818927) isolated from *G. max* (Fig 9).

3.9.3. Phylogenetic analysis of the Bradyrhizobium strains on the basis of *nodA-nifH* gene phylogeny

The symbiotic genes phylogeny was found to be congruent with the housekeeping gene phylogeny suggesting the vertical inheritance was seen to occur in *Bradyrhizobium* strains. The phylogenetic tree with *nodA-nifH* genes, a concatenated tree was constructed (Fig 10) and percentage similarity with the type strains was calculated (Table 6).

Table 6. NCBI GenBank accession numbers of strains isolated from *D. polycarpum* and *S. ciliata*.

Strains	16S rRNA	*recA*	*atpD*	*glnII*	*nodA*	*nifH*
GenBank accession numbers of strains isolated from *D. polycarpum*						
NEHU-DP 7	OP673531	OP604030	OP603967	OP603976	OP604003	OP603993
NEHU-DP 8	-	OP604031	OP603968		OP604004	OP603994
NEHU-DP 19	-	OP604032	-	-	-	-
NEHU-DP 22	-	OP604033	-	-	-	-
NEHU-DP 23	-	OP604034	-	-	-	-
NEHU-DP 24	-	OP604035	-	-	-	-
GenBank accession numbers of strains isolated from *S. ciliata*						
NEHU-SC 6	-	OP603984	-	-	OP604005	OP603995
NEHU-SC 8	-	-	-	-	-	-
NEHU-SC 12	-	OP603985	OP603969	OP603977	OP604006	OP603996
NEHU-SC 13	OP673532	OP603986	OP603970	OP603978	OP604007	OP603997
NEHU-SC 15	OP673533	OP603987	-	-	OP604008	-
NEHU-SC 16	OP673534	OP603988	OP603971	OP603979	OP604009	OP603998
NEHU-SC 20	-	OP603989	OP603972	OP603980	OP604010	OP603999
NEHU-SC 21	-	OP603990	OP603973	OP603981	OP604011	OP604000
NEHU-SC 23	OP673535	OP603991	OP603974	OP603982	OP604012	OP604001
NEHU-SC 28	OP673536	OP603992	OP603975	OP603983	OP604013	OP604002

The strains NEHU-SC12, NEHU-SC23, NEHU-DP8 and NEHU-SC16 isolated from Mawthadraishan (West Khasi Hills), Umling (Ri-Bhoi), Mawthadraishan and Nongstoiñ (West Khasi Hills) clustered together to form a clade divergent from *B. elkanii* USDA 76T (AM117554) isolated from *G. max*, USA.

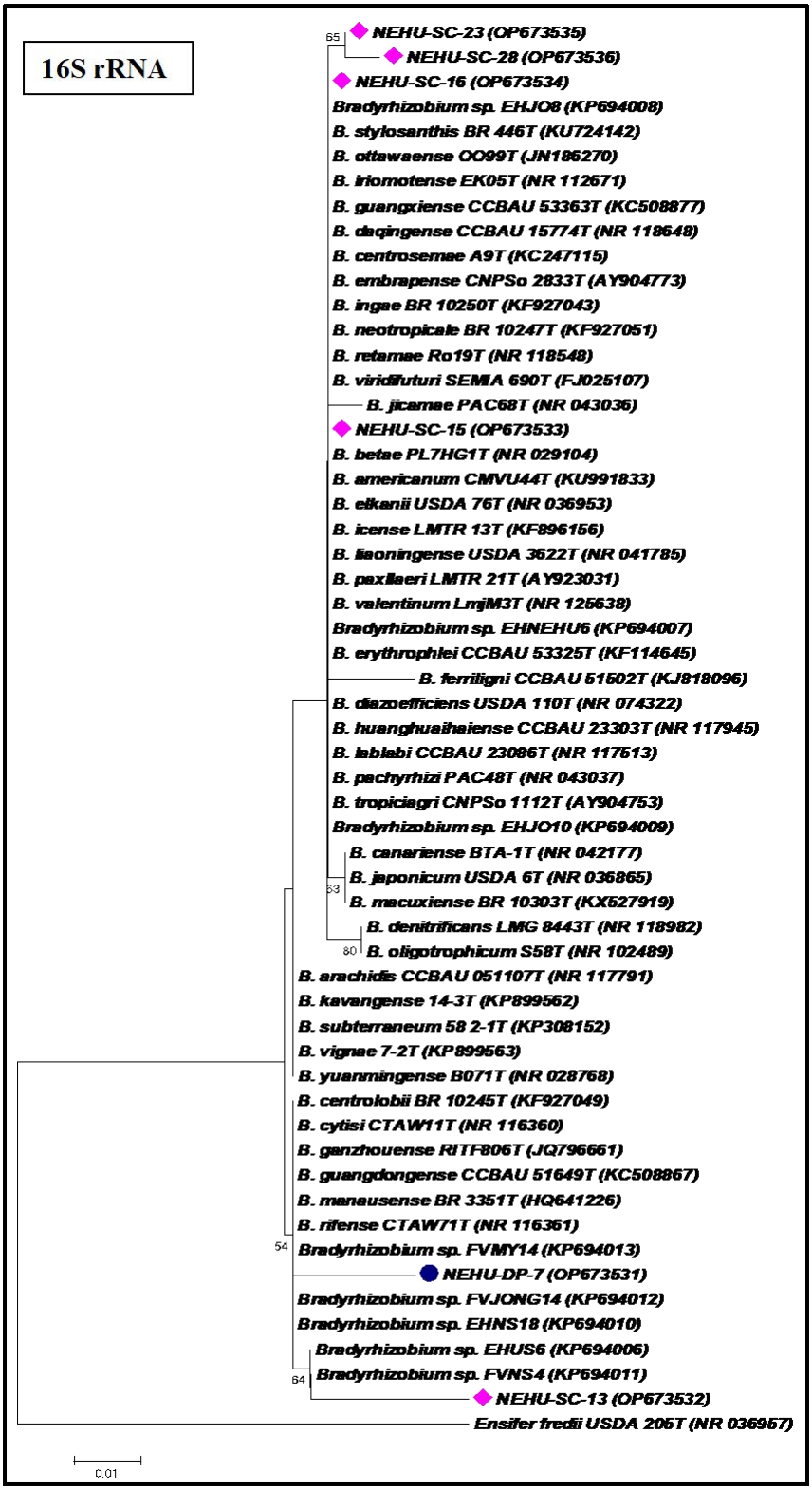

Fig 2. Phylogenetic analysis of **16S rRNA** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 1% substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

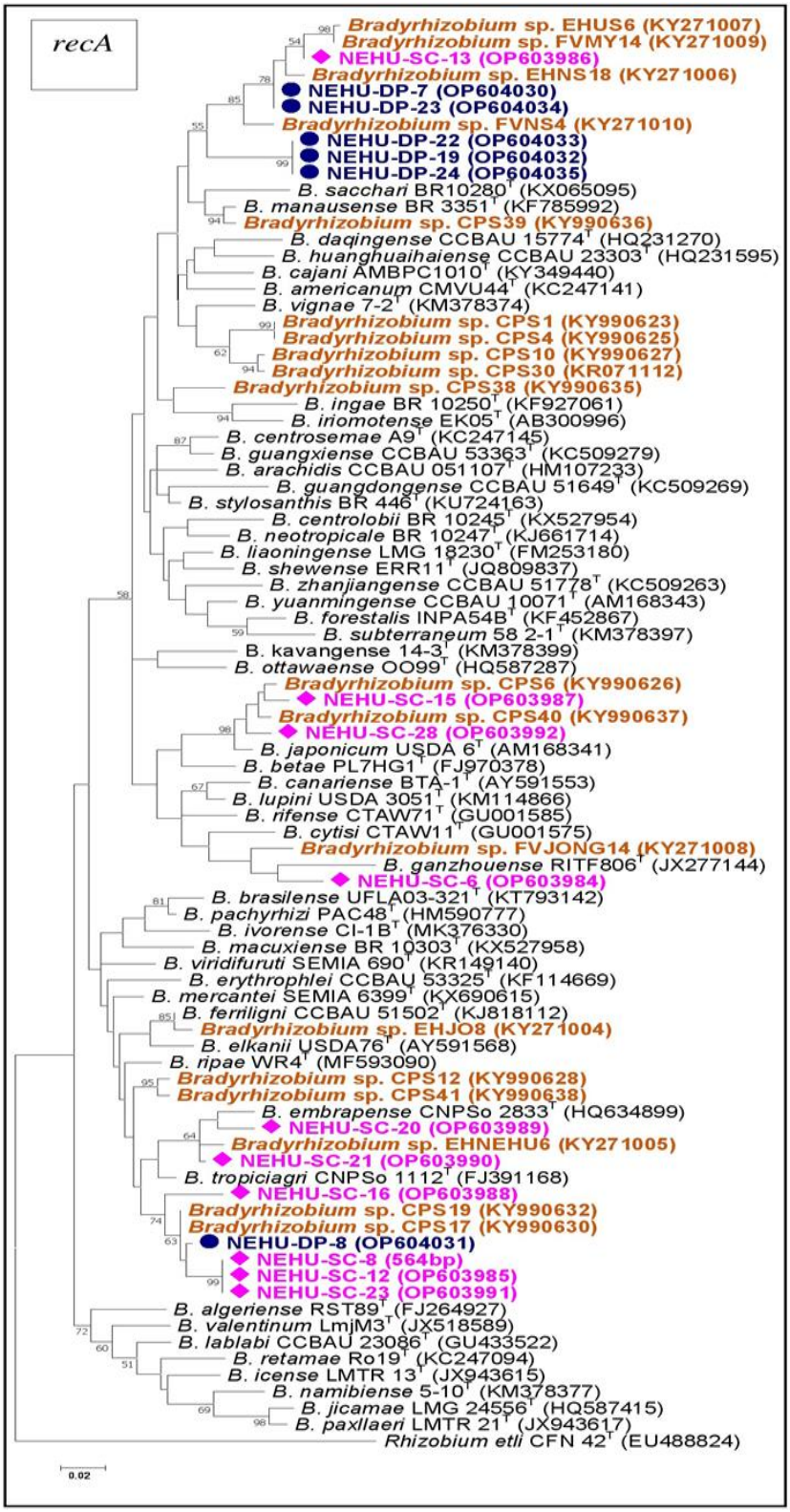

Fig 3. Phylogenetic analysis of ***recA*** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 2% substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

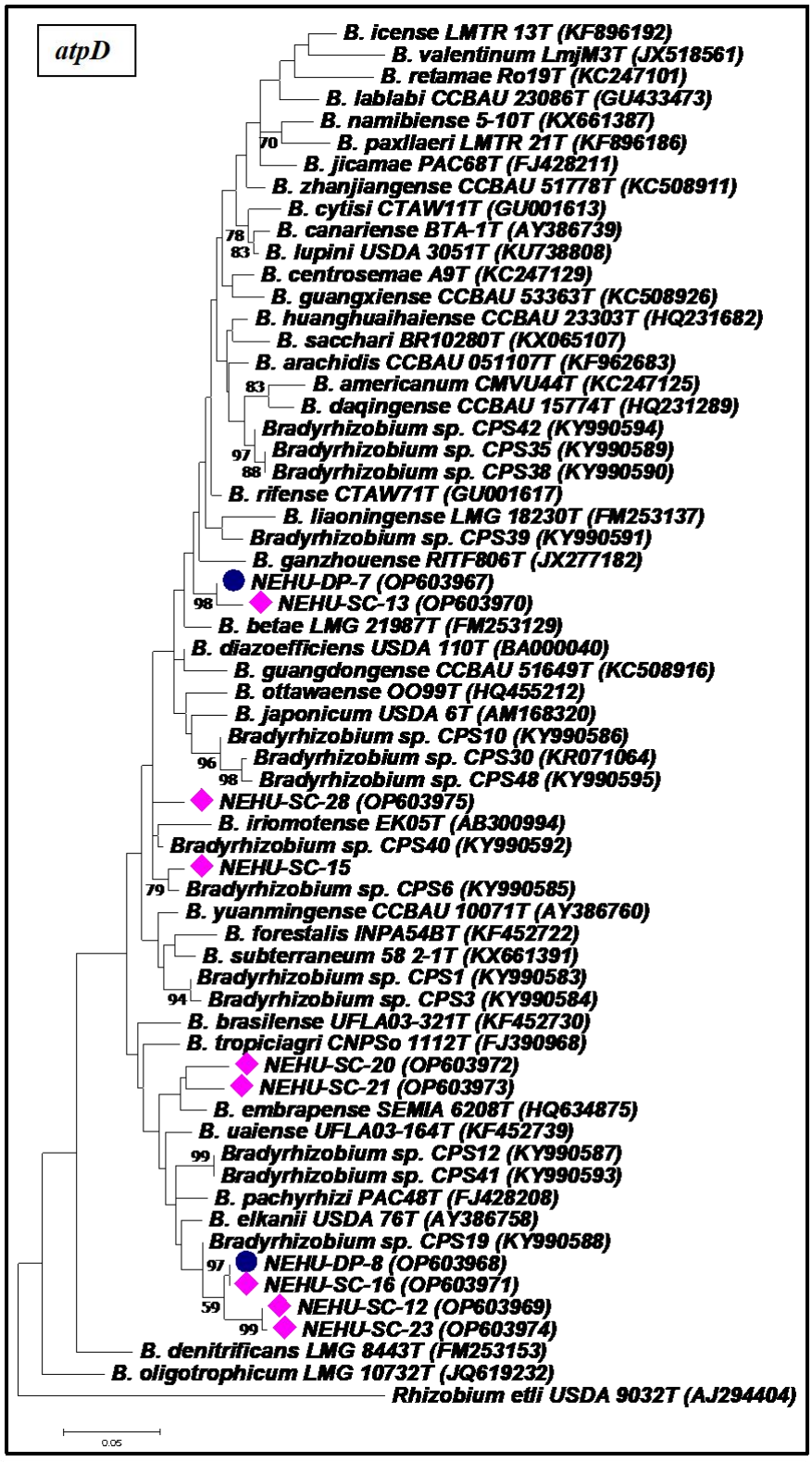

Fig 4. Phylogenetic analysis of ***atpD*** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 5% substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

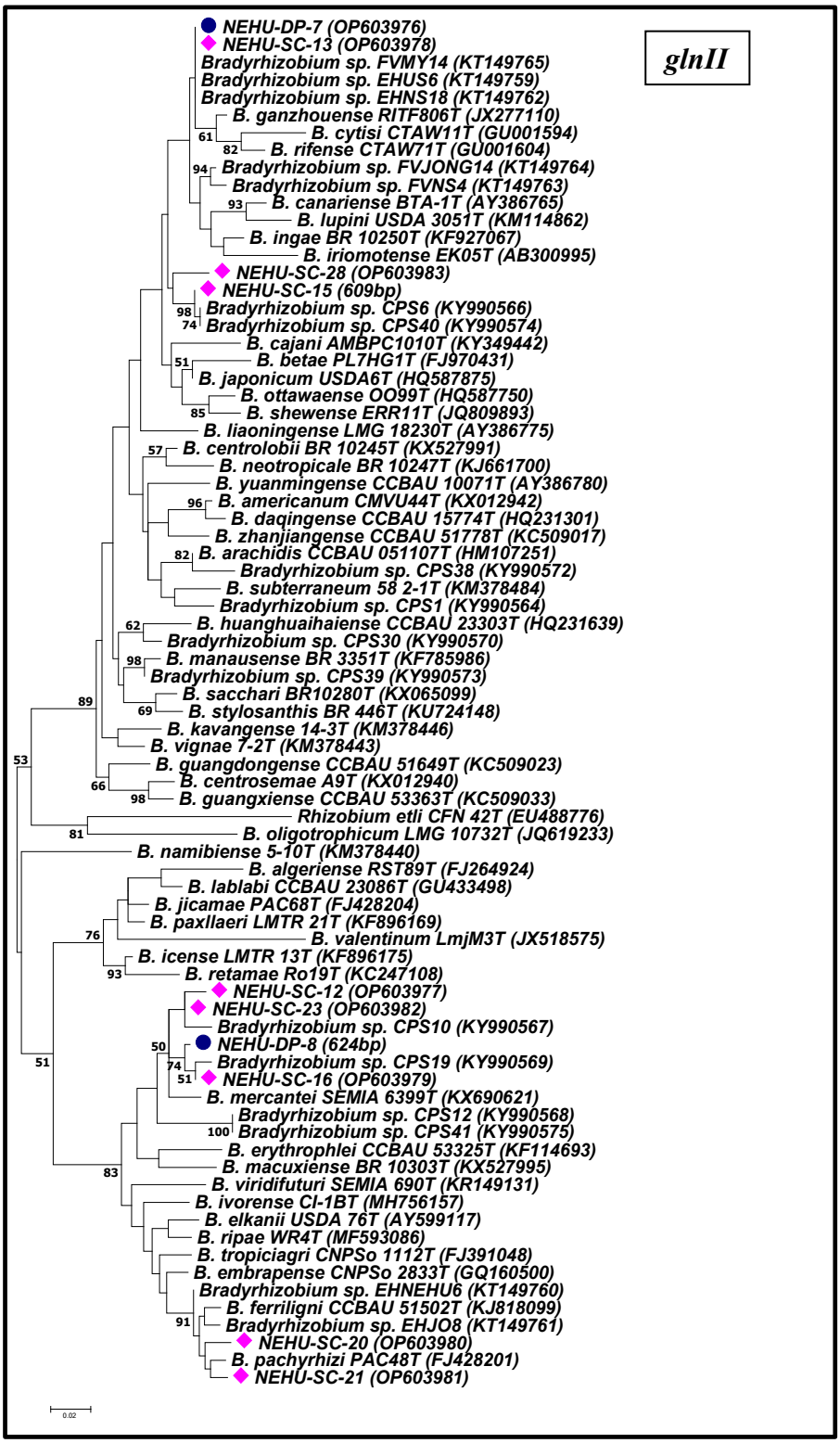

Fig 5. Phylogenetic analysis of ***glnII*** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 2% substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

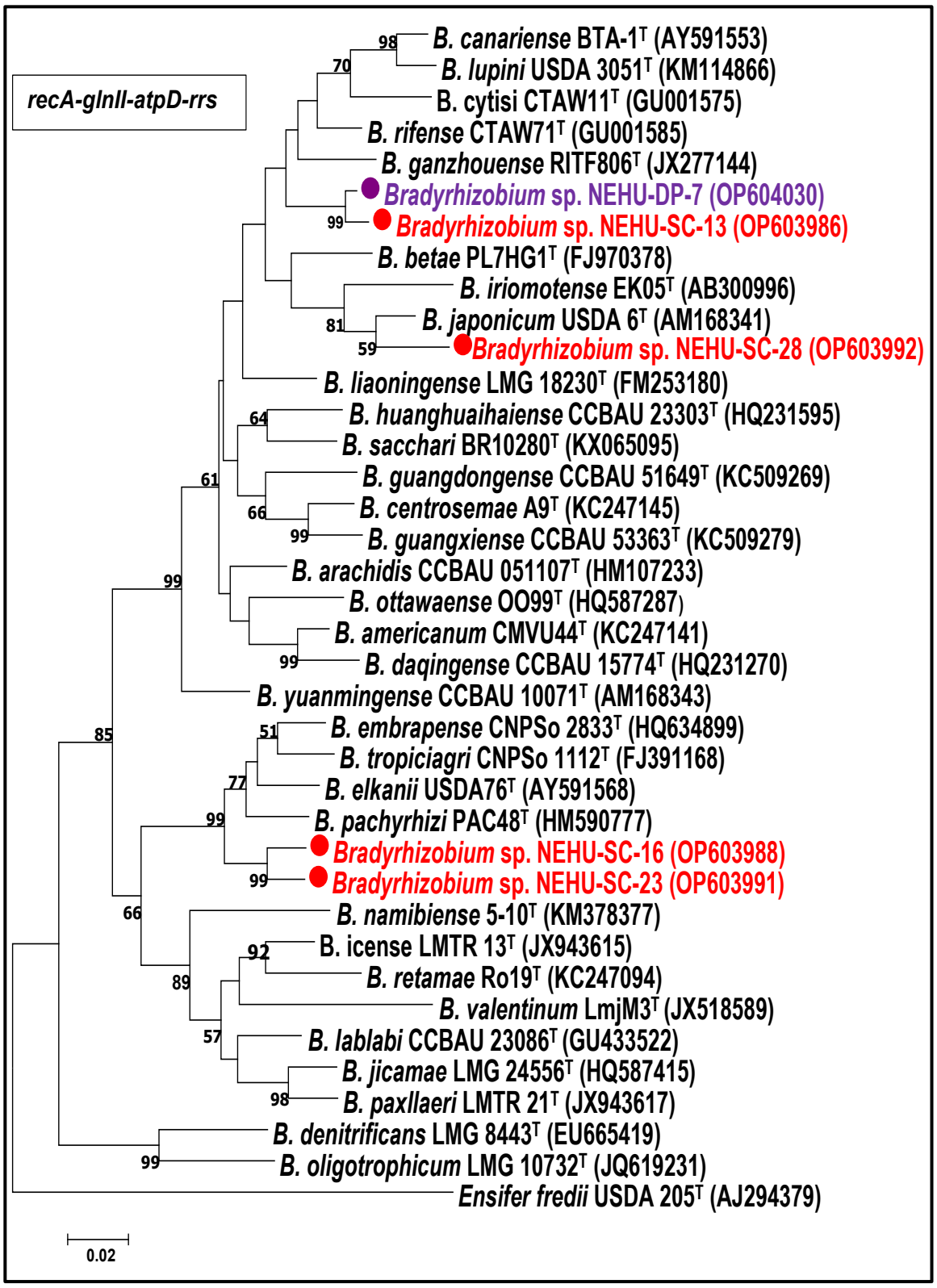

Fig 6. Comparative phylogenetic analysis of concatenated ***recA-glnII-atpD-rrs*** gene of *Bradyrhizobium* strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 2 % substitutions are indicated on the scale bar. Accession numbers obtained are in the parenthesis.

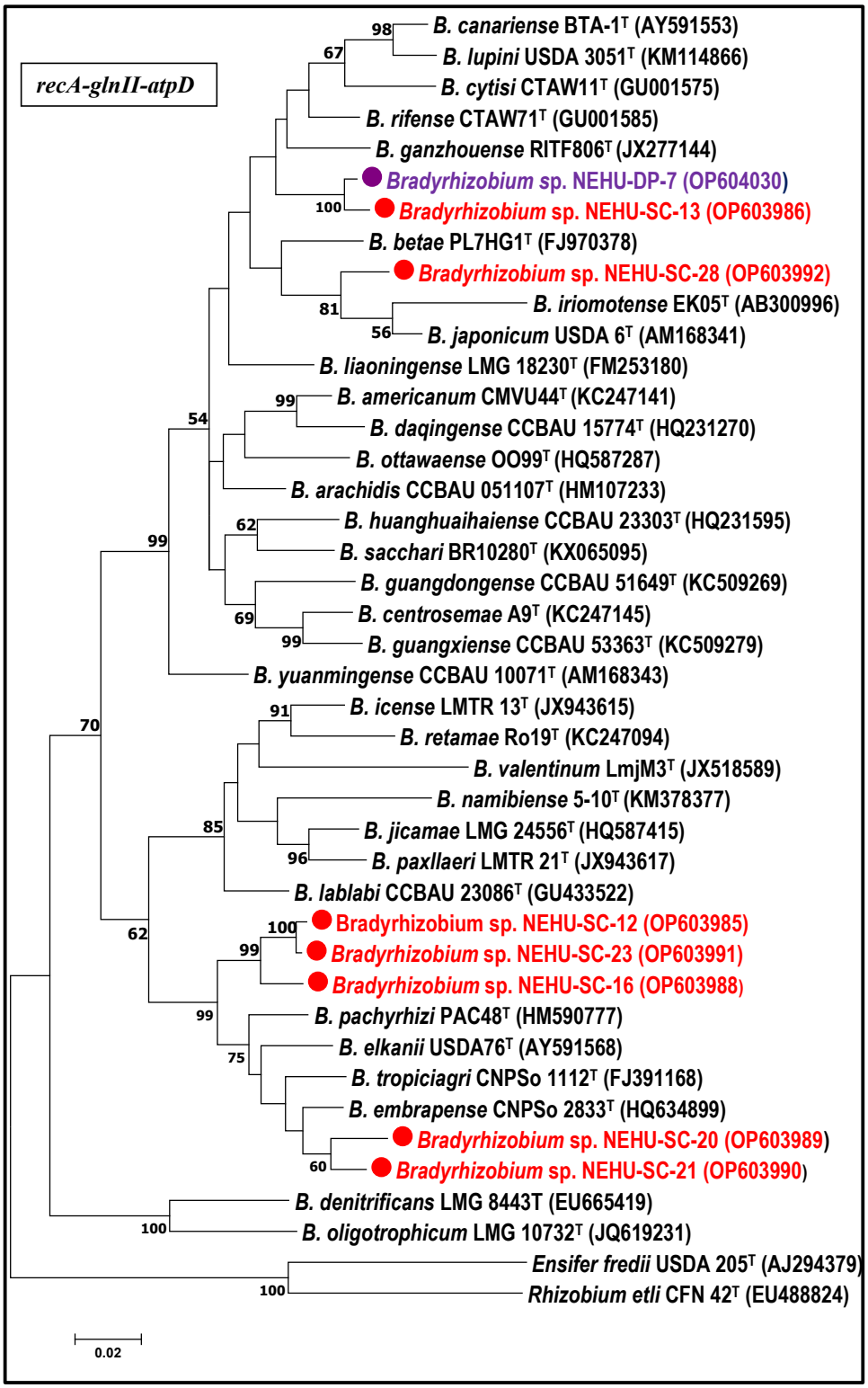

Fig 7. Comparative phylogenetic analysis of concatenated ***recA-glnII-atpD*** gene of *Bradyrhizobium* strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 2 % substitutions are indicated on the scale bar. Accession numbers obtained are in the parenthesis

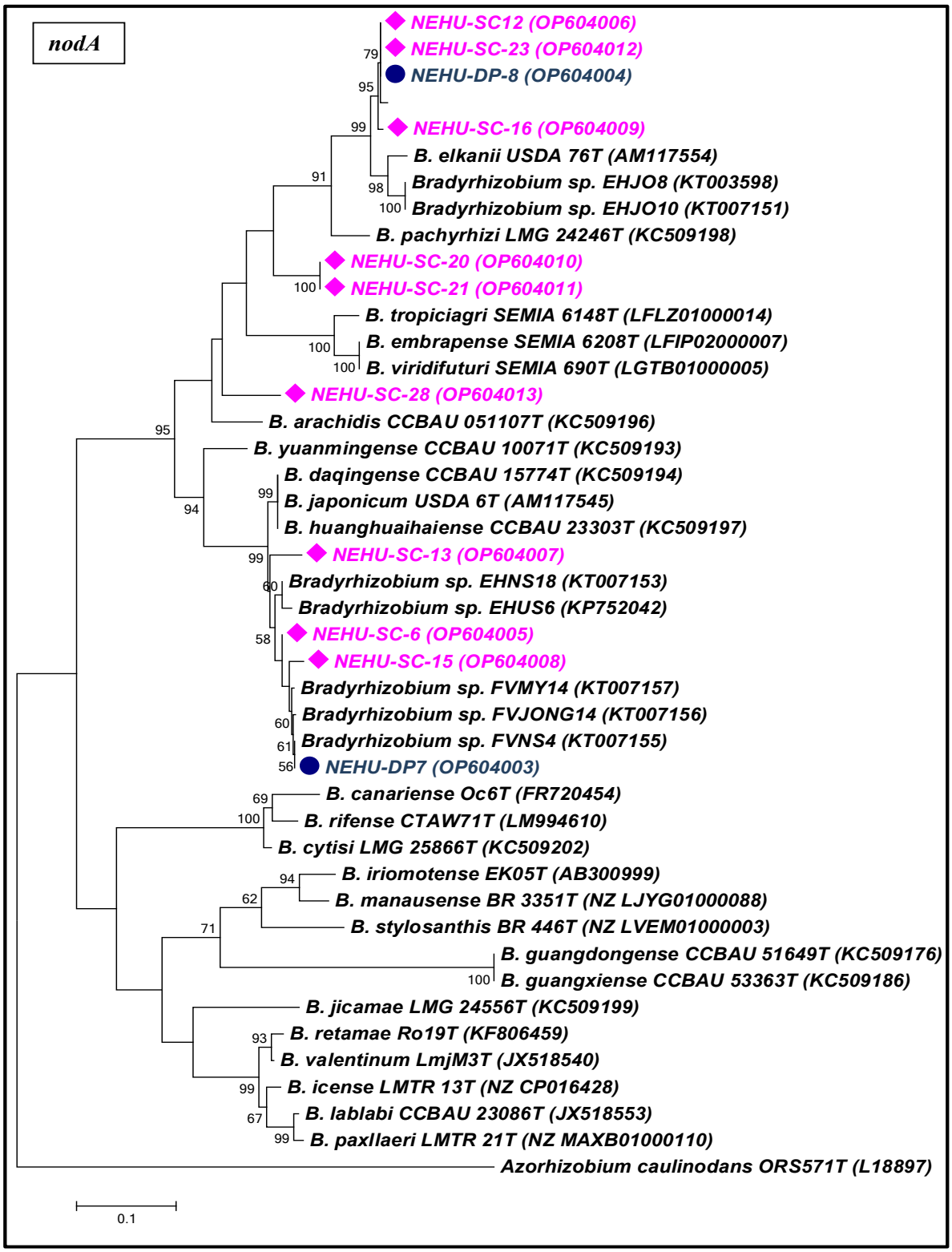

Fig 8. Phylogenetic analysis of ***nodA*** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 1% substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

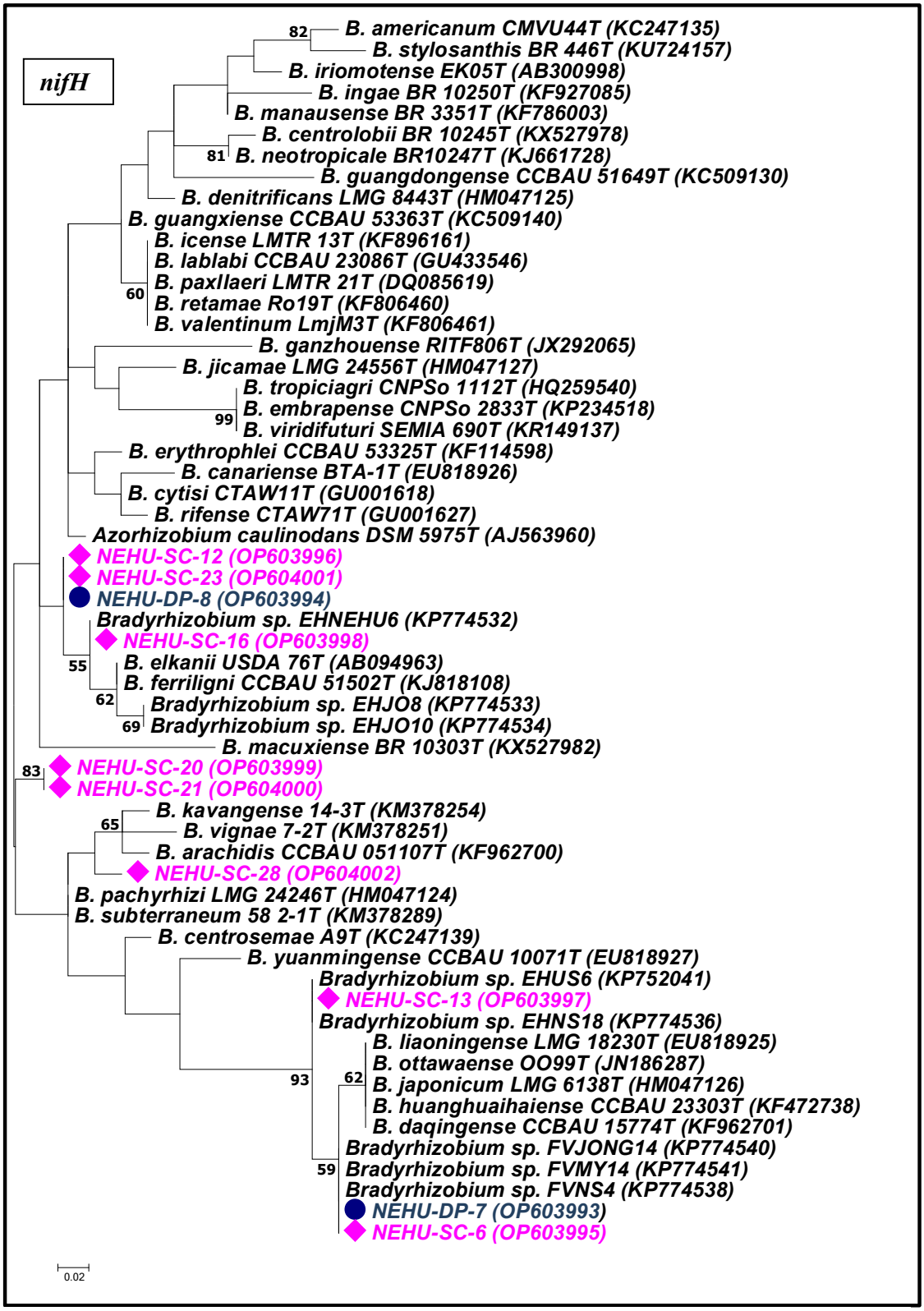

Fig 9. Phylogenetic analysis of ***nifH*** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 2% substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

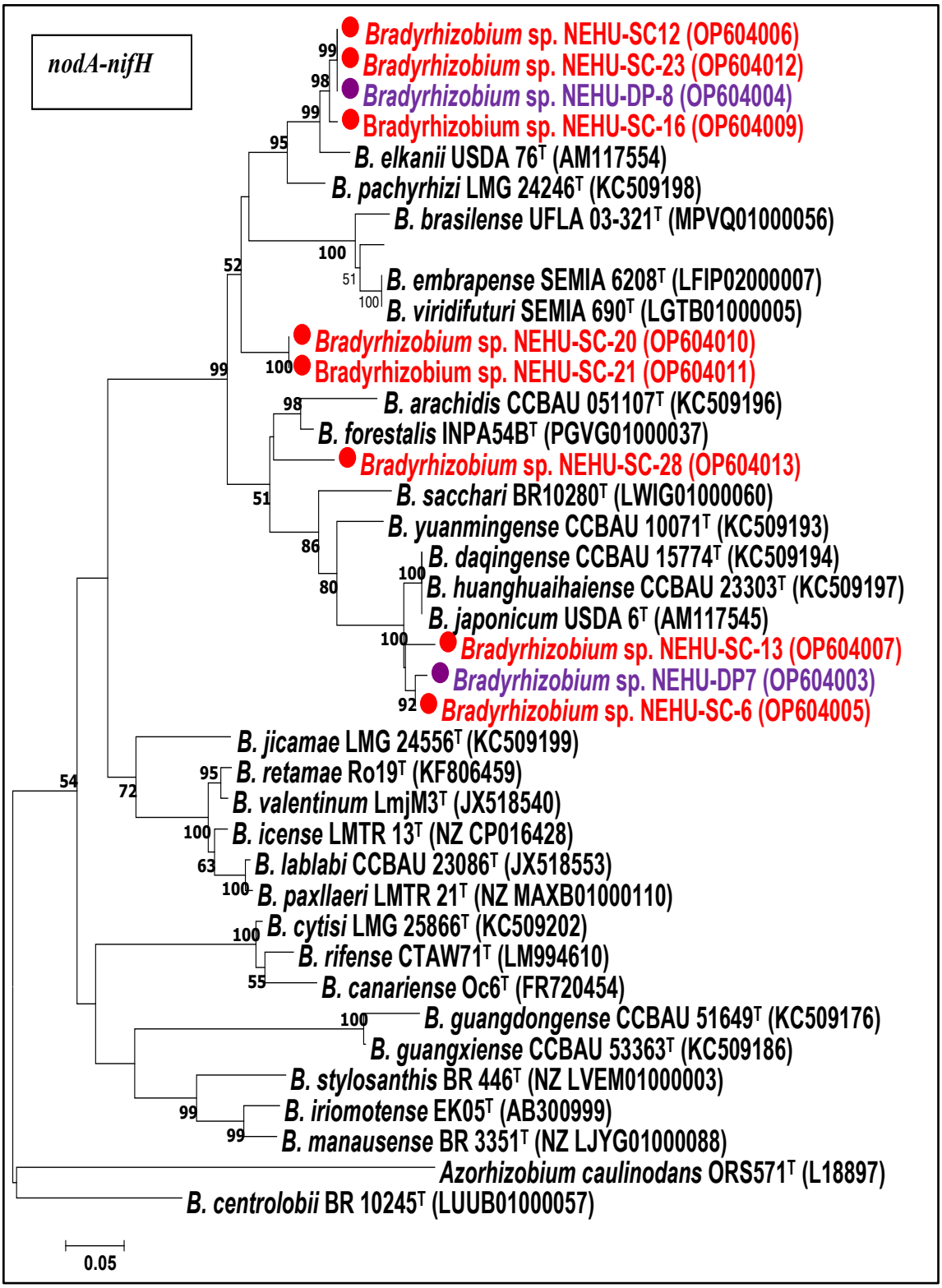

Fig 10. Phylogenetic analysis of ***nodA-nifH*** gene of *Bradyrhizobium* strains with other type strains using Maximum Likelihood method. Bootstrap values are indicated at the internodes for 1000 replications and 5 % substitutions are indicated on the scale bar. Accession numbers obtained are shown in parenthesis.

Similarly, strains NEHU-SC20 and NEHU-SC21, both were isolated from Khliehriat (Jaiñtia Hills) clustered together to form a clade divergent from *B. viridifuturi* SEMIA 690T (LGTB01000005) and the strains NEHU-DP7 and NEHU-SC6 isolated from Kynshi (West Khasi Hills) and NEHU (East Khasi Hills) respectively, clustered together divergent from *B. japonicum* USDA 6T (AM117545) isolated from *G. max*, Japan. The strains NEHU-SC28 and NEHU-SC13 isolated from Umroi (Ri-Bhoi) and Mawthadraishan (West Khasi Hills), each formed a separate lineage divergent from *B. forestalis* INPA54BT (PGVG01000037) and *B. japonicum* USDA 6T (AM117545) isolated from *G. max*, Japan, respectively. The NCBI accession numbers provided by GenBank for the housekeeping genes and symbiotic genes are given in Table 6.

4. Conclusion

In the present study, the BLASTN sequence similarity results of the *recA* gene of the genetically diverse strains isolated from *D. polycarpum* and *S. ciliata* revealed that all strains may belong to the genus *Bradyrhizobium*. The *recA* gene phylogenetic analysis of the *Bradyrhizobium* strains isolated from Meghalaya gives an insight into the wide distribution of other similar *Bradyrhizobium* strains that were isolated from different regions of the world and their biological origins. The strains NEHU-SC13 (L1-TI), NEHU-DP7 and NEHU-DP23 (C1-TII), NEHU-DP22, NEHU-DP19 and NEHU-DP24 (C2-TIII) clustered in a separate clade close to *B. sacchari* BR10280T (KX065095) isolated from Brazil. Interestingly, the *Bradyrhizobium* strains from *D. polycarpum* (NEHU-DP7 and NEHU-DP23 (C1-TII), NEHU-DP22, NEHU-DP19 and NEHU-DP24 (C2-TIII) and *S. ciliata* (NEHU-SC13 (L1-TI), in the present study, were also found to be close to or clustered with the Bradyrhizobial strains that was isolated from nodules of *E. chinense* and *F. vestita* (Ojha et al., 2017) and divergent from the *Bradyrhizobium* strains isolated from *C. pumila* (Rathi et al., 2018) that are native to Meghalaya, in *recA* phylogeny. The phylogeny of the symbiotic genes (*nodA* and *nifH*) of the *Bradyrhizobium* strains isolated from the two native legumes, *D. polycarpum* and *S. ciliata* growing in acidic soils were congruent with their house-keeping gene phylogeny.

The *nifH* gene derived phylogenetic tree was congruent with that of the *nodA* genes-based information. The strains of *nodA* group/type-I (NEHU-SC12, NEHU-SC23 and NEHU-DP8) and strain NEHU-SC16 that formed a single lineage close to, but divergent of *B. elkanii* USDA 76T isolated from *G. max*, USA. The strains NEHU-SC6 and NEHU-SC15 clustered together forming a novel clade (C3) and strains NEHU-SC13 and NEHU-DP7 formed a single lineage close to, but divergent from *B. japonicum* USDA 6T isolated from *G. max*, Japan. In the present study, the phylogenetic analysis of symbiotic genes indicated that the vertical transfer of symbiotic and fixation genes occurred in the *Bradyrhizobium* strains isolated from *D. polycarpum* and *S. ciliata* which is supported by the previous result with a wide range of bradyrhizobia from various host and environments (Moulin et al., 2004; Ojha et al., 2017; Rathi et al., 2018).

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Conflict of Interest: The authors declares that there is no conflict of interest

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