# Great diverse rhizobial community nodulating *Astragalus mongholicus* in the northeastern region of China

## Supplementary materials

**Supplementary Figure S1.** Neighbor-Nets tree are constructed based upon concatenated core genes (*recA, atpD, glnII*).

**Supplementary Figure S2.** Neighbor-Nets tree are constructed based upon symbiotic gene *nodC*.

**Supplementary Table S1.** Representative rhizobia of *Astragalus mongolicus* Bunge from NEC (34 strains with red color) and NWC (18 strains with blue color) for this study.

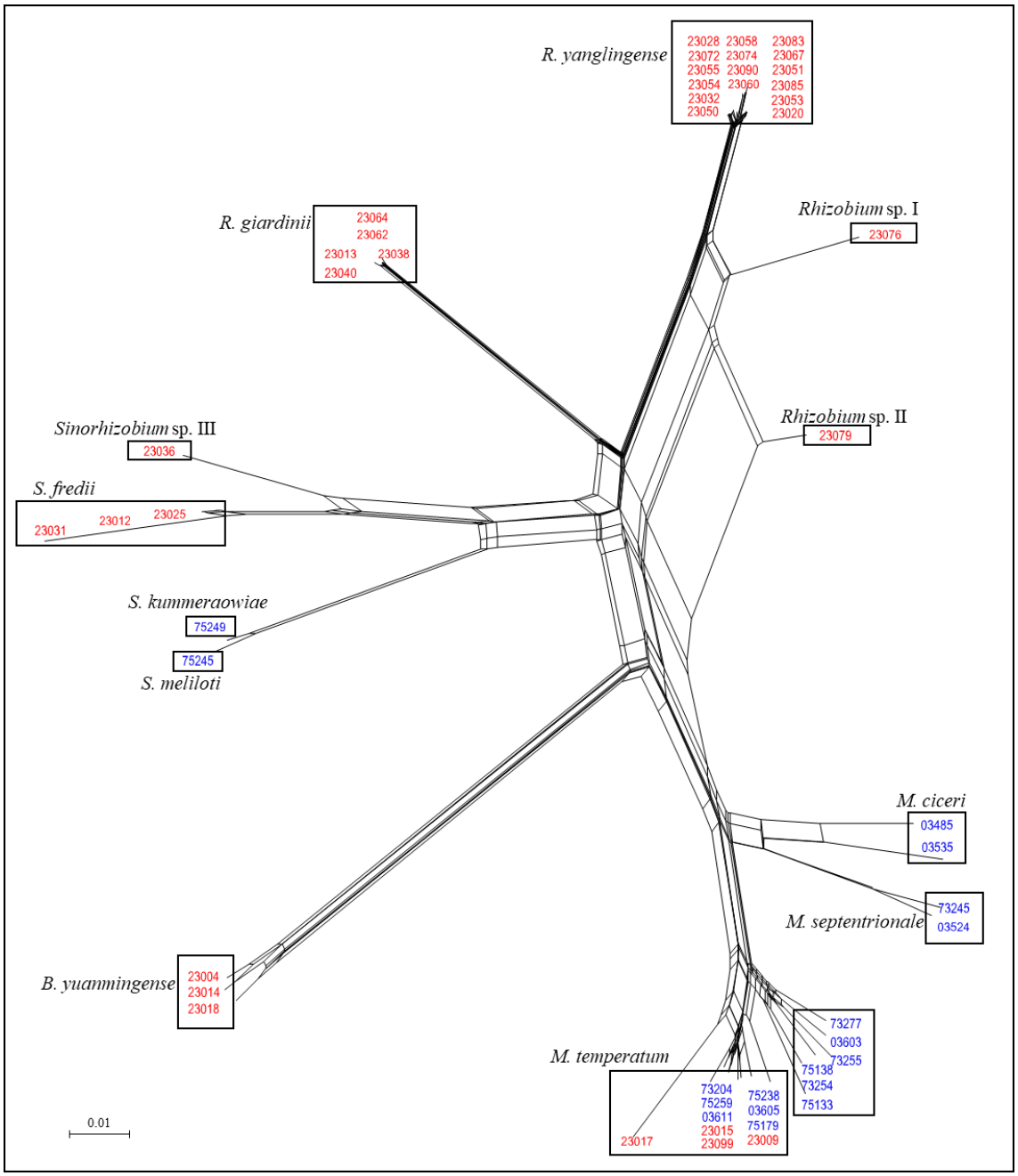
**Supplementary Table S2.** Primers for these genes and annealing temperature of them (Ta, °C) in PCR amplification.

**Supplementary Table S3.** Nucleotide polymorphism of representative rhizobia of *Astragalus mongolicus* Bunge from NEC and NWC.

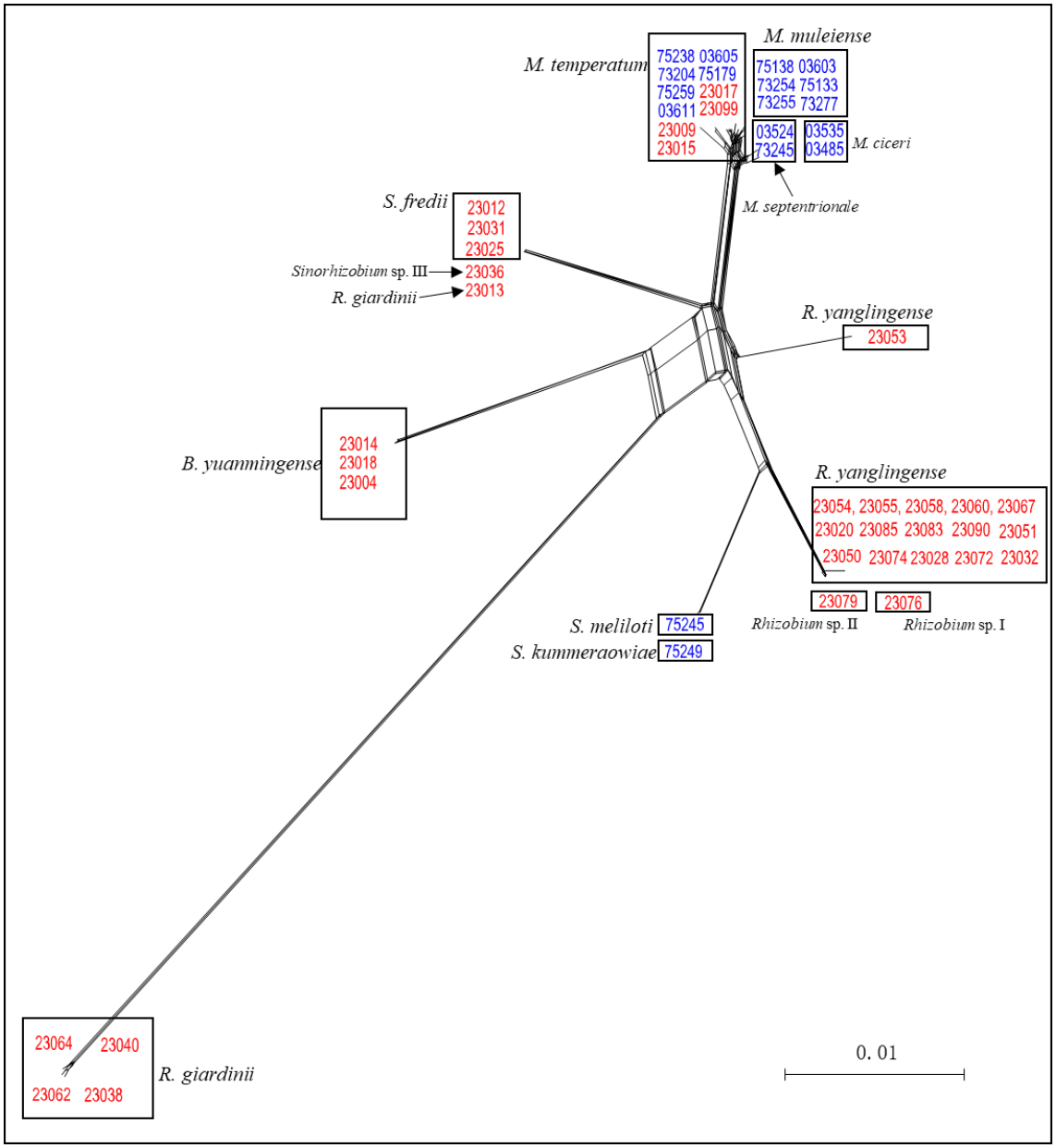
**Supplementary Table S4.** Genetic differentiation (presented as *Dxy*) and gene flow (presented as *Nm*) in representative rhizobia of *Astragalus mongolicus* Bunge from NEC and NWC.

**Supplementary Table S5.** Accession numbers of the genes obtained in NEC (34 strains with red color) and NWC (18 strains with blue color) and deposited in GenBank.

**Supplementary Figure S1. Neighbor-Nets tree are constructed based upon concatenated core genes (*recA, atpD, glnII*)**. These representative rhizobia of *Astragalus mongolicus* Bunge, with IMUNJ before the numbers isolated from NEC and CCBAU before the numbers isolated from NWC, were highlighted with red and blue colours, respectively. The length of *recA*, *atpD* and *glnII* sequences are 440, 425 and 503 bp, respectively.



**Supplementary Figure S2. Neighbor-Nets tree are constructed based upon symbiotic gene *nodC*.** These representative rhizobia of *Astragalus mongolicus* Bunge, with IMUNJ before the numbers isolated from NEC and CCBAU before the numbers isolated from NWC, were highlighted with red and blue colours, respectively. The length of *nodC* sequence is 429 bp.



**Supplementary Table S1**. Representative rhizobia of *Astragalus mongolicus* Bunge from NEC (34 strains with red color) and NWC (18 strains with blue color) for this study.

| Strains (No.) | Species | Areas | Collectors | Provinces | Host plants | Longitude | Latitude | |
|---|---|---|---|---|---|---|---|---|
| IMUNJ 23004 | *B. yuanmingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23009 | *M. temperatum* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23012 | *S. fredii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23013 | *R. giardinii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23014 | *B. yuanmingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23015 | *M. temperatum* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23017 | *M. temperatum* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23018 | *B. yuanmingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23020 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23025 | *S. fredii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23028 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23031 | *S. fredii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23032 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23036 | *Sinorhizobium* sp. III | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23038 | *R. giardinii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23040 | *R. giardinii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23050 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23051 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23053 | *M. temperatum* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23054 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23055 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23058 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23060 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23062 | *R. giardinii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23064 | *R. giardinii* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23067 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23072 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23074 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23076 | *Rhizobium* sp. I | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| IMUNJ 23079 | *Rhizobium* sp. II | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23083 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23085 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23090 | *R. yanglingense* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| IMUNJ 23099 | *M. temperatum* | NEC(AreaA) | Zhaojun Ji | Inner Mongolia | *A. mongholicus* | 120°45′35″ | 42°34′45″ | This study |
| CCBAU 03485 | *M. ciceri* | NWC(AreaB) | Hui Yan | Shanxi | *A. mongholicus* | 113°45′30.462″ | 39°25′57.585″ | Previous (Yan et al., 2016) |
| CCBAU 03524 | *M. septentrionale* | NWC(AreaB) | Hui Yan | Shanxi | *A. mongholicus* | 113°45′30.462″ | 39°25′57.585″ | Previous (Yan et al., 2016) |
| CCBAU 03535 | *M. ciceri* | NWC(AreaB) | Hui Yan | Shanxi | *A. mongholicus* | 113°45′30.462″ | 39°25′57.585″ | Previous (Yan et al., 2016) |
| CCBAU 03603 | *M. muleiense* | NWC(AreaB) | Hui Yan | Shanxi | *A. mongholicus* | 112°36′58.271″ | 36°59′59.582″ | Previous (Yan et al., 2016) |
| CCBAU 03605 | *M. temperatum* | NWC(AreaB) | Hui Yan | Shanxi | *A. mongholicus* | 112°36′58.271″ | 36°59′59.582″ | Previous (Yan et al., 2016) |
| CCBAU 03611 | *M. temperatum* | NWC(AreaB) | Hui Yan | Shanxi | *A. mongholicus* | 112°36′58.271″ | 36°59′59.582″ | Previous (Yan et al., 2016) |
| CCBAU 73204 | *M. temperatum* | NWC(AreaC) | Hui Yan | Gansu | *A. mongholicus* | 104°24′42.556″ | 35°04′36.277″ | Previous (Yan et al., 2016) |
| CCBAU 73245 | *M. septentrionale* | NWC(AreaC) | Hui Yan | Gansu | *A. mongholicus* | 104°03′54.723 | 34°32′45.599″ | Previous (Yan et al., 2016) |
| CCBAU 73254 | *M. muleiense* | NWC(AreaC) | Hui Yan | Gansu | *A. mongholicus* | 104°03′54.723 | 34°32′45.599 | Previous (Yan et al., 2016) |
| CCBAU 73255 | *M. muleiense* | NWC(AreaC) | Hui Yan | Gansu | *A. mongholicus* | 104°03′54.723″ | 34°32′45.599 | Previous (Yan et al., 2016) |
| CCBAU 73277 | *M. muleiense* | NWC(AreaC) | Hui Yan | Gansu | *A. mongholicus* | 104°03′22.441″ | 34°24′14.061″ | Previous (Yan et al., 2016) |
| CCBAU 75133 | *M. muleiense* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 105°51′44″ | 35°33′37″ | Previous (Yan et al., 2016) |
| CCBAU 75138 | *M. muleiense* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 105°51′44″ | 35°33′37″ | Previous (Yan et al., 2016) |
| CCBAU 75179 | *M. temperatum* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 105°58′04″ | 35°34′57″ | Previous (Yan et al., 2016) |
| CCBAU 75238 | *M. temperatum* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 106°15′09.129″ | 37°46′59.535″ | Previous (Yan et al., 2016) |
| CCBAU 75245 | *S. meliloti* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 106°15′09.129″ | 37°46′59.535″ | Previous (Yan et al., 2016) |
| CCBAU 75249 | *S. kummerowiae* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 106°15′09.129″ | 37°46′59.535″ | Previous (Yan et al., 2016) |
| CCBAU 75259 | *M. temperatum* | NWC(AreaD) | Hui Yan | Ningxia | *A. mongholicus* | 106°15′09.129″ | 37°46′59.535″ | Previous (Yan et al., 2016) |

**Supplementary Table S2.** Primers for these genes and annealing temperature of them (Ta, °C) in PCR amplification.

| Genes | Complete name or function | Preferred primers (5'→3', forward/reverse) | Ta (°C) |
|---|---|---|---|
| *atpD* | $F_0F_1$ ATP synthase subunit beta | GCTSGGCCGCATCMTSAACGTC/ GCCGACACTTCMGAACCNGCCTG | 58 |
| *glnII* | Glutamine synthetase II protein | YAAGCTCGAGTACATYTGGCT/ TGCATGCCSGAGCCGTTCCCA | 58 |
| *recA* | Recombinase A | TTCGGCAAGGGMTCGRTSATG/ ACATSACRCCGATCTTCATGC | 54 |
| *nodC* | N-acetylglucosaminyltransferase, NodC | TGATYGAYATGGARTAYTGGCT/ CGYGACARCCARTCGCTRTTG | 52 |

**Supplementary Table S3.** Nucleotide polymorphism of representative rhizobia of *Astragalus mongolicus* Bunge from NEC and NWC.

| Site (Strain No.) | Length (bp) | *S* | *Eta* | *h/Hd* | $\pi$ | $\pi_S$ | $\pi_N$ | $\pi_N/\pi_S$ |
|---|---|---|---|---|---|---|---|---|
| **Concatenated core genes** | | | | | | | | |
| NEC (34) | 1375 | 482 | 582 | 23/0.963 | 0.10000 | 0.12711 | 0.09032 | 0.71056 |
| NWC (18) | 1375 | 332 | 382 | 18/1.000 | 0.06727 | 0.08744 | 0.09549 | 1.09206 |
| **Nodulation gene *nodC*** | | | | | | | | |
| NEC (34) | 429 | 318 | 420 | 15/0.793 | 0.24096 | 0.21447 | 0.25557 | 1.19163 |
| NWC (18) | 429 | 114 | 126 | 14/0.954 | 0.05925 | 0.04146 | 0.06482 | 1.56343 |

**Note:** *S*, segregating sites or number of polymorphic (segregating) sites; *Eta*, total number of mutations. *h*, haplotype number; *Hd*, haplotype diversity; $\pi$, average number of nucleotide differences per site between two sequences; $\pi_S$, nucleotide diversity for synonymous substitutions; $\pi_N$, nucleotide diversity for nonsynonymous substitutions.

**Supplementary Table S4.** Genetic differentiation (presented as *Dxy*) and gene flow (presented as *Nm*) in representative rhizobia of *Astragalus mongolicus* Bunge from NEC and NWC.

| *Dxy* \ *Nm* | NWC | NEC |
|---|---|---|
| **Concatenated core genes** | | |
| NEC | | 0.83*** |
| NWC | 0.13410*** | |
| **Nodulation genes *nodC*** | | |
| NEC | | 0.97*** |
| NWC | 0.22712*** | |

**Note:** Number of migrants (*Nm*) and average nucleotide divergence between groups (*Dxy*) are shown in the upper and lower triangular of the table. ***, $P<0.01$, *, $P<0.05$, ns, $P>0.05$.

**Supplementary Table S5.** Accession numbers of the genes obtained in NEC (34 strains with red color) and NWC (18 strains with blue color) and type strains (14 strains with black color) and deposited in GenBank.

| NO. | **strain** | ***recA* accession** | ***atpD* accession** | ***glnII* accession** | ***nodC* accession** |
|---|---|---|---|---|---|
| 1 | IMUNJ 23004 | PQ247471 | PQ247505 | PQ247539 | PQ279588 |
| 2 | IMUNJ 23009 | PQ247472 | PQ247506 | PQ247540 | PQ279589 |
| 3 | IMUNJ 23012 | PQ247473 | PQ247507 | PQ247541 | PQ279590 |
| 4 | IMUNJ 23013 | PQ247474 | PQ247508 | PQ247542 | PQ279591 |
| 5 | IMUNJ 23014 | PQ247475 | PQ247509 | PQ247543 | PQ279592 |
| 6 | IMUNJ 23015 | PQ247476 | PQ247510 | PQ247544 | PQ279593 |
| 7 | IMUNJ 23017 | PQ247477 | PQ247511 | PQ247545 | PQ279594 |
| 8 | IMUNJ 23018 | PQ247478 | PQ247512 | PQ247546 | PQ279595 |
| 9 | IMUNJ 23020 | PQ247479 | PQ247513 | PQ247547 | PQ279596 |
| 10 | IMUNJ 23025 | PQ247480 | PQ247514 | PQ247548 | PQ279597 |
| 11 | IMUNJ 23028 | PQ247481 | PQ247515 | PQ247549 | PQ279598 |
| 12 | IMUNJ 23031 | PQ247482 | PQ247516 | PQ247550 | PQ279599 |
| 13 | IMUNJ 23032 | PQ247483 | PQ247517 | PQ247551 | PQ279600 |
| 14 | IMUNJ 23036 | PQ247484 | PQ247518 | PQ247552 | PQ279601 |
| 15 | IMUNJ 23038 | PQ247485 | PQ247519 | PQ247553 | PQ279584 |
| 16 | IMUNJ 23040 | PQ247486 | PQ247520 | PQ247554 | PQ279585 |
| 17 | IMUNJ 23050 | PQ247487 | PQ247521 | PQ247555 | PQ279602 |
| 18 | IMUNJ 23051 | PQ247488 | PQ247522 | PQ247556 | PQ279603 |
| 19 | IMUNJ 23053 | PQ247489 | PQ247523 | PQ247557 | PQ279604 |
| 20 | IMUNJ 23054 | PQ247490 | PQ247524 | PQ247558 | PQ279605 |
| 21 | IMUNJ 23055 | PQ247491 | PQ247525 | PQ247559 | PQ279606 |
| 22 | IMUNJ 23058 | PQ247492 | PQ247526 | PQ247560 | PQ279607 |
| 23 | IMUNJ 23060 | PQ247493 | PQ247527 | PQ247561 | PQ279608 |
| 24 | IMUNJ 23062 | PQ247494 | PQ247528 | PQ247562 | PQ279586 |
| 25 | IMUNJ 23064 | PQ247495 | PQ247529 | PQ247563 | PQ279587 |
| 26 | IMUNJ 23067 | PQ247496 | PQ247530 | PQ247564 | PQ279609 |
| 27 | IMUNJ 23072 | PQ247497 | PQ247531 | PQ247565 | PQ279610 |
| 28 | IMUNJ 23074 | PQ247498 | PQ247532 | PQ247566 | PQ279611 |
| 29 | IMUNJ 23076 | PQ247499 | PQ247533 | PQ247567 | PQ279612 |
| 30 | IMUNJ 23079 | PQ247500 | PQ247534 | PQ247568 | PQ279613 |
| 31 | IMUNJ 23083 | PQ247501 | PQ247535 | PQ247569 | PQ279614 |
| 32 | IMUNJ 23085 | PQ247502 | PQ247536 | PQ247570 | PQ279615 |
| 33 | IMUNJ 23090 | PQ247503 | PQ247537 | PQ247571 | PQ279616 |
| 34 | IMUNJ 23099 | PQ247504 | PQ247538 | PQ247572 | PQ279617 |
| 35 | CCBAU 03485 | KJ556389 | KJ556432 | KJ556475 | KJ729181 |
| 36 | CCBAU 03524 | KJ556392 | KJ556435 | KJ680396 | KJ729183 |
| 37 | CCBAU 03535 | KJ556393 | KJ556436 | KJ556478 | KJ729184 |
| 38 | CCBAU 03603 | KJ556395 | KJ556438 | KJ556480 | KJ729185 |
| 39 | CCBAU 03605 | KJ556396 | KJ556439 | KJ556481 | KJ729186 |
| 40 | CCBAU 03611 | KJ556397 | KJ556440 | KJ556482 | KJ729187 |
| 41 | CCBAU 73204 | KJ556400 | KJ556443 | KJ556485 | KJ729190 |
| 42 | CCBAU 73245 | KJ556402 | KJ556445 | KJ556487 | KJ729192 |
| 43 | CCBAU 73254 | KJ556404 | KJ556447 | KJ556489 | KJ729194 |
| 44 | CCBAU 73255 | KJ556405 | KJ556448 | KJ556490 | KJ729195 |
| 45 | CCBAU 73277 | KJ556406 | KJ556449 | KJ556491 | KJ729196 |
| 46 | CCBAU 75133 | KJ556414 | KJ556457 | KJ556499 | KJ729204 |
| 47 | CCBAU 75138 | KJ556415 | KJ556458 | KJ556500 | KJ729205 |
| 48 | CCBAU 75179 | KJ556418 | KJ556461 | KJ556503 | KJ729208 |
| 49 | CCBAU 75238 | KJ953902 | KJ953897 | KJ953898 | KJ953901 |
| 50 | CCBAU 75245 | KJ556428 | KJ556471 | KJ556512 | KM373710 |

| | | | | | |
|---|---|---|---|---|---|
| 51 | CCBAU 75249 | KJ556430 | KJ556473 | KJ556514 | KM373711 |
| 52 | CCBAU 75259 | KJ556424 | KJ556467 | KJ556509 | KJ729214 |
| 53 | **SH22623$^{T}$** | AY907359 | AY907373 | AY929462 | ns |
| 54 | **USDA 1844$^{T}$** | AY907358 | AY907372 | AY929453 | GQ507367 |
| 55 | **MSDJ1109$^{T}$** | AB253203 | HM142762 | EU488785 | ns |
| 56 | **USDA 9039$^{T}$** | AJ 294372 | AJ 294396 | AF169583 | ns |
| 57 | **H152$^{T}$** | HQ394251 | HQ394216 | EU488778 | AF217267 |
| 58 | **CCBAU 10071$^{T}$** | AY591566 | AY386760 | AY386780 | ns |
| 59 | **SDW014$^{T}$** | EF639843 | DQ345070 | EU249387 | GQ167237 |
| 60 | **USDA 3383$^{T}$** | AJ 294367 | AJ 294395 | AF169580 | ns |
| 61 | **LMG 17148$^{T}$** | AM182157 | AM418768 | AF169578 | ns |
| 62 | **CCBAU 83963$^{T}$** | HQ316782 | HQ316724 | HQ316739 | HQ316752 |
| 63 | **SDW018$^{T}$** | EF639844 | D0659499 | DO345073 | EU130395 |
| 64 | **CCBAU 71714$^{T}$** | AB253216 | GU994044 | GU994062 | GU994071 |
| 65 | **USDA 1002$^{T}$** | AJ 294382 | AJ 294400 | DQ767676 | ns |
| 66 | **USDA 205$^{T}$** | AJ 294379 | AJ 294402 | AF169591 | GU994072 |

Note: ns means that the *nodC* accession number has not been deposited in GenBank.