

Definition of the rhizobial symbiovars *caraganae*, *robiniae* and *sophorae* within *Mesorhizobium* and *albiziae* within *Neomesorhizobium*

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Rhizobia are bacteria that induce nodule formation on the roots (or rarely stems) of legumes (plant family *Fabaceae*) where they fix atmospheric nitrogen (N₂) [1]. The term symbiovar (sv.) describes strains of the same or different rhizobial species that are able to establish symbiosis with distinct legume species and can be distinguished by specific symbiosis (*sym*) gene phylogenies [2]. Specific *sym* genes, but *nodC* in particular, have been shown to be closely correlated with symbiovar – legume host specificity [3, 4].

Recently, in this journal, the *International Committee for the Systematics of Prokaryotes* Subcommittee for Rhizobia and Agrobacteria published a set of guidelines for the naming of new symbiovars [5]. It was proposed that a new symbiovar name must be accompanied by a discussion of the evidence that it is novel and distinct, including information on legume host specificity and on gene sequences. The phylogeny of the *nodC* gene should be included in the description of all new symbiovars and details of other *sym* genes should be given where appropriate. Also, each symbiovar must have a representative strain, and the genome sequence of that strain must be publicly available so that all the potential host specificity determinants can be explored. Referring to these guidelines, 62 symbiovars were listed along with their associated bacterial species, legume species host(s), a representative strain with genome accession number, *nodC* accession number where available and relevant reference(s) [5]. It was acknowledged that the names sv. *albiziae*, sv. *astragalus/caraganae*, sv. *robiniae* and sv. *sophorae* had been used to label clades in *nodC* phylogenies presented in different studies [4, 6–8], but none of these studies described the potential symbiovars in the text, and consequently, they were not included in the list of recognized symbiovars. Here, we describe these potential symbiovars and assess if they should be included in this list.

The *nodC* sequence labelled sv. *albiziae* in phylogenetic trees of different studies was obtained from *Mesorhizobium albiziae* CCBAU 61158^T and clearly separated from other *nodC* sequences [4, 6–9]. The *nifH* sequence for this strain was also clearly separated from those of other symbiovars (4). *M. albiziae* CCBAU 61158^T induced N₂ fixing nodules on *Albizia julibrissin*, *Glycine max*, *Leucaena leucocephala* and *Phaseolus vulgaris* but did not nodulate *Astragalus laxmannii*, *Glycyrrhiza glabra*, *Lotus corniculatus*, *Melilotus suaveolens*, *Pisum sativum*, *Robinia pseudoacacia*, *Trifolium repens*, *Vigna radiata* and *Vigna unguiculata* [9]. The complete genome sequence for *M. albiziae* CCBAU 61158^T is available, as is required for a symbiovar description. In a recent phylogenomic analysis of 61 strains in the *Mesorhizobium* complex, *M. albiziae* CCBAU 61158^T was clearly separated from all type strains and was classified within *Neomesorhizobium* gen. nov. [10]. *Neomesorhizobium albiziae* CCBAU 61158^T sv. *albiziae* meets the requirements to be the reference strain of a new symbiovar *albiziae* within the published guidelines (5).

There were five type strains (*Mesorhizobium caraganae* CCBAU 11299^T, *Mesorhizobium sangaii* SCAU7^T, *Mesorhizobium septentrionale* SDW 014^T, *Mesorhizobium shangrilense* CCBAU 65327^T and *Mesorhizobium temperatum* SDW 018^T) within the *nodC* clade labelled sv. *astragalus/caraganae* [6]. Considerable nodulation and genetic information are available for all five type strains, but no evidence was presented arguing that sv. *astragalus/caraganae* was a novel and distinct symbiovar. However, these five type strains, along with *Mesorhizobium gobiense* CCBAU 83330^T, *Mesorhizobium silamurunense* CCBAU 1550^T, *Mesorhizobium tamadayense* Ala-3^T and *Mesorhizobium tianshanense* CCBAU 33061^T, were encompassed within the *nodC* clade labelled sv. *caraganae* in separate studies [4, 7, 8]. Evidence was presented that sv. *caraganae* should be considered a new symbiovar within the genus *Mesorhizobium* and as a consequence, this name should be accepted in substitution of the name ‘*astragalus/caraganae*’ mentioned by Martinez-Romero *et al.* [5].

The *nodC* sequence labelled sv. *robiniae* in phylogenetic trees of different studies was obtained from *Mesorhizobium robiniae* CCNWYC 115^T and clearly separated from other *nodC* sequences [4, 6, 8, 11]. Strain CCNWYC 115^T was representative of five strains isolated from *R. pseudoacacia* that had similar *nodC*, *nodA* and *nifH* sequences separated from those of other strains [4, 11]. *M. robiniae* CCNWYC 115^T induced N₂ fixing nodules on *R. pseudoacacia* but did not nodulate *Melilotus officinalis*,

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Abbreviations: N₂, Nitrogen fixation; sv., symbiovar.

P. vulgaris, *Sophora alopecuroides*, *Sophora flavescens*, *Trifolium pratense*, *Vicia cracca* and *Vigna sesquipedoides* [11]. These data provide a strong case that *M. robiniae* strain CCNWC 115^T represents a distinct symbiotype, but its genome has not been sequenced. However, the genome of this strain deposited in the DSMZ culture collection as DSM 100022^T is available in GenBank (NZ_JBEPMC000000000) and the *nodC* gene of this strain (NZ_JBEPMC01000014.1) is identical to that of strain CCNWC 115^T (MH787225.1). *M. robiniae* DSM 100022^T sv. *robiniae* meets the requirements to be the reference strain of a new symbiotype *robiniae* within the published guidelines (5).

The name 'sv. *sophorae*' [4, 6, 8] was associated with *Mesorhizobium calcicola* ICMP 19560^T, *Mesorhizobium cantuariense* ICMP 19515^T, *Mesorhizobium kowhii* ICMP 19512^T, *Mesorhizobium newzealandense* ICMP 19545^T, *Mesorhizobium sophorae* ICMP 19535^T, *Mesorhizobium waimense* ICMP19557^T and *Mesorhizobium waitakense* ICMP 19523^T, all of which were isolated from, and shown to nodulate, New Zealand native *Sophora* spp. [12, 13]. The strains also nodulated *Clanthus puniceus* but not *Amorpha fruticosa*, *Astragalus sinicus*, *Cicer arietinum* or *L. corniculatus* [14]. The *nodC*, *nodA* and *nifH* gene sequences were highly similar/identical for all seven strains and clearly separated from those of other symbiotypes. The complete genome sequence for all seven *Mesorhizobium* spp. is available and all were included in the phylogenomic analysis of 61 strains in the *Mesorhizobium* complex [10]. Thus, sv. *sophorae* meets the requirements of a new symbiotype description within the published guidelines [5]. *M. sophorae* ICMP 19535^T sv. *sophorae* is the representative strain [15].

In summary, sv. *albisiae*, sv. *robiniae* and sv. *sophorae* meet the requirements of a new symbiotype description within the published guidelines, while strains labelled 'astragalus/caraganae' have been included in the recognized sv. *caraganae*.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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