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## Chapter

# Phylogenomic Review of Root Nitrogen-Fixing Symbiont Population Nodulating Northwestern African Wild Legumes

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## Abstract

The present review discusses the phylogenomic diversity of root nitrogen-fixing bacteria associated to wild legumes under North African soils. The genus *Ensifer* is a dominant rhizobium lineage nodulating the majority of the wild legumes, followed by the genus *Rhizobium* and *Mesorhizobium*. In addition, to the known rhizobial genera, two new *Microvirga* and *Phyllobacterium* genera were described as real nodulating and nitrogen-fixing microsymbiotes from *Lupinus* spp. The promising rhizobia related to nitrogen fixation efficiency in association with some legumes are shared. Phylogenetic studies are contributing greatly to our knowledge of relationships on both sides of the plant-bacteria nodulation symbiosis. Multiple origins of nodulation (perhaps even within the legume family) appear likely. However, all nodulating flowering plants are more closely related than previously suspected, suggesting that the predisposition to nodulate might have arisen only once. The origins of nodulation, and the extent to which developmental programs are conserved in nodules, remain unclear, but an improved understanding of the relationships between nodulin genes is providing some clues.

**Keywords:** rhizobia, North Africa, symbiosis, legumes, phylogenomic

## 1. Introduction

Africa has a vast array of indigenous legumes, ranging from large rain forest trees to small annual herbs [1]. However, in recent years, there has been a tendency in agriculture and forestry to use exotic species for crops and wood. As has been pointed out several times over nearly 30 years, most recently [2], by the US National Academy of Sciences, this ignores the potential of the native species, which are arguably better adapted to their environment. For this review, the nodulated indigenous legume genera in Northwestern Africa with known uses have been selected to illustrate the problems and potential for their better exploitation.

The wild legume flora in Northwestern Africa is rich, with great specific and infraspecific diversity [3]. The overgrazing and expansion of agriculture has gradually

led to the regression and extinction of many pastoral and forage species. In addition, desertification causes disturbance of plant-microbe symbioses, which are a critical ecological factor in helping further plant growth in degraded ecosystems [4]. In this context, the establishment of indigenous pastoral legume species associated with their appropriate symbiotic bacterial partners may be of increased value for success in soil fertility restoration. Biological N<sub>2</sub> fixation (BNF) is the major way for N input into desert ecosystems. Rhizobium-legume symbioses represent the major mechanism of BNF in arid lands, compared with the N<sub>2</sub>-fixing heterotrophs and associative bacteria [5, 6] and actinorhizal plants [7, 8]. Deficiency in mineral N often limits plant growth, and so symbiotic relationships have evolved between plants and a variety of N<sub>2</sub>-fixing organisms [9]. The symbiotically fixed N<sub>2</sub> by the association between rhizobium species and the legumes represents a renewable source of N for agriculture. Values estimated for various legume crops and pasture species are often impressive [10]. In addition to crop legumes, the nodulated wild (herb and tree) legumes have potential for nitrogen fixation and reforestation and to control soil erosion [11]. It has been reported that a novel, suitable wild legume-rhizobia associations are useful in providing a vegetational cover in degraded lands [12].

Considering the major ecological importance of many wild legumes such as *Retama* sp., *Acacia* sp., *Lotus* sp., *Lupinus* sp., *Medicago* sp., etc. in Northwest Africa by their important role in soil fertility maintenance, coverage, and dune stabilization, the present chapter proposes to review the phylogenomic diversity of root nitrogen-fixing symbiont population nodulating Northwestern African wild legumes listed in the bibliography, some of which are common and play important ecological and pastoral roles, but others are rare and endangered. As well as the host legumes, the nodule endosymbionts also vary widely in Africa and include newly described members of both  $\alpha$  and  $\beta$  branches of the Proteobacteria, now often referred to as  $\alpha$ - or  $\beta$ -rhizobia, even though they do not have “rhizobium” as part of their generic names [13].

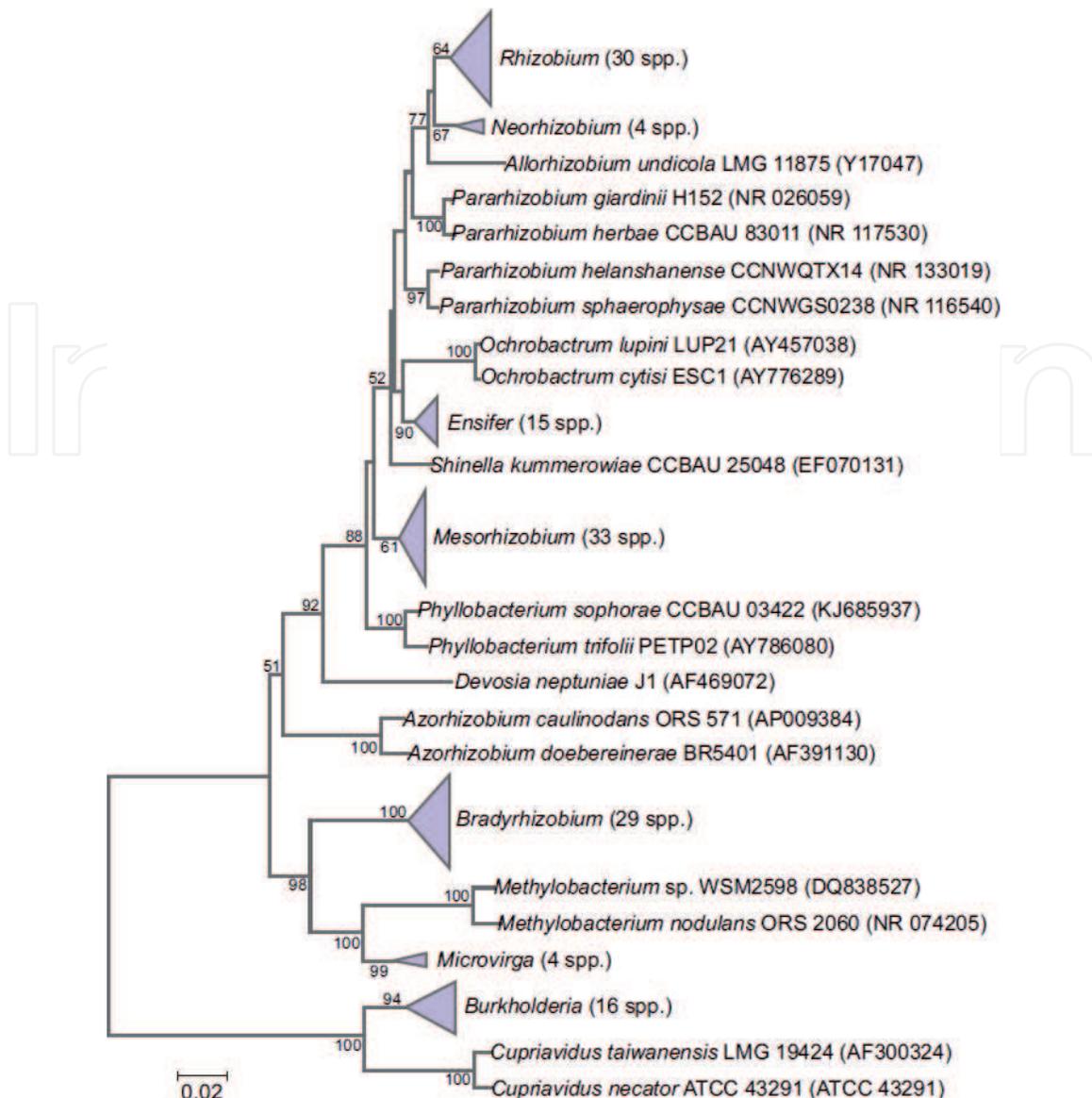
Therefore, understanding the nature of indigenous populations of rhizobia-nodulating wild legumes is of considerable agricultural significance. It is also of interest to identify a wider variety of bacterial strains in a bid to define new strains for the production of inoculants for smallholder farms.

## 2. Genetics and functional genomics of legume nodulation

The interaction between rhizobia and legumes in root nodules is an essential element in sustainable agriculture, as this symbiotic association is able to enhance biological fixation of atmospheric nitrogen (N<sub>2</sub>) and is also a paradigm in plant-microbe signaling [14–16]. The knowledge of the whole genome would allow the specific features of each rhizobium to be identified. The prominent feature of this group of bacteria is their molecular dialog with plant hosts, an interaction that is enabled by the presence of a series of symbiotic genes encoding for the synthesis and export of signals triggering organogenetic and physiological responses in the plant [17, 18]. In recent years, significant progress has been made in resolving the complex exchange of signals responsible for nodulation through genome assembly, mutational and expression analysis, and proteome characterization of legumes [14, 19, 20] and rhizobia [15, 21–23].

## 3. Phylogenomic of wild legume root nitrogen-fixing symbionts

The known diversity of rhizobia increases annually and is the subject of several reviews, the most recent and comprehensive being that of [24]. It is not our intention to revisit this subject nor the genetic basis of nodulation [25, 26], the horizontal



**Figure 1.**  
 Phylogenetic tree showing the relationships of currently described genera and species of alpha- and beta-rhizobia, based on aligned sequences of the 16S rRNA gene (1341-bp internal region) (adapted from [28]).

transfer of symbiosis-related genes [23], or the symbiovar concept [27] but instead to attempt to link, where possible, rhizobial genotypes with their geographical locations and/or legume tribes/genera. At the time of writing, rhizobia consist of a diverse range of genera in the Alphaproteobacterial and Betaproteobacterial classes and are termed “alpha-rhizobia” and “beta-rhizobia,” respectively (**Figure 1**).

### 3.1 The genus *Bradyrhizobium* (*Bradyrhizobiaceae*)

The *Bradyrhizobium* genus was described by Jordan in 1982 [29]. It currently consists of nine rhizobia species.

For the *Loteae* tribe, previous studies found that *Lotus palustris* and *L. purpureus* species from Algeria were nodulated by *Bradyrhizobium lupini*, and *L. pedunculatus* by *B. japonicum* [30]. However, *L. creticus* ssp. *maritimus* is nodulated by both [30]. At Tunisia, *L. roudairei* microsymbiont is closely related to *B. japonicum* [31]. For the *Acacieae* tribe, two studies reported that rhizobial strains associated to the *Acacia saligna*, an Australian introduced species, belonged to the genus *Bradyrhizobium* genus under Algerian and Moroccan soils [32–34]. For the *Genisteae* tribe, it has been noticed that *Bradyrhizobium* is the dominant genus of

symbiotic nitrogen-fixing bacteria associated with *Retama* species in North Africa: *Retama monosperma*, *R. raetam*, and *R. sphaerocarpa* (Algeria: [35, 36]; Morocco: [37]). Recently, the novel *B. retamae* species, in which groups with *B. elkanii* and *B. pachyrhizi* and related *B. lablabi* and *B. jicamae* type strains are included in *Bradyrhizobium* group II [38], has been isolated from *R. sphaerocarpa* and *R. monosperma* in Morocco [37]. For the genus *Cytisus*, two studies reported that *Cytisus villosus* is nodulated by *B. cytisi* sp. nov. and *B. rifense* sp. nov. in Morocco [39, 40] and by genetically diverse *Bradyrhizobium* strains in Algeria belonging to *B. japonicum* and *B. canariense* and to new lineage within the *Bradyrhizobium* genus [41]. Fifty-two strains isolated from root nodules of the Moroccan shrubby legume *Cytisus triflorus* were genetically characterized, and results showed that it is nodulated by *Bradyrhizobium* strains, with 99% homology with *Bradyrhizobium* genosp. AD [42]. For the genus *Lupinus*, some endosymbiotic bacteria of *L. luteus* and *L. micranthus* from Tunisia and Algeria belonged to *B. lupini*, *B. canariense*, *B. valentinum*, *B. cytisi*/*B. rifense*, *B. japonicum*, *B. elkanii*, and *B. retamae* [43–45].

### 3.2 The genus *Mesorhizobium* (*Phyllobacteriaceae*)

The genus *Mesorhizobium* was described by Jarvis et al. [46]. Several *Rhizobium* species were transferred to this genus. It currently consists of 21 rhizobia species.

For subtribe *Astragalinae* (Coluteinae Clade), Guerrouj et al. [37] reported that rhizobial symbiont of *Astragalus gombiformis* in Eastern Morocco is closely related to *M. camelthorni*. A polyphasic approach analysis indicated that bacterial strains isolated from the pasture legume *Biserrula pelecinus* growing in Morocco belong to the genus *Mesorhizobium*. At Tunisia, Mahdhi et al. [47] showed that five strains isolated from *Astragalus corrugatus* were phylogenetically related to *M. temperatum* and to *Mesorhizobium* sp. From the tribe *Galegeae* (subtribe *Coluteinae*), Ourarhi et al. [48] reported that *Colutea arborescens* is nodulated by diverse rhizobia in Eastern Morocco, among them, the genus *Mesorhizobium*. For the *Loteae* tribe, *M. alhagi* as well as *M. temperatum* were isolated, at Tunisia, from *Lotus creticus* [49–51]. Zakhia et al. [31] reported that *Lotus argenteus* microsymbiotes are closely related to *M. mediterraneum* in the infra-arid zone of Tunisia. Roba et al. [52] reported that *M. delmotii* and *M. prunaredense* are two new rhizobial species nodulating *Anthyllis vulneraria* growing on Tunisian soils. From the *Acacieae* tribe, Boukhatem et al. [33] reported that rhizobial strains associated to the *Acacia saligna*, an Australian introduced species, to *A. ehrenbergiana* and *F. albida* belonged to *M. mediterraneum* under Algerian soils. From the *Genisteeae* tribe, the genetic diversity of *Genista saharae* microsymbionts in the Algerian Sahara reported that they belonged to *M. camelthorni* [53]. For the *Mimoseae* tribe, root-nodulating bacteria associated to *Prosopis farcta* growing in the arid regions of Tunisia were assigned to the genus *Mesorhizobium* [54]. From the *Hedysareae* tribe, Zakhia et al. [31] reported that one strain isolated from *Ebenus pinnata* root nodules is closely related to *M. ciceri* in the infra-arid zone of Tunisia.

### 3.3 The genus *Rhizobium* (*Rhizobiaceae*)

The genus *Rhizobium* was the first named (from Latin meaning “root living”), and for many years this was a “catch all” genus for all rhizobia. Some species were later moved in to new genera based on phylogenetic analyses [65]. It currently consists of 49 rhizobial species.

For *Galegae* tribe, Zakhia et al. [31] reported that rhizobial symbionts of *Astragalus gombiformis*, *A. armatus*, and *A. cruciatus* are closely related to *Rhizobium mongolense*, *R. leguminosarum*, and *R. galegae*, in the infra-arid zone of Tunisia. From

*Genisteae* tribe, it was shown that strains from Tunisia nodulating *Argyrolobium uniflorum* are closely affiliated to *R. giardinii*, *Calicotome villosa* to *R. mongolense*, and *Genista microcephala* to *R. mongolense* and *R. leguminosarum* [31]. Mahdi et al. [55–57] reported that strains nodulating *Genista saharae* and *Retama retam* are members of the genus *Rhizobium*. Nonetheless, there are reports indicating that members of the genus *Rhizobium* nodulate *Adenocarpus decorticans* and *Cytisus arboreus* at Morocco [58]. For the *Loteae* tribe, *R. leguminosarum* and *R. mongolense* were isolated, at Tunisia, from *Anthyllis henoniana*, *R. leguminosarum* from *Coronilla scorpioides*, and *R. mongolense* from *Lotus creticus* [31]. Rejili et al. [51] reported that *Lotus creticus* microsymbiotes are closely related to *R. huautlense* in the arid areas of Tunisia. Bacterial strains isolated from root nodules of *Scorpiurus muricatus* sampled from different regions of western Algeria are affiliated to *R. vignae*, *R. radiobacter*, and *R. leguminosarum* [59]. For the *Trifolieae* tribe, *R. galegae* species was isolated, in Tunisia and Algeria, from *Medicago marima* and *M. truncatula* [31]. In Algeria, Merabet et al. [60] reported that *Medicago ciliaris* and *M. polymorpha* are nodulated by *Rhizobium* sp. Similarly, genetic diversity of rhizobia from annual *Medicago orbicularis* showed that they are affiliated to *Rhizobium tropici* [61]. For the *Vicieae* tribe, *R. leguminosarum* species was isolated from *Lathyrus numidicus* [31]. Mahdhi et al. [62] reported that *Vicia sativa* isolates from Tunisia had 16S rDNA type identical to that of the reference *R. leguminosarum*. From *Acacieae*, Boukhatem et al. [33] reported that bacteria-nodulating *Acacia saligna* and *A. seyal* under Algerian soils are affiliated to the *R. tropici* clade and *R. sullae* clade, respectively. On the other hand, the same study mentioned that five bacterial isolates, all from *A. saligna*, formed a separate clade in the vicinity of the *R. galegae*-*R. huautlense*-*R. loessense* branch [33]. The same authors showed that the *R. leguminosarum* reference strain was represented by five *A. karroo* isolates and five *A. seyal* isolates [33]. At Tunisia, the genetic diversity of root nodule bacteria associated to *Hedysarum coronarium* (*sulla*), from *Hedysareae* tribe, showed that they are closely related to *R. sullae* [84]. Similarly, Ezzakkioui et al. [85] indicated that the strains from the Moroccan *Hedysarum flexuosum* legume had 99.75–100% identity with *R. sullae*.

### 3.4 The genus *Ensifer* (*Sinorhizobium*) (*Rhizobiaceae*)

The genera *Sinorhizobium* and *Ensifer* were recently recognized as forming a single phylogenetic clade [63, 64] and are now united, and all species of the genus *Sinorhizobium* have been transferred to the genus *Ensifer*, in line with rule 38 of the Bacteriological Code [66, 67]. The genus currently consists of 17 species.

Bacteria belonging to *Ensifer* genus are widely distributed in arid regions of Tunisia. From the *Loteae* tribe, *E. meliloti* and *E. numidicus* were isolated, at Tunisia, from *Lotus creticus* [49–51, 67] and *Rhizobium* sp. from *Hippocrepis areolata* [47]. From the *Acacieae* tribe, genetic characterization of rhizobial bacteria-nodulating *Acacia tortilis* subsp. *raddiana*, *A. gummifera*, *A. cyanophylla*, *A. karroo*, *A. ehrenbergiana*, and *A. horrida* in Tunisia, Algeria, and Morocco reported that they belonged to the species *E. meliloti*, *E. garamanticus*, and *E. numidicus* and *Ensifer* sp. [31, 33, 68–70, 86]. At Algeria, isolates from four different host species, namely, *A. karroo*, *A. ehrenbergiana*, *A. saligna*, and *A. tortilis*, were closely related to *E. fredii*, *E. teranga*, and *E. kostiense* reference strains [33]. For the *Mimoseae* tribe, 40 isolates associated to *Prosopis farcta* growing in the arid regions of Tunisia belonged to *E. meliloti*, *E. xinjiangense*/*E. fredii*, and *E. numidicus* species [54]. For the *Trifolieae* tribe, strains nodulating different *Medicago* species in Tunisia, Algeria, and Morocco such as *M. sativa*, *M. arborea*, *M. truncatula*, *M. ciliaris*, *M. laciniata*, *M. polymorpha*, *Medicago arabica*, *M. marima*, *Medicago littoralis*, and *M. scutella* are associated to *E. meliloti*, *E. medicae*, or *E. garamanticus* [31, 60, 61, 67, 71–76]. Similarly, *Ononis*

Subfamily tribe	Genus	Species	Symbiont	Geographic origin	
<b>Mimosoideae</b>					
Acacieae	<i>Acacia</i>	<i>A. cyanophylla</i>	<i>E. meliloti</i> , <i>E. fredii</i> , <i>Ensifer</i> sp.	Tunisia, Morocco	
		<i>A. gummifera</i>	<i>E. meliloti</i> , <i>E. garamanticus</i> , <i>E. numidicus</i> , <i>Ensifer</i> sp.	Tunisia, Morocco	
		<i>A. horrida</i>	<i>E. meliloti</i> , <i>E. garamanticus</i> , <i>E. numidicus</i> , <i>Ensifer</i> sp.	Tunisia, Morocco	
		<i>A. tortilis raddiana</i>	<i>E. meliloti</i> , <i>E. garamanticus</i> , <i>E. numidicus</i> , <i>Ensifer</i> sp.	Tunisia, Morocco	
		<i>A. saligna</i>	<i>Bradyrhizobium</i> sp., <i>Mesorhizobium</i> sp., <i>Rhizobium</i> sp., <i>Ensifer</i> sp.	Algeria, Morocco	
		<i>A. ehrenbergiana</i>	<i>Mesorhizobium</i> sp., <i>Ensifer</i> sp.	Algeria	
		<i>A. karroo</i>	<i>Rhizobium</i> sp., <i>Ensifer</i> sp.	Algeria	
		<i>A. nilotica</i>	<i>Rhizobium</i> sp.	Algeria	
		<i>A. seyal</i>	<i>Rhizobium</i> sp.	Algeria	
		<i>F. albida</i>	<i>Mesorhizobium</i> sp.	Algeria	
Mimosae	<i>Prosopis</i>	<i>P. farcta</i>	<i>Mesorhizobium</i> sp., <i>E. meliloti</i> , <i>E. xinjiangense</i> , <i>E. fredii</i> , <i>E. numidicus</i>	Tunisia	
<b>Papilionoideae</b>					
Galegae	<i>Astragalus</i>	<i>A. armatus</i>	<i>R. mongolense</i> , <i>R. leguminosarum</i> , <i>R. galegae</i>	Tunisia	
		<i>A. cruciatus</i>	<i>R. mongolense</i> , <i>R. leguminosarum</i> , <i>R. galegae</i>	Tunisia	
		<i>A. corrugatus</i>	<i>M. temperatum</i> , <i>Mesorhizobium</i>	Tunisia	
		<i>A. gombiformis</i>	<i>M. camelthorni</i> , <i>R. mongolense</i> , <i>R. leguminosarum</i> , <i>R. galegae</i>	Morocco, Tunisia	
		<i>Biserrula</i>	<i>B. pelecinus</i>	<i>Mesorhizobium</i>	Morocco
	<i>Colutea</i>	<i>C. arborescens</i>	<i>Mesorhizobium</i>	Morocco	
Genisteae	<i>Argyrolobium</i>	<i>A. uniflorum</i>	<i>R. giardinii</i>	Tunisia	
		<i>Adenocarpus</i>	<i>A. decorticans</i>	<i>Rhizobium</i>	Morocco
		<i>Calicotome</i>	<i>C. villosa</i>	<i>R. mongolense</i>	Morocco
		<i>Cytisus</i>	<i>C. arboreus</i>	<i>Bradyrhizobium</i> sp.	Morocco
			<i>C. triflorus</i>	<i>Bradyrhizobium</i>	Morocco
				<i>C. villosus</i>	<i>B. cytisi</i> , <i>B. rifense</i> , <i>B. japonicum</i> , <i>B. canariense</i>
	<i>Lupinus</i>	<i>L. luteus</i>	<i>B. lupini</i> , <i>B. canariense</i> , <i>B. valentinum</i> , <i>B. cytisi</i> , <i>B. rifense</i> , <i>B. japonicum</i> , <i>B. elkanii</i> , <i>B. retamae</i> , <i>Microvirga</i>	Algeria, Tunisia	

Subfamily tribe	Genus	Species	Symbiont	Geographic origin
		<i>L. micranthus</i>	<i>B. lupini</i> , <i>B. canariense</i> , <i>B. valentinum</i> , <i>B.</i> <i>cytisi</i> , <i>B. rifense</i> , <i>B.</i> <i>japonicum</i> , <i>B. elkanii</i> , <i>B. retamae</i> , <i>Microvirga</i> , <i>Phyllobacterium</i>	Algeria, Tunisia
	<i>Genista</i>	<i>G. microcephala</i>	<i>R. mongolense</i> , <i>R.</i> <i>leguminosarum</i> , <i>Rhizobium</i>	Tunisia
		<i>G. saharae</i>	<i>M. camelthorni</i>	Algeria
	<i>Retama</i>	<i>R. monosperma</i>	<i>B. retamae</i>	Algeria, Morocco
		<i>R. raetam</i>	<i>B. retamae</i> , <i>Rhizobium</i>	Algeria, Tunisia, Morocco
		<i>R. sphaerocarpa</i>	<i>B. retamae</i>	Algeria, Morocco
Hedysareae	<i>Hedysarum</i>	<i>H. carnosum</i>	<i>E. meliloti</i>	Tunisia
		<i>H. flexuosum</i>	<i>R. sullae</i>	Morocco
		<i>H. coronarium</i>	<i>R. sullae</i>	Tunisia
		<i>H. spinosissimum</i>	<i>E. meliloti</i>	Tunisia
	<i>Ebenus</i>	<i>E. pinnata</i>	<i>M. ciceri</i>	Tunisia
Loteae	<i>Anthyllis</i>	<i>A. henoniana</i>	<i>R. leguminosarum</i> , <i>R.</i> <i>mongolense</i>	Tunisia
		<i>A. vulneraria</i>	<i>M. delmotii</i> , <i>M.</i> <i>prunaredense</i>	Tunisia
	<i>Coronilla</i>	<i>C. scorpioides</i>	<i>R. leguminosarum</i>	Tunisia
	<i>Hippocrepis</i>	<i>H. areolata</i>	<i>Rhizobium</i>	Tunisia
		<i>H. bicontorta</i>	<i>E. meliloti</i>	Tunisia
	<i>Lotus</i>	<i>L. argenteus</i>	<i>M. mediterraneum</i>	Tunisia
		<i>L. creticus</i>	<i>B. lupini</i> , <i>B.</i> <i>japonicum</i> , <i>M. alhagi</i> , <i>M. temperatum</i> , <i>R. mongolense</i> , <i>R.</i> <i>huautlense</i> , <i>E. meliloti</i> , <i>E.</i> <i>numidicus</i>	Algeria, Tunisia
		<i>L. palustris</i>	<i>B. lupini</i>	Algeria
		<i>L. pedunculatus</i>	<i>B. japonicum</i>	Algeria
		<i>L. purpureus</i>	<i>B. lupini</i>	Algeria
		<i>L. pusillus</i>	<i>M. alhagi</i> , <i>M.</i> <i>temperatum</i> , <i>E. meliloti</i>	Tunisia
		<i>L. roudairei</i>	<i>B. japonicum</i>	Tunisia
	<i>Scorpiurus</i>	<i>S. muricatus</i>	<i>R. vignae</i> , <i>R. radiobacter</i> , <i>R. leguminosarum</i>	Algeria
Trifolieae	<i>Medicago</i>	<i>M. arabica</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Morocco
		<i>M. arborea</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Morocco

Subfamily tribe	Genus	Species	Symbiont	Geographic origin
		<i>M. ciliaris</i>	<i>Rhizobium</i> , <i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Algeria
		<i>M. marima</i>	<i>R. galegae</i>	Algeria, Tunisia
		<i>M. laciniata</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Tunisia
		<i>M. littoralis</i>	<i>E. meliloti</i> , <i>E. medicae</i> , and <i>E. garamanticus</i>	Tunisia
		<i>M. orbicularis</i>	<i>R. tropici</i>	Tunisia, Algeria, Morocco
		<i>M. polymorpha</i>	<i>Rhizobium</i> , <i>E. meliloti</i>	Tunisia, Algeria, Morocco
		<i>M. sativa</i>	<i>E. meliloti</i> , <i>E. medicae</i>	Tunisia, Algeria, Morocco
		<i>M. scutella</i>	<i>E. meliloti</i>	Algeria, Tunisia
		<i>M. truncatula</i>	<i>R. galegae</i> , <i>E. meliloti</i> , <i>E. medicae</i>	Tunisia, Algeria, Morocco
	<i>Melilotus</i>	<i>M. indicus</i>	<i>E. meliloti</i>	Algeria
	<i>Ononis</i>	<i>O. natrix</i> ssp. <i>filifolia</i>	<i>E. meliloti</i>	Tunisia
	<i>Trigonella</i>	<i>T. maritima</i>	<i>E. meliloti</i>	Tunisia
Vicieae	<i>Lathyrus</i>	<i>L. numidicus</i>	<i>R. leguminosarum</i>	Tunisia
	<i>Vicia</i>	<i>V. sativa</i>	<i>R. leguminosarum</i>	Tunisia

**Table 1.**

Recapitulative results of root nodule symbionts from Northwestern African wild legumes.

*natrix* and *Trigonella maritima* are nodulated by *E. meliloti* [31, 62]. Nodule rhizobia of *Melilotus indicus* growing in the Algerian Sahara are affiliated to *E. meliloti* [75]. *E. meliloti* and *E. numidicus* strains were isolated from the Genisteeae tribe such as *Argyrolobium uniflorum*, *Retama raetam*, and *Genista saharae* [55–57, 67]. For *Galegae* tribe, Mahdhi et al. [47] reported that rhizobial symbionts of *Astragalus corrugatus* are closely related to *E. meliloti* under Tunisian soils. From the *Hedysareae* tribe, Mahdhi et al. [35] reported that strains isolated from *Hedysarum spinosissimum* root nodules are closely related to *E. meliloti* in the infra-arid zone of Tunisia.

### 3.5 The genus *Neorhizobium* (*Rhizobiaceae*)

The genus *Neorhizobium* was proposed by Mousavi et al. [77] as an alternative to solve the issue of grouping the members of this genus with *Agrobacterium* and *Rhizobium* genera. The genetic diversity of the Algerian legume *Genista saharae* isolates was assessed, and results reported that they are affiliated to *Neorhizobium alkalisoli*, *N. galegae*, and *N. huautlense* [53]. Several studies reported that *N. galegae* is isolated from different legumes in Tunisia such as *Astragalus* sp. [31, 54], *Argyrolobium*

*uniflorum* [31], *Anthyllis henoniana* [31], *Lotus creticus* [31, 50], *Medicago marima*, and *M. truncatula* [31]. Rejili et al. [51] reported that *Lotus creticus* is also nodulated by *N. huautlense* in the arid areas of Tunisia. For *Galegae* tribe, Mahdhi et al. [47] reported that rhizobial symbionts of *Astragalus corrugatus* are closely related to *N. galegae* under Tunisian soils.

### 3.6 The genus *Phyllobacterium* (*Phyllobacteriaceae*)

The *Phyllobacterium* genus comprises of bacteria that are well-known for their epiphytic and endophytic associations with plants [78]. Nonetheless, root-nodulating and nitrogen-fixing *Phyllobacterium* was described in Tunisia, in the nodules of genistoid legume *Lupinus micranthus* [44, 45]. Prior to this finding, endophytic *Phyllobacterium* strains were identified on the nodules of the Tunisian legumes *Genista saharae*, *Lotus creticus*, and *L. pusillus* [51, 56], but they are lacking the ability to form nodules.

### 3.7 The genus *Microvirga* (*Methylobacteriaceae*)

The genus *Microvirga* which comprises soil and water saprophytes was included in the alphaproteobacterial lineage of root-nodule bacteria only in 2012, although the first symbiotic strains were detected in nodules of *Lupinus texensis* [79–81]. Recently, *Microvirga* strains were only isolated from *L. micranthus* and *L. luteus* in Tunisia, belonging to the *Genisteeae* tribe [44, 45].

**Table 1** shows the root nodule symbionts from Northwestern African wild legumes.

## 4. Promising nitrogen-fixing rhizobia

The root nodule symbiosis established between legumes and rhizobia is an exquisite biological interaction responsible for fixing a significant amount of nitrogen in terrestrial ecosystems. The success of this interaction depends on the recognition of the right partner by the plant within the richest microbial ecosystems on Earth, the soil. Recent metagenomic studies of the soil biome have revealed its complexity, which includes microorganisms that affect plant fitness and growth in a beneficial, harmful, or neutral manner. In this complex scenario, understanding the molecular mechanisms by which legumes recognize and discriminate rhizobia from pathogens, but also between distinct rhizobia species and strains that differ in their symbiotic performance, is a considerable challenge.

By symbiotic efficiency and properties, strains isolated from wild legumes varied in their symbiosis effectiveness with their host plant of origin. A great diversity among and within isolates was reported by many authors. This symbiotic diversity within and between isolates growing in diverse geographical areas was also defined by Tinick and Hadobas [82] for other legume plants. All strains were capable of nodulation. Mahdhi et al. [55, 83] reported that two *Retama raetam* isolates RB3 and RM4 (*Rhizobium*) gave the highest nodule numbers per plant, 26 ( $\pm 2.053$ ) and 27 ( $\pm 0.997$ ), respectively. The effective strain LAC765 (*Ensifer*) was isolated from *Lotus creticus* with a 91.46 ( $\pm 0.01\%$ ) dry biomass of the  $T_N$  control [50]. The dry matter of the aerial part is considered a criterion for assessing the efficiency of a given strain; a highly significant correlation between these two parameters has been reported. Results related to symbiotic efficiency showed that among 45 tested isolates, 20 isolates are highly efficient (relative effectiveness  $\geq 70\%$ ), 20 isolates are partially effective ( $60\% \leq$  relative effectiveness  $< 70\%$ ), and 5 isolates are inefficient (relative

effectiveness <60%). The strain GN29 isolated from *Genista saharae*, affiliated to *Rhizobium* genus, is considered inefficient (relative effectiveness = 32.29%). Among the 20 isolates considered highly efficient, 5 isolates were isolated from *Retama retam*, five from *Lotus* sp., 4 from *Genista saharae*, 3 from *Vicia sativa*, 2 from *Argyrolobium uniflorum* and 2 from *Trigonella maritima*. From the 20 highly efficient isolates, 13 isolates belong taxonomically to *Ensifer* sp., 6 to *Rhizobium* sp., and one to *Mesorhizobium* sp.

## 5. Conclusion

The Mediterranean basin is a hotspot place of legume diversity and the center of diversification of many of them. Our review contributes to enlarge our knowledge on the LNB-legume symbioses. We evidenced the biodiversity among bacteria-nodulating wild legumes in Northwestern Africa and unknown associations were found. Several groups may represent new genospecies to be further characterized to assess their taxonomical status. This work thus opens further interesting perspectives and makes new models available for evolutionary studies and for understanding mechanisms involved in nitrogen-fixing symbiosis.

## Conflict of interest

The authors declare that they have no conflict of interest.

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