



Emergence of β -rhizobia as new root nodulating bacteria in legumes and current status of the legume–rhizobium host specificity dogma

Ahmed Idris Hassen¹ · Sandra C. Lamprecht² · Francina L. Bopape¹

Received: 29 November 2019 / Accepted: 12 February 2020 / Published online: 24 February 2020
© Springer Nature B.V. 2020

Abstract

Recent developments in the legume rhizobium symbiotic interaction particularly those related to the emergence of novel strains of bacteria that nodulate and fix nitrogen in legumes is gaining momentum. These novel strains of bacteria were mostly isolated from the root nodules of indigenous and invasive legumes belonging to the sub families Papilionoideae and Mimosoideae in South Africa, South America and South East China. These rhizobia are phylogenetically and taxonomically different from the traditional ‘alpha rhizobia’ and are termed ‘ β -rhizobia’ as they belong to the β -sub class of Proteobacteria. There are also new reports of novel species of root nodulating bacteria from the α -Proteobacteria, not known for several decades since the discovery of rhizobia. However, in this review focus is given to the emerging β -rhizobia isolated from the indigenous Papilionoid legumes in the Cape Floristic regions in South Africa and the indigenous and invasive Mimosoid legumes in South America and South East Asia respectively. The nodulation of the indigenous South African Papilionoid legumes including that of *Aspalathus linearis* (rooibos) is discussed in a bit detail. Previous reports indicated that *A. linearis* is very specific in its rhizobium requirement and was reported to be nodulated by the slow growing *Bradyrhizobium* spp. This review however summarizes that the bacteria associated with the root nodules of *A. linearis* belong to members of both the alpha (α) Proteobacteria that include *Mesorhizobium*, *Rhizobium* and *Bradyrhizobium* spp. and the beta (β) Proteobacteria represented by the genus *Burkholderia* (now reclassified as *Paraburkholderia*). In addition, the occurrence of *Paraburkholderia* as the newly emerging root nodule symbionts of various other legumes has been discussed. In doing so, the review highlights that nodulation is no longer restricted to the traditional ‘rhizobia’ group following the emergence of the new beta rhizobia as potential nodulators of various indigenous legumes. It thus provides some insights on the status of the legume–rhizobium host specificity concept and the loss of this specificity in several symbiotic associations that puts the long held dogma of host specificity of the legume rhizobium symbiosis in a dilemma.

Keywords Nodulation · Beta-rhizobia · *Paraburkholderia* · *Burkholderia* · Indigenous legumes · Host specificity · *Aspalathus linearis*

Introduction

Ever since Frank (1889) had first described the bacterial genus *Rhizobium*, the legume rhizobium symbiotic interaction has been studied for almost 130 years now owing to its huge significance in sustainable agricultural productivity,

soil fertility and ecological benefits. Until 27 years ago, the bacteria that are involved in nodulating the majority of legume species were all classified under a single genus *Rhizobium* (Gyaneshwar et al. 2011). A breakthrough in bacterial phylogenies based on sequences of the conserved small sub unit of the 16S ribosomal RNA changed the ‘one genus *Rhizobium*’ concept (Young and Haukka 1996). This resulted in the division of the rhizobia into the well-known genera *Rhizobium*, *Bradyrhizobium*, *Ensifer* (Syn: *Sinorhizobium*), *Mesorhizobium*, *Allorhizobium* and *Azorhizobium* all belonging to the alpha subclass of Proteobacteria (alpha-proteobacteria) (Graham 2008; Willems 2006; Velazquez et al. 2010). Due to the ecological significance and economic importance, the rhizobia-legume symbiosis has since been

✉ Ahmed Idris Hassen
HassenA@arc.agric.za

¹ Agricultural Research Council-Plant Health and Protection (ARC-PHP), P. Bag X134, Queenswood, Pretoria 0121, South Africa

² ARC-PHP, P. Bag X5017, Vredenberg, Stellenbosch, South Africa

studied extensively and currently, the genera that contains the root nodule forming bacteria has risen to 14 (Deng et al. 2011). The new inclusions are the genera *Methylobacterium* (Sy et al. 2001), *Devosia* (Rivas et al. 2002), *Ochrobactrum* (Ngom et al. 2004), and *Shinella* (Li et al. 2008) that belong to the Alphaproteobacteria and *Burkholderia* (Moulin et al. 2001) and *Cupriavidus* (Chen et al. 2001) from the Betaproteobacteria (Fig. 1). The newly discovered rhizobia particularly those belonging to the beta-subclass of Proteobacteria are the subject of this review that will briefly discuss some recent studies, which disclosed the emergence of new group of bacteria as root nodulating symbionts of certain indigenous and exotic legumes.

Overview on the rhizobium-legume host specificity

Host specificity refers to the phenomenon whereby a given rhizobium species or strain nodulates (i.e. its ability to form root nodules) only a specific or limited number of legume species. Few famous examples of such high host specificity include the symbiosis between *Rhizobium leguminosarum* (bv. trifolii) and *Trifolium* spp. and the symbiosis between *Rhizobium etli* (bv. phaseoli) and *Phaseolus vulgaris* (Jiao et al. 2015). Other examples of host specificity include *Mesorhizobium muleine* CCBAU83963 for *Cicer arietinum*

(Zhang et al. 2012) and *Sinorhizobium meliloti* for only three genera: *Medicago*, *Melilotus* and *Trigonella* (Roche et al. 1996; Albrecht et al. 1999). The mutualistic interaction between the two symbiotic partners results in the formation of a new plant organ, the root nodule. The rhizobia within nodules (which now become the bacteroides) convert atmospheric nitrogen into ammonia, a form that can be directly used by the legume plant (Wang et al. 2012). However, prior to the formation of nodules and the subsequent biological process that converts atmospheric nitrogen into a usable form, the legume and its micro symbiont need to establish a successful interaction. This interaction requires a signal recognition between the two symbiotic partners, which signifies that symbiotic specificity evolves because of the interaction of both the rhizobia and the host legume genes (Wang et al. 2012, 2018).

The first step in the legume rhizobium symbiosis is a ‘molecular dialogue’ between the two partners which starts after the legume secretes special molecules called *flavonoids* that immediately diffuse across the bacterial membrane. Moments after the bacteria perceives the flavonoid signals, it results in the activation of the bacterial nodulation (nod) genes, which encode the enzyme needed for the synthesis of the rhizobial *Nod factors*. These are lipochitooligosaccharides with four or five 1,4-linked *N*-acetyl-glucosamine residues that carry a fatty acyl chain of varying length attached to the C-2 position of the non-reducing end

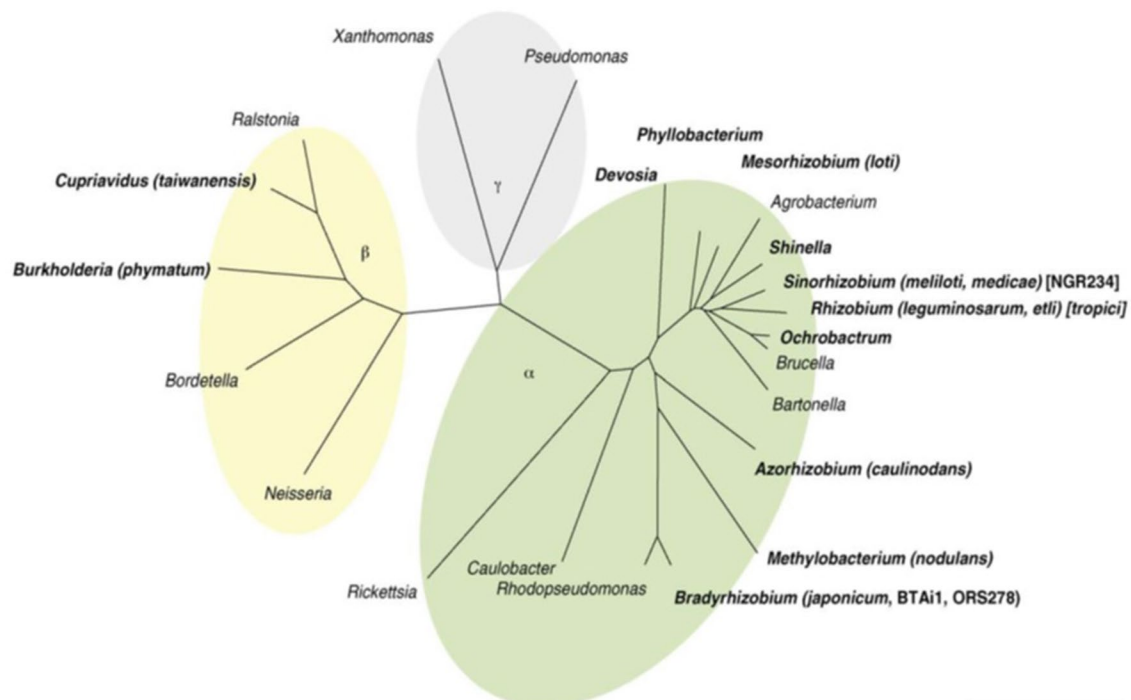


Fig. 1 Phylogenetic tree of Proteobacteria constructed from the 16S rRNA gene sequences. The genera containing rhizobia are indicated in bold (adapted from Barreda and Fikri-Benbrahim 2014 with permission)

and various species-specific chemical decorations at both the reducing and non-reducing ends (Wang et al. 2018). Generally, Nod factors are the signal molecules essential for initiating symbiotic development in the legumes and are required for root hair curling, induction of nodulation and entry of the rhizobia into the roots (Pueppke and Broughton 1999; Oldroyd et al. 2011). Only a particular type of Nod factor allows a given rhizobial strain to nodulate a certain legume host, giving rise to the first rhizobial determinant of *host specificity* (Fauvart and Michiels 2008). A typical example could be the variation in the Nod factors between *Sinorhizobium meliloti*, a root nodulating nitrogen fixing symbiont of *Medicago* spp. and *Rhizobium leguminosarum* bv. *viciae* a nitrogen fixing symbiont of *Vicia faba* (Fig. 2). These two rhizobia spp. have different legume host preference for nodulation mainly due to the variation in the nod factors, which differ at the reducing terminal sugar unit and

by the structure of the acyl chain. *S. meliloti* Nod factors are characterized by four glucosamine units and an acyl chain of 16 C-atoms in length with two unsaturated bonds, an acetyl group at the non-reducing end and a sulfate group at the reducing terminal end in the nod factor. On the other hand, the *R. leguminosarum* nod factor consists of a mixture of factors that contain several compounds, and the length of the glucosamine backbone is four or five units that carry an acyl chain of 18 C-atoms with either one or four unsaturated bonds (Albrecht et al. 1999).

The idea of Nod factors being the major determinants in the rhizobium-legume host specificity is still in debate. In a report published by Perret et al. (2000), the molecular basis of host specificity and symbiotic promiscuity has been dealt with in detail. The report, supported with few evidences, states that the Nod factors produced by rhizobia and the legumes they nodulate have no strict correlation. For instance,

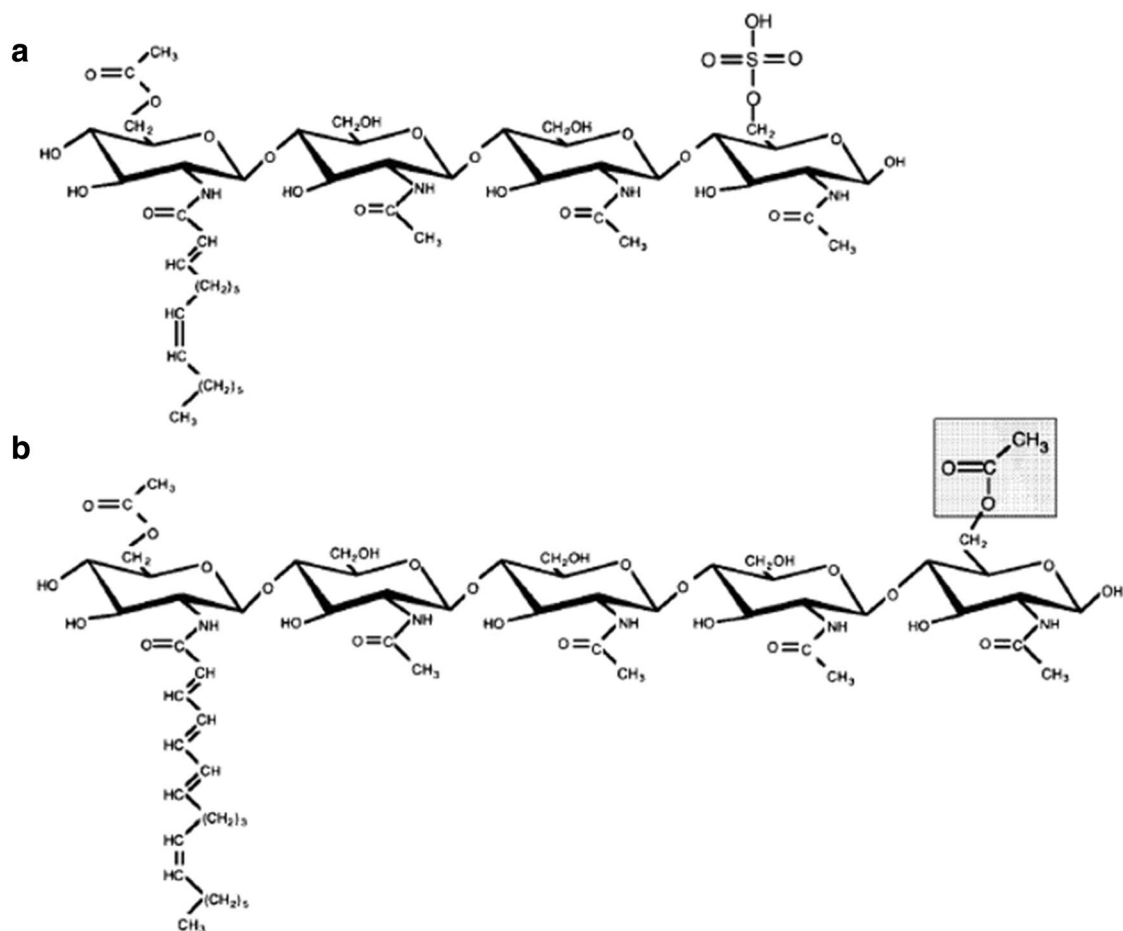


Fig. 2 Nod factor variation in *Sinorhizobium meliloti* (a) and *Rhizobium leguminosarum* bv. *viciae* (b) that determine host specificity for nodulation. The two Nod factors differ at the reducing terminal sugar unit and by the structure of the acyl chain. The *S. meliloti* Nod factor contains four glucosamine units, an acyl chain of 16 C-atoms in length with two unsaturated bonds, an acetyl group at the non-reduc-

ing end and a sulfate group at the reducing terminal sugar residue. In contrast, *R. leguminosarum* bv. *Vicia* Nod factor contains glucosamine backbone of four or five units carrying an acyl chain of 18 C-atoms with either one or four unsaturated bonds which could be O-acetylated at the non-reducing terminal sugar residue (adapted from Albrecht, the *EMBO Journal* with permission)

although the Nod factors produced by *Rhizobium etli* and *Rhizobium loti* (now *Mesorhizobium loti*) are identical, the two rhizobia have distinct host ranges in which *M. loti* nodulates *Lotus* spp. whereas *R. etli* is specific to *Phaseolus* spp. (Cardenas et al. 1995). In yet another example, *R. leguminosarum* bv. *viciae* produce Nod factors which are the same as that of *R. leguminosarum* bv. *trifolii*, but the two rhizobium biovars nodulate different legume hosts (Orgambide et al. 1995; Spaink et al. 1995).

On the other hand, several recent investigations back up the idea that Nod factors are still the major determinants in legume host specificity where each rhizobium produces a particular mix of Nod factors characterized by chemical substitutions at certain positions that give specificity to the signalling (Wang et al. 2012, 2018; Clua et al. 2018). In a decade long study since 1990, a concerted research by scientists that involved microbiologists, geneticists, plant cell biologists, biochemists and analytical chemists from known universities globally resulted in the isolation and characterization of Nod factors from several species of *Rhizobium* (Long 2001). The outcome of this work showed that diverse *Rhizobium* species produce Nod factors with similar chito-oligo backbones, but different side chains that include sugars, acetyl or carbamyl residues and modified lipids. This difference in the side chains of the Nod factors provides host specificity in the legume nodulation.

Nodulation of legumes by both alpha and beta rhizobia

First report of legume nodulation by β -rhizobia

For almost more than a century, it was believed that all legumes are nodulated only by *Rhizobium* and its relatives in the alpha sub class of Proteobacteria such as *Bradyrhizobium*, *Ensifer* (*Sinorhizobium*) species. However, over the last 20 years, sufficient evidence has accumulated that indicate the emergence of new members of root nodulating bacteria belonging to the genera, *Methylobacterium*, *Devosia*, *Ochrobactrum*, *Shinella*, *Burkholderia* and *Cupriavidus*. The latter two genera fall into the sub class of β -Proteobacteria and are collectively termed as 'beta-rhizobia' (Moulin et al. 2001; Gyaneshwar et al. 2011). In many parts worldwide, unlike the food legumes soybean (*Glycine max* L.), cowpea (*Vigna unguiculata* (L.) Walp, common beans (*Phaseolus vulgaris* L.) to mention a few, the symbiotic properties of several other indigenous legumes not directly used for human consumption have not been investigated thoroughly. A few examples include the indigenous South African legumes such as *Aspalathus*, *Cyclopia*, *Lotononis*, *Desmodium* as well as the medicinally important *Crotalaria* species, which still

warrant such studies to elaborate on their symbiotic properties. Although recent reports on the nodulation properties of *Cyclopia* and *Aspalathus* are gaining momentum recently, there is still a big gap in the elucidation of the diversity of the rhizobia and their taxonomic and phylogenetic relatedness, which warrants more work in this regard. For instance, members of the genus *Aspalathus* including the popular *A. linearis* have been considered to be nodulated by the slow growing *Bradyrhizobium* species until recently. It was repeatedly reported that members of the genus *Aspalathus* are specific in their *Bradyrhizobium* requirements (Staphorst and Strijdom 1975; Deschodt and Strijdom 1976). These reports were however based solely on cultural and growth properties of the bacteria without the availability of any molecular data (Hassen et al. 2012).

Moulin et al. (2001) was the first to disclose the occurrence of nodulation of legumes by members of the β -subclass of Proteobacteria. In this pioneer discovery, the researchers showed that the ability of rhizobia to establish a symbiotic interaction with legumes is more widespread than has been anticipated to date. The study revealed that two bacteria isolated from the nodules of *Aspalathus* (strain STM678) and *Machaerium* (strain STM815) were taxonomically found to be very distant from known rhizobia. For quite a long time before this discovery, strain STM678 originally isolated from the South African *Aspalathus carnosa* was thought to belong to the slow growing *Bradyrhizobium* species (Boone et al. 1999; Dakora 1998). Nevertheless, the study by Moulin et al. (2001) using phylogenetic analysis of the gene sequences of the 16S ribosomal RNA showed that the strains do not belong to any of the major branches of rhizobia described and not even to the α -subclass of Proteobacteria, but rather belong to the β -subclass of Proteobacteria. Further phylogenetic analysis of partial sequence of the 23S *rRNA* gene and the *dnaK* gene encoding the chaperon heat shock protein were consistent with the 16S rRNA analysis that unambiguously positioned these strains in the *Burkholderia* genus within the β -subdivision of Proteobacteria.

The discovery of nodulating bacteria other than the traditional rhizobia belonging to the Alphaproteobacteria prompted extensive work on the phylogeny and taxonomy of the newly nodulating strains of β -Proteobacteria. This resulted in the description of the two new nodulating strains as *Burkholderia tuberum* STM678 and *Burkholderia phy-matum* STM815 (Vandamme et al. 2002). Later, Chen et al. (2003) reported that legume nodulation and nitrogen fixation by the β -Proteobacteria is widespread in nature. Following the pioneer study in 2001, research in the past two decades has accumulated ample evidences worldwide about the nodulation and nitrogen fixation of native and exotic legumes by the newly emerging ' β -rhizobia' group.

Nodulation of Papilionoid legumes by both the α and β -Proteobacteria

Nodulation of *Aspalathus linearis* burm. f.

Several works are available that report about the nodulation of legumes belonging to the sub family Papilionoideae. However, there was very little information regarding the taxonomy of rhizobia that nodulate *Aspalathus* species until the work by (Moulin et al. 2001; Hassen et al. 2012) who first reported the nodulation of *Aspalathus carnososa* and *A. linearis* by members of the β -sub class of Proteobacteria respectively. A few years later after the discovery of legume nodulation by the beta group of rhizobia, the nodulation properties of the South African indigenous legume, *A. linearis* burm f. and their associated symbionts was studied. *A. linearis* burm. f. commonly referred to as 'rooibos', derived from the Afrikaans *roy boss* (=red bush), is an indigenous South African legume normally found in the winter rainfall area of the Cederberg region of the Western Cape province. It is a legume in the family Leguminosae, tribe Crotalaria of the sub family Papilionoideae. In South Africa and many other countries, *A. linearis* is used to make rooibos tea, a popular herbal tea that has several health benefits (Joubert and de Beer 2011; Hassen et al. 2012).

Like several other legumes, *A. linearis* forms a symbiotic relationship with certain group of *Rhizobia* that form root nodules and fix well over 100 kg nitrogen ha⁻¹ annually (Sprenst et al. 2009; Hassen et al. 2012). *A. linearis* is generally characterized by the formation of *indeterminate* nodules; elongated nodules with persistent meristem that continuously give rise to new nodule cells similar to that of *Medicago sativa* or *Pisum sativum* (Gage 2004) (Fig. 3).

These are different from *determinate* nodules, which are circular in shape and without meristem that contain homogeneous population of symbiotic cells (Maroti and Kondorosi 2014) such as those in soybeans and cowpea. The study by Hassen et al. (2012) reported for the first time that this indigenous Papilionoid legume is nodulated by members of both the α - and β -sub class of Proteobacteria. These workers initially isolated the bacteria using soil trap technique in the glasshouse from soil samples collected from the rhizosphere of wild growing *A. linearis* at various sites in the Cederberg mountain areas in the Western Cape. The study evaluated the ability of the isolated bacteria to nodulate the original legume host. Their results indicated that the majority of the trapped isolates were able to nodulate their original legume host.

Unlike the previous thoughts and reports that *A. linearis* have a specific *Bradyrhizobium* requirement for their nodulation (Boone et al. 1999; Dakora 1998), the root nodule isolates from this indigenous legume displayed variations in growth rate, colony size, colour and cultural morphology (Fig. 4). Analysis of the taxonomic positions and phylogeny using nucleotide sequence of the 16S ribosomal RNA revealed that the majority of the nodule isolates from *A. linearis* belong to three genera (*Mesorhizobium*, *Rhizobium*, *Burkholderia*) (Fig. 5). The finding of this work outlines that contrary to the long-time belief that *A. linearis* are specifically nodulated *Bradyrhizobium* species, only 2% of the nodule isolates were closely related to *Bradyrhizobium* spp. In a previous work by Muofhe (1997), the absence of appropriate bacterial strains that nodulate and fix atmospheric nitrogen within the Cape region and which could constraint nodulation and growth of *A. linearis* was reported. This is contrary to what was found out by Hassen et al. (2012) in which they

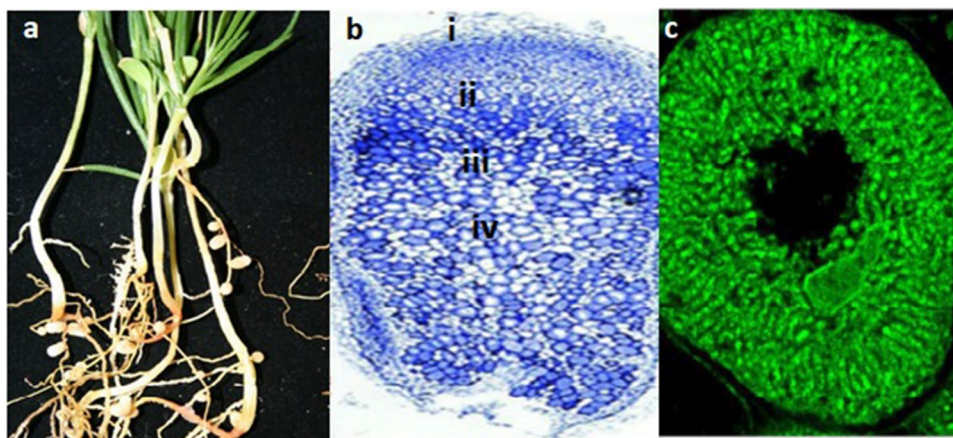


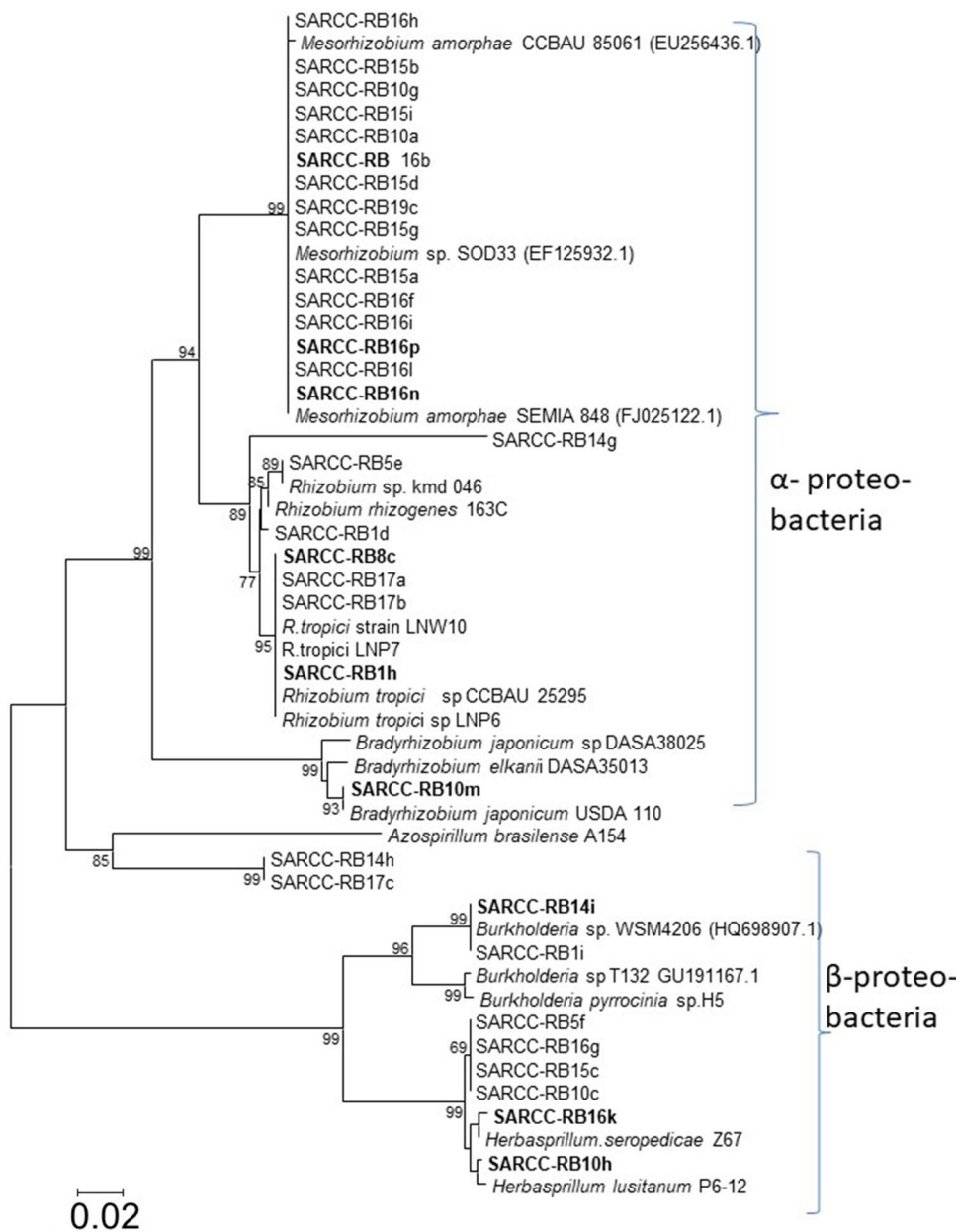
Fig. 3 **a** Indeterminate, elongated pink nodules of *Aspalathus linearis*. **b** Different nodule zones in the mature longitudinal root nodule section formed in *S. meliloti*, *M. truncatula* symbiosis showing the meristem (*i*), infection zone (*ii*), nitrogen fixation zone (*iii*) and senescence zone (*iv*). **c** Symbiotic cells containing the differentiating endo-

symbionts stained with green fluorescence and the host cytoplasm fully packed with long nitrogen-fixing bacteroides (**c**). Adapted from (**a**) Hassen et al. (2012); (**b**, **c**) Maroti and Kondorosi (2014), *Frontiers in Microbiology*, with permission



Fig. 4 Morphologically different strains of rhizobia isolated from the root nodules of *Aspalathus linearis* (rooibos): **a** *Rhizobium* sp., **b** *Burkholderia* and *Mesorhizobium* spp. and **c** *Bradyrhizobium* and *Herbasprillum* spp. adapted from Hassen et al. (2012)

Fig. 5 Neighbour Joining 16S rRNA phylogenetic tree of rhizobial isolates that nodulated *Aspalathus linearis*. The tree shows the taxonomic placements of the characterized isolates that contain representative species from both the alpha and beta Proteobacteria. Adapted from Hassen et al. (2012)



reported the formation of an average of 3–12 nodules per plant by rhizobia. More than one species of rhizobia including those belonging to both the alpha and beta rhizobia nodulated *A. linearis* (Fig. 6). The rhizobia also resulted in statistically significant increase in plant biomass (dry weight and fresh weight) indicating that the Cape floristic region is not poor in its microbial diversity that effectively colonize and form nodules in *Aspalathus* species.

The isolation of *Mesorhizobium* spp. (alpha rhizobia) and *Burkholderia* spp. (beta rhizobia) which both nodulated *A. linearis* can be attributed to many factors, one of which is that both genera occur in similar acidic and very poor nutrient soils (Lamaire et al. 2015). However, the vast majority of the nodule symbionts detected in *A. linearis*, like in many other legumes, are the alpha-rhizobia, in this particular case *Mesorhizobium* followed by *Rhizobium* spp. (Fig. 5). One possible reason, amongst several other hypotheses, could be that shortly after the transfer of the *nod* genes from beta to alpha-rhizobia, there was a rapid expansion of legumes into environments that favoured alpha-rhizobia more. For instance, *Burkholderia* strains mainly occur as symbionts in acidic and very poor soil, but they could be outcompeted by other symbionts (e.g. *Mesorhizobium* spp.) when the concentration of soil nutrients in particular nitrogen increases (Sprent et al. 2017).

Nodulation of *Lebeckia* spp.

Another South African indigenous legume for which extensive study on the symbiotic performance and the rhizobium associated with it has been conducted was *Lebeckia* spp., a legume of the Leguminosae family and the tribe Crotalariae mainly indigenous to the Southern and Western Cape regions of South Africa (Phalane 2008). This genus falls under the sub family Papilionoideae and consists of close

to 35 species that are mainly indigenous to the Western and Southern regions of South Africa. In the study to determine the diversity and taxonomy different *Lebeckia* spp. (Phalane 2008), used various species of the indigenous *Lebeckia* collected from nine localities in the western and northern Cape regions. These workers isolated several morphologically different rhizobia which were then characterized to infer the phylogenetic positions at molecular level by PCR amplification using *Nif* gene derived primer RPO1 (Richardson et al. 1995) and the 16S rRNA primers 16f 27 and 16r 1485 (Lane 1991) followed by sequencing. The results from this analysis indicated that the various species in the genus *Lebeckia* are nodulated by both the alpha (*Mesorhizobium*, *Bradyrhizobium* and *Sinorhizobium* spp.) and rhizobia of the beta Proteobacteria group. This work presented the first report on the taxonomic identities of the rhizobia associated with the root nodules of this unique legume indigenous to South Africa. The essential finding of this work was the nodulation of all the needle shaped leaf *Lebeckia* spp. by the newly emerging beta sub group of rhizobia (*Burkholderia*) and the promiscuous nodulation of *Lebeckia sericea* by both *Mesorhizobium* and *Bradyrhizobium* species.

Despite the report by Phalane (2008) that the alpha (α)-Proteobacteria including *Mesorhizobium*, *Rhizobium* and *Bradyrhizobium* spp. are reported as the predominant symbionts of *Lebeckia* species, later studies indicated that the indigenous *Lebeckia* spp. are mainly nodulated by *Burkholderia* species (De Meyer et al. 2013, 2014). The study on the symbiotic properties of South African fynbos legumes spanned from 2002 until recently with an aim to domesticate herbaceous South African legumes such as *Rhynchosia* and *Lebeckia* as perennial pasture legumes in Australian agricultural systems exposed to drying climate (Howieson et al. 2013). Several novel *Paraburkholderia* species have since been isolated and characterized from

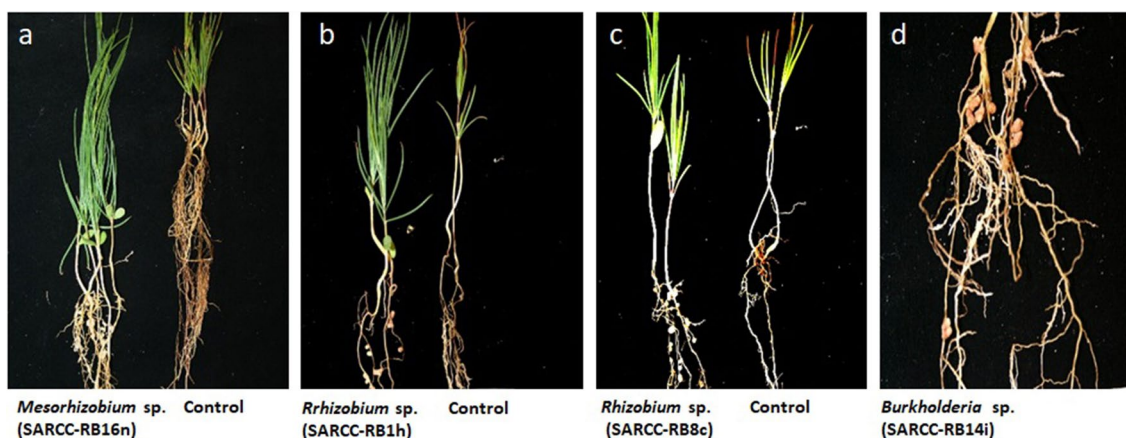


Fig. 6 *Aspalathus linearis* is nodulated by different species of bacteria belonging to *Mesorhizobium* (a), *Rhizobium* (b, c) and *Burkholderia* species (d). In all cases, the nodules are indeterminate and pink. Adapted from Hassen et al. (2012)

different *Lebeckia* spp. of which *Lebeckia ambigua*, a perennial suffrutescent legume of the fynbos in South Africa, has been extensively studied. One of such studies investigated the symbiotic properties and taxonomic positions of 23 bacterial strains isolated from this legume (Howieson et al. 2013). According to the 16S rRNA phylogeny study, most of the rhizobia isolates belonged to the genus *Paraburkholderia* having high sequence similarity with *P. graminis*, *P. caledonica* and *P. tuberum*. These findings prompted for more detailed studies using polyphasic approaches including 16S rDNA, *gyrB*, *recA* gene phylogenies, DNA-DNA hybridizations, MALDI-TOF MS analysis as well as physiological and biochemical tests. These studies helped to describe new species of *Paraburkholderia* such as *P. sprentiae* sp. nov. (De Meyer et al., 2013), *P. dilworthii* sp. nov. (De Meyer et al. 2014) and *P. fynbosensis* sp. nov. (De Meyer et al. 2018) from the previously characterized 23 strains isolated from *Lebeckia ambigua* (Fig. 7). Unlike most of the previously studied *Paraburkholderia* mainly in South East China and the Americas, *Paraburkholderia* strains isolated within South Africa are

primarily associated with Papilionoid legumes rather than the *Mimosa* species (De Meyer et al. 2015).

Nodulation of other Papilionoideae legumes

Cyclopia spp. are the other endemic Papilionoid legumes that represent part of the fynbos biome of the South African Cape region. Two of the species *C. intermedia* and *C. subternata* are predominantly used in the production of a local beverage drink known as *honey bush tea* with several health benefits as well as economic value on a foreign market. The diversity of the root nodule rhizobia associated with several *Cyclopia* species has been studied in South Africa using molecular characterization techniques that involved the 16S–23S IGS-RFLP analysis (Kock 2003). Both alpha and beta rhizobia were detected from the nodules as nitrogen fixing symbionts, but the majority belonged to members of the beta rhizobia (*Burkholderia*) most of which were phylogenetically related to *Burkholderia tuberum*. Then, Elliott et al. (2007) demonstrated the two *Burkholderia tuberum* strains STM678 and DUS833 described earlier can nodulate

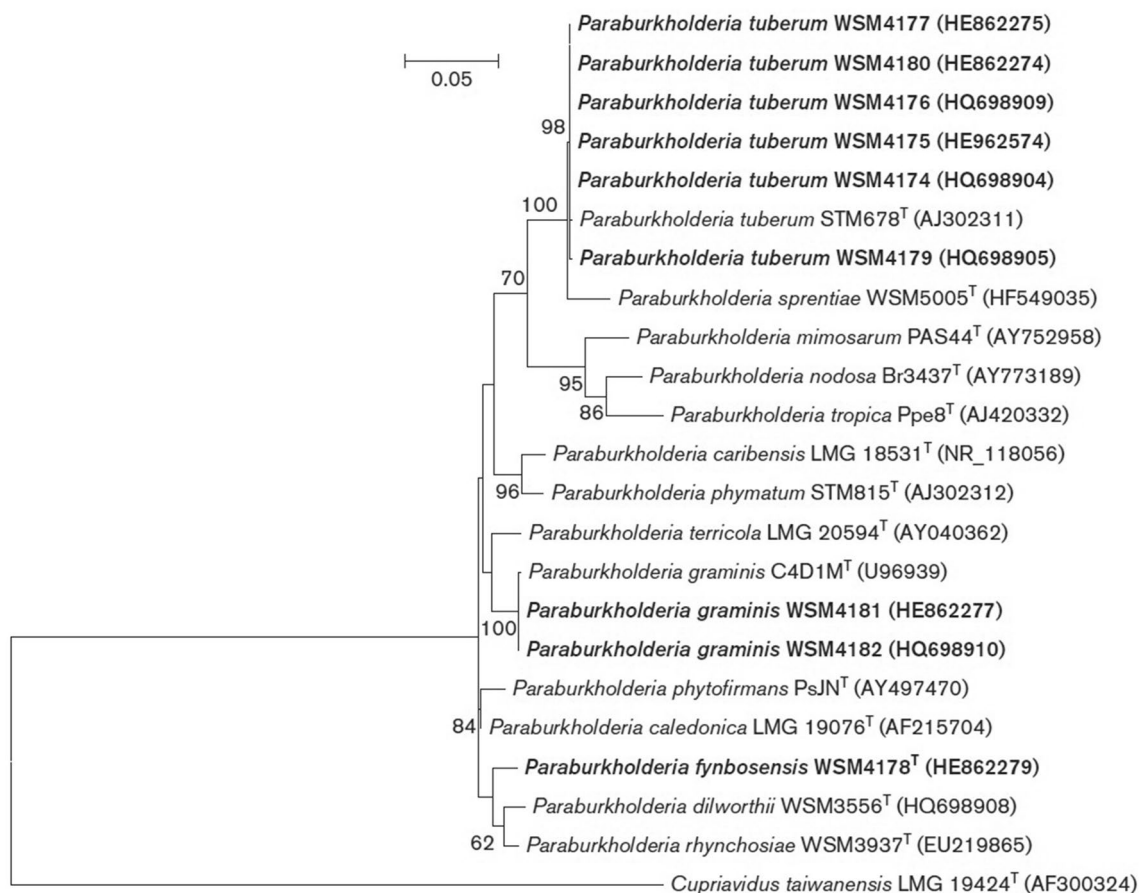


Fig. 7 Phylogenetic tree showing the evolutionary relationship and taxonomic positions of *Paraburkholderia* species including the newly described novel isolates from *Lebeckia ambigua* nodules. The

tree construction was based on analysis of the 16S ribosomal RNA sequences. Adapted from De Meyer et al. (2018), with permission

Cyclopia species and other Papilionoid legumes from wildly different tribes. Nodules formed by *B. tuberum* ST678 or DUS833 on *Cyclopia genistoides* were typically intermediate with invasion and nitrogen fixing zones. Beukes et al. (2013) also confirmed that the majority of these *Burkholderia* isolates from *Cyclopia* are conspicuous to *B. tuberum* that could expand the host range of *Cyclopia* species for this species of *Burkholderia*.

Parallel with the disclosure of the nodulation of the Papilionoid legumes *Aspalathus* and *Cyclopia* spp. by both the alpha and beta-sub group of Proteobacteria (Moulin et al. 2001; Kock 2003; Hassen et al. 2012), several new β -rhizobia that nodulate other Papilionoid legumes have been described. In an effort to gather more evidences that South African Papilionoid legumes are nodulated by *Paraburkholderia* strains different from those nodulating *Mimosa* spp. in China and the Americans, Garau et al. (2009) conducted a similar study on the indigenous herbaceous legume *Rhynchosia ferulifolia*. Two root nodule strains *Burkholderia* sp. WSM3937 and WSM3930 isolated from this legume were studied for their symbiotic performance using the same host in the glasshouse and confirmed that both nodulated *Rhynchosia* effectively. Using analysis of the 16S rRNA gene as well as intragenic sequences of *nodA* and *nifH* the nodule isolates from *R. ferulifolia*, belong to the genus *Burkholderia* with substantial similarity with *Burkholderia tuberum* STM678T, the β -rhizobium from South Africa, but have been found to have a distant relationship with South American *Mimosa*-nodulating β -rhizobia. This study by Garau et al. (2009) was of course the first to report N-fixation between β -rhizobia and an herbaceous, Papilionoid legume from which the strains were originally isolated.

Later, Beukes et al. (2013), reported the occurrence of diverse *Burkholderia* spp. in the nodules of the endemic Papilionoid legumes of the tribe Hypocalypteae and Podalyrieae from Cape Floristic Region in South Africa. Based on the analysis of molecular techniques that involved 16S rRNA and *recA* housekeeping gene, as well as analysis of the genes that encode for *nifH* and *nodA*, there exists a horizontal gene transfer that determines their symbiotic performance and their evolutionary origins were by far distinct from *Burkholderia* from other parts of the world. Similar reports of nodulation of legumes by the newly emerging beta rhizobia that mainly include *Paraburkholderia* (formerly *Burkholderia*) is gaining momentum in the past decades. Very recently, Beukes et al. (2019a) investigated and reported the nodulation of an indigenous legume tree *Vachellia karroo* (formerly *Acacia karroo*), by both members of the alpha and beta Proteobacteria. The study, which used a 16S rRNA sequence analysis as well as *recA*-based phylogenetic analyses, identified nodule isolates from *V. karroo* down to species level many of which belonged to *Paraburkholderia* spp. In a separate study, the taxonomic

status of rhizobia that nodulate the indigenous South African fynbos legume *Hypocalyptus sophoroides* was elucidated using a genealogical concordance analysis that include six loci (16S rRNA, *atpD*, *recA*, *rpoB*, *lepA* and *gltB*) as well as comparison of average nucleotide identity. The result of this study disclosed that these nodulating strains are novel strains of *Paraburkholderia* which were divergent from their closest relatives and were classified as *Paraburkholderia strydomina* sp. nov. and *Paraburkholderia steynii* sp. nov. (Beukes et al. 2019b). Recently, *Paraburkholderia* species that nodulate soybean have been identified by means of multilocus sequence typing analysis in Venezuela (Ramírez et al. 2019). This is despite the fact that there is very little evidence that demonstrates the occurrence of *Burkholderia* and *Paraburkholderia* as the predominant soybean rhizobia in agricultural fields. The authors argue that such occurrence of soybean nodulating *Paraburkholderia* in Venezuela soils located in different climatic and topographical regions could have resulted through horizontal gene transfer.

Nodulation of Mimosoideae legumes by β -rhizobia

The genus *Mimosa* is one of the largest genera of legumes (500 species) belonging to the family Leguminosae, sub family Mimosoideae most of which form a symbiotic association with rhizobia (Sprent et al. 2009; Elliott et al. 2007). The principal centre of diversification for *Mimosa* are the Americans including Central Brazil, Mexico and Sub tropical South America. However, three main invasive *Mimosa* spp. (*M. diplotricha*, *M. pigra* and *M. pudica*) have recently become widespread in tropical and subtropical areas in South East Asia (Liu et al. 2012). In terms their symbiotic properties, several investigations revealed that the β -rhizobia are the common nodulating symbionts of the tribe *Mimosa* (Chen et al. 2003, 2005; Platero et al. 2016; Liu et al. 2012). The two major beta-rhizobia that are the preferred symbionts of *Mimosa* spp. are *Burkholderia* (now *Paraburkholderia*) and *Cupriavidus* spp. Chen et al. (2001) were the first to isolate bacteria from *Mimosa* nodules, which were taxonomically different from the conventional 'Rhizobia' belonging to α -Proteobacteria. The nodule isolates obtained from *M. diplotricha* and *M. pudica* were very similar to the genus *Ralstonia* (syn = *Wautersia*) in the β -Proteobacteria group and were later confirmed to belong to a new species *Ralstonia taiwanensis* (Chen et al. 2001) which was later re-described as *Cupriavidus taiwanensis* (Vandamme and Coenye 2004). *C. taiwanensis* strain LMG19424 has been reported to be the most effective nitrogen fixing symbiont of the invasive *Mimosa* shrubs in Taiwan. Taxonomic characterization of several symbiotic isolates from the nodules of *M. pudica* and *M. diplotricha* throughout the entire island in Taiwan revealed that 94% were β -rhizobia of which 93% belong to *C. taiwanensis*, which suggests that *C. taiwanensis*

may be the preferred symbionts of these *Mimosa* species (Chen et al. 2005). However, subsequent investigations in later years confirmed that invasive *Mimosa* spp. have colonized large areas of southern China and *Burkholderia* and *Cupriavidus* spp. were reported to be the dominant symbionts of the three invasive *Mimosa* spp. (Liu et al. 2012). According to this report, a more accurate representation of the origins of the symbiotic relationship established by rhizobia with both the native and exotic legumes was obtained by examining the symbiosis related genes such as the N₂-fixation (*nifH*) and nodulation (*nodA*) genes. Both *Burkholderia* and *Cupriavidus* spp. nodulating the invasive *Mimosa* legumes harbour these symbioses related genes which are thought to be acquired through lateral gene transfer from rhizobia and/or one of the relatives in the α -Proteobacteria (Chen et al. 2005).

Prior to the studies on the nodulation properties of invasive *Mimosa* spp. mainly in China and Taiwan, and before the discovery of the β -rhizobia as their predominant symbionts, little was known about the symbionts of *Mimosa* species in their native South American environments. Although several strains of β -rhizobia have been isolated since the first report of Moulin et al. (2001) on *Burkholderia* strains STM815 and STM678, the majority of them were isolated from *Mimosa* spp. in Asia, with only very few in the Americas (Barrett and Parker 2005). The works of Chen et al. (2005) provided a very conclusive proof for the nodulation of native South American *Mimosa* spp. by novel strains of *Burkholderia* spp. In this study, 16S rRNA sequence analysis and RFLP of amplified 16S rRNA genes of several *Mimosa* nodulating strains collected from Brazil and Venezuela revealed that *Burkholderia* strains form effective symbiosis with native *Mimosa* spp. in South America.

More recent works have since been reported on the nodulation of the native South American *Mimosa* spp. by the β -rhizobia with *Burkholderia* (*Paraburkholderia*) being the preferred major symbionts. The first demonstration of biological nitrogen fixation of *Mimosa* by β -rhizobial symbionts was held under field conditions in two of the major centres of diversity of *Mimosa* spp. in Brazil, Cerrado and Caatinga (dos Reis Jr et al. 2010). The major finding of this study was that *Burkholderia* spp. were the predominant symbionts in these native soils, and no single *Cupriavidus* sp. was isolated suggesting that the native South American Cerrado and Caatinga *Mimosa* spp. prefer *Burkholderia* as their major symbiont. However, in contrast to the reports for the major centres of diversification of the genus *Mimosa* (Brazil and Mexico) as being nodulated mainly by *Burkholderia* spp., the native Uruguayan *Mimosa* spp. are exclusively nodulated by β -rhizobia belonging to the genus *Cupriavidus* (Platero et al. 2016). An interesting part of this investigation is that all the five tested Uruguayan native species of *Mimosa* effectively nodulated in the field with rhizobia belonging

to the genus *Cupriavidus* but none of them belonged *C. taiwanensis*.

Later, Lammel et al. (2015) conducted the symbiotic properties of native woody *Mimosa* spp. in Brazil using *Mimosa scabrella* and *Mimosa bimucronata* as trap legumes, previously tested and verified to be nodulated and fix nitrogen with *Burkholderia* spp. (Chen et al. 2006, 2007). More recently, Agnol et al. (2017) conducted similar work in the Atlantic Forest (Mata Atlantica) which is the centre of *Mimosa* diversity and that represents one of the largest and richest biomass in Brazil. In this study, rhizobia were trapped from several soil samples collected at different locations of the Atlantic Forest using *Mimosa pudica* as the trap legume. The genetic diversity of the trapped isolates in the nodules of *M. pudica* from the soils in the different sites was determined using a concatenated 16S rRNA-*recA* phylogeny and it was found that the majority of the isolates trapped in the nodules belonged to the *Paraburkholderia* species. These findings of course supported the results of previous and initial work by Bontemps et al. (2010) who characterized nodule isolates from 47 *Mimosa* spp. based on concatenated 16S rDNA and *recA* sequences. The concatenation revealed that these nodulating isolates were related to the new β -rhizobia species including *Burkholderia nodosa* and *Burkholderia tuberum*, but were in several distinct clades that indicated they could probably be new *Paraburkholderia* species. It is noteworthy that the root nodulating beta-rhizobia species described as '*Burkholderia*' throughout this review are reclassified as the genus *Paraburkholderia* based on analysis of their phylogenetic positions and beneficial traits to separate them from plant and/or animal pathogenic *Burkholderia* strains (Dobritsa and Samadpour 2016).

Concluding summary

Reports of novel strains of root nodulating β -Proteobacteria, mainly that of *Paraburkholderia* which nodulate and fix nitrogen with several indigenous legumes adapted to acidic and nutrient poor soils is increasing in the past few years (Moulin et al. 2001; Chen et al. 2003; Garau et al. 2009; Elliott et al. 2007). These successive findings of the nodulation of legumes by the β -rhizobia has changed the long held dogma that only rhizobia, bacteria belonging to the Alphaproteobacteria, are able to nodulate legumes. The phenomenon that a single legume plant is nodulated by more than one species of rhizobia has been a point of debate with regard to the legume–rhizobium host specificity. A good example of such phenomena could be the fact that *Mesorhizobium* and *Burkholderia* spp. are both capable of nodulating *A. linearis* (Hassen et al. 2012) and probably other related legumes. One possible argument is that horizontal gene transfer of nod genes from one species to another

could have resulted in the loss of specificity of many rhizobia to their legume host (Donate-Correa et al. 2007). This loss of specificity and the emergence of new group of root nodulating rhizobia (both α - and β -Proteobacteria) can also be attributed to the plasticity of the nodulation traits. For instance, in *Rhizobium* and *Sinorhizobium*, the nodulation traits are easily transferable plasmid borne genes whereas in the genus *Mesorhizobium*, there is a mobile symbiosis island in the chromosome (Gyaneshwar et al. 2011). Another possibility of the observed promiscuity (being nodulated by more than one species of rhizobia) in *A. linearis* or any other legume that nodulates promiscuously could be associated with the presence of more than one type of flavonoids, nod gene inducers, released by the same legume which warrants future investigation.

Coupled with the aforementioned traits and genomic features of the rhizobia, the spread of crop plants beyond the range of their native ancestors might also have resulted in the selection of a less stringent symbiotic specificity (Mutch and Young 2004). It is also argued that the nodulation by the newly emerging β -rhizobia belonging to the genus *Burkholderia* therefore seem to be associated more with a physical niche, such as acid, sandy, infertile soils, than with a particular legume phylogeny, hence not abiding by the principles of the legume host specificity for nodulation. The driving force for legume host specificity in the nodulation process is still debatable since, in the past two decades, several novel species of rhizobia that nodulate more than one legume host were detected. Moreover, several indigenous legumes, for example the South African indigenous Papilionoid legume *A. linearis*, are nodulated by more than one rhizobium species including both alpha and beta rhizobia. Therefore, whether Nod factors are generally the decisive molecular signals for legume–rhizobium host specificity is yet to be fully understood to further elucidate the mechanisms behind long held legume–rhizobium host specificity dogma. Rhizo-biologists have now a concern (Howieson et al. 2013) that the emergence of new alpha rhizobia and the discovery of several novel species of *Paraburkholderia* (β -rhizobia) from root nodules could be the microbiological challenge facing the domestication of legumes to promote sustainable agriculture in the future.

Acknowledgements Due acknowledgement goes to the Agricultural Research Council—Plant Health and Protection for the material and moral assistance rendered in the publication of this work.

References

- Agnol RFD, Bournaud C, Faria SM, Bena G, Moulin M, Hungria M (2017) Genetic diversity of symbiotic *Paraburkholderia* species isolated from nodules of *Mimosa pudica* (L.) and *Phaseolus vulgaris* (L.) grown in soils of Brazilian Atlantic Forest (Mata Atlântica). *FEMS Microbiol Ecol* 93:1–15
- Albrecht C, Guerts R, Bisseling T (1999) Legume nodulation and mycorrhizae formation; two extremes in host specificity meet. *EMBO J* 18:281–288
- Barreda H, Fikri-Benbrahim K (2014) Taxonomy of rhizobia: current perspectives. *Br Microbiol Res J* 4:616–639
- Barrett CF, Parker MA (2005) Prevalence of *Burkholderia* sp. nodule symbionts on four mimosoid legumes from Barro Colorado Island, Panama. *Syst Appl Microbiol* 28(1):57–65
- Beukes CW, Venter SN, Law IJ, Phalane FL, Steenkamp ET (2013) South African Papilionoid legumes are nodulated by diverse *Burkholderia* with unique nodulation and nitrogen-fixation loci. *PLoS ONE* 8:1–13
- Beukes CW, Boshoff FS, Phalane FL, Hassen AI, le Roux MM, Steenkamp ET (2019a) Both alpha and beta-rhizobia occupy the root nodules of *Vachellia karro* in South Africa. *Front Microbiol* 10:1195. <https://doi.org/10.3389/fmicb.2019.01195>
- Beukes CW, Steenkamp ET, van Zyl E, Avontuur J, Chan WY, Hassen AI, Palmer M, Mthombeni LS, Phalane FL, Sereme TK, Venter SN (2019b) *Paraburkholderia strydomiana* sp. nov. and *Paraburkholderia steynii* sp. nov.: rhizobial symbionts of the fynbos legume *Hypocalyptus sophoroides*. *Antonie Van Leeuwenhoek*. <https://doi.org/10.1007/s10482-019-01269-5>
- Bontemps C, Elliott GN, Simon MF, dos Reis FB, Jr GE, Lawton RC, Neto NE, Loureiro MF, de Faria SM, Sprent JI, James EK, Young JPW (2010) *Burkholderia* species are ancient symbionts of legumes. *Mol Ecol* 19:44–52
- Boone CM, Olsthroon MMA, Dakpra FD, Spaink HP, Thomas-Oats JE (1999) Structural characterization of lipo-chitin oligosaccharides isolated from *Bradyrhizobium aspalati*, microsymbionts of commercially important South African legumes. *Carbohydr Res* 317:155–163
- Cárdenas L, Domínguez J, Quinto C, López-Lara IM, Lugtenberg BJ, Spaink HP, Rademaker GJ, Haverkamp J, Thomas-Oates JE (1995) Isolation, chemical structures and biological activity of the lipo-chitin oligosaccharide nodulation signals from *Rhizobium etli*. *Plant Mol Biol* 29(3):453–464
- Chen WM, Laevens S, Lee TM, Coeney T, de Vos P, Mergeay M, Vandamme P (2001) *Ralstonia taiwanensis* sp. nov. isolated from root nodules of Mimosa species and spatum of a cystic fibrosis patient. *Int J Syst Evol Microbiol* 51:1729–1735
- Chen W-M, Mouline L, Bontemps C, Vandamme P, Bena G, Bovine-Masson C (2003) Legume symbiotic nitrogen fixation by β -Proteobacteria is widespread in nature. *J Bacteriol* 185:7266–7272
- Chen W-M, James EK, Chou J-H, Sheu SY, Yang SZ, Sprent JI (2005) β -Rhizobia from *Mimosa pigra*, a newly discovered invasive plant in Taiwan. *New Phytol* 168:661–675
- Chen WM, James EK, Coenye T, Chou J-H, Barrios E, de Faria SM, Elliott GN, Sheu S-Y, Sprent JI, Vandamme P (2006) *Burkholderia mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp. from Taiwan and South America. *Int J Syst Evol Microbiol* 56:1847–1851
- Chen WM, Faria SM, James EK, Elliott GN, Kuan-Yin Lin K-Y, Jui-Hsing Chou J-H, Shih-Yi Sheu S-Y, Cnockaert M, Sprent JI, Vandamme P (2007) *Burkholderia nodusa* sp. no., isolated from root nodules of the woody Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. *J Syst Evol Microbiol* 57:1055–1059
- Clua J, Roda C, Zaneti ME, Blanco FA (2018) Compatibility between legumes and rhizobia for the establishment of a successful nitrogen fixing symbiosis. *Genes* 9:125
- Dakora FD (1998) Nodulation specificity of *Aspalathus linearis* subsp. *linearis*, a shrub tea legume indigenous to the Western Cape. In:

- Elmerich C, Kondorosi A, Newton WE (eds) Biological nitrogen fixation for the 21st century. Kluwer, Dordrecht, pp 671–672
- De Meyer S, Cnockaert M, Ardley JK, Maker J, Yates R, Howieson JG, Vandamme P (2013) *Burkholderia sprentiae* sp. nov., isolated from *Lebeckia ambigua* root nodules. *Int J Syst Evol Microbiol* 63:3950–3957
- De Meyer S, Cnockaert M, Ardley JK, Van Wyk B-E, Vandamme PA, Howieson JG (2014) *Burkholderia dilworthii* sp. nov., isolated from *Lebeckia ambigua* root nodules. *Int J Syst Evol Microbiol* 64:1090–1095
- De Meyer SE, Tian R, Seshadri R, Reddy TBK, Markowitz V, Ivanova N, Pati A, Woyke T, Kyripides N, Ron Yates R, Howieson J, Reeve W (2015) High-quality permanent draft genome sequence of the *Lebeckia ambigua*-nodulating *Burkholderia* sp. strain WSM4176. *Stand Genomic Sci* 10:79
- De Meyer SE, Cnockaert M, Moulin L, Howieson JG, Vandamme P (2018) Symbiotic and non-symbiotic *Paraburkholderia* isolated from South African *Lebeckia ambigua* root nodules and the description of *Paraburkholderia fynbosensis* sp. nov. *Int J Syst Evol Microbiol* 68:2607–2614
- Deng ZS, Zhao FL, Kong ZY, Yang WQ, Lindstrom K, Wang ET, Wei GH (2011) Diversity of endophytic bacteria within nodules of the *Spherophysa salsula* in different regions of Loess Plateau in China. *FEMS Microbiol Ecol* 76:463–475
- Deschodt CC, Strijdom BW (1976) Effective nodulation of *Aspalathus linearis* sub. spp. linearis by rhizobia from other *Aspalathus* species. *Phytophylactica* 8:103–104
- Dobritsa AP, Samadpour M (2016) Transfer of eleven species of the genus *Burkholderia* to the genus *Paraburkholderia* and proposal of *Caballeronia* gen. nov. to accommodate twelve species of the genera *Burkholderia* and *Paraburkholderia*. *Int J Syst Evol Microbiol* 66:2836–2846
- Donate-Correa J, Leon-Barrios M, Hernandez M, Perez-Galdona R, del Arco-Aguilar M (2007) Different *Mesorhizobium* species sharing the same symbiotic gene nodulate the shrub legume *Angaris latifolia*. *Syst Appl Microbiol* 30:615–623
- dos Reis Jr FB, Simon MF, Gross E, Boodey RM, Elliott GN, Netto NE, de Loureiro MF, de Querzo LP, Scotti MR, Chen W-M, Noren A, Rubio MC, de Faria SM, Bontemps C, Goi SR, Young JPW, Sprent JI, James EK (2010) Nodulation and nitrogen fixation by *Mimosa* spp. in the Cerrado and Caatinga biomes of Brazil. *New Phytol* 186:934–946
- Elliott GN, Chen WM, Bontemps C, Chou JH, Young JPW, Sprent JI, James EK (2007) Nodulation of *Cyclopia* spp. (Leguminosae, Papilionoideae) by *Burkholderia tuberum*. *Ann Bot* 100:1403–1411
- Fauvert M, Michiels J (2008) Rhizobial secreted proteins as determinants of host specificity in the rhizobium-legume symbiosis. *FEMS Microbiol Lett* 285:1–9
- Frank B (1889) Über die Pilzsymbiose der Leguminosen. *Ber Dtsch Bot Ges* 7:332–346
- Gage DJ (2004) Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. *Microbiol Mol Biol Rev* 68:280–300
- Garau G, Yates RJ, Deiana P, Howieson JG (2009) Novel strains of *Burkholderia* have a role in nitrogen fixation with papilionoid herbaceous legumes adapted to acid, infertile soils. *Soil Biol Biochem* 41:125–134
- Graham PH (2008) Ecology of the root nodule bacteria of legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Nitrogen fixing legume symbioses. Springer, Dordrecht, pp 23–58
- Gyaneshwar P, Hirsh AM, Moulin L, Chen W-M, Elliott GN, Bontemps C, Estrada-de Los Santos P, Gross E, dos Reis B, Jr F, Sprent JI, Young JPW, James EK (2011) Legume nodulating Betaproteobacteria: diversity, host range, and future prospects. *Mol Plant Microbe Interact* 24:1276–1288
- Hassen AI, Bopape FL, Habig J, Lamprecht SC (2012) Nodulation of rooibos (*Aspalathus linearis* Burm. f), an indigenous South African legume by members of both the α -Proteobacteria and β -Proteobacteria. *Biol Fert Soils* 48:295–303
- Howieson JG, De Meyer SE, Vivas-Marfisi A, Ratnayake S, Ardley JK, Yates RJ (2013) Novel *Burkholderia* bacteria isolated from *Lebeckia ambigua* – A perennial suffrutescent legume of the fynbos. *Soil Biol Biochem* 60:55–64
- Jiao YS, Li YH, Yan H, Wang ET, Tian CF, Chen WX, Guo BL, Chen WF (2015) Rhizobial diversity and nodulation characteristics of the extremely promiscuous legume *Sophora flavescens*. *Mol Plant Microbe Interact* 28:1338–1352
- Kock MM (2003) Diversity of root nodulating bacteria associated with *Cyclopia* species. PhD Thesis, University of Pretoria, Pretoria
- Joubert E, de Beer D (2011) Rooibos (*Aspalathus linearis*) beyond the farm gate: from herbal tea to potential phytopharmaceutical. *S Afr J Botany* 77(4):869–886
- Lamaire B, Dlodlo O, Chimpango S, Stirton C, Schrire B, Boarwright JS, Honny O, Smets E, Sprent J, James EK, Muasya AM (2015) Symbiotic diversity, specificity and distribution of rhizobia in native legumes of the core Cape subregion (South Africa). *FEMS Microbiol Ecol* 91:1–17
- Lammel DR, Cruz LM, Mescolotti D, Sturmer SL, Cardoso EJBN (2015) Woody *Mimosa* species are nodulated by *Burkholderia* in ombrophylous forest soils and their symbiosis are enhanced by arbuscular mycorrhizal fungi (AMF). *Plant Soil* 393:123–135
- Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–175
- Li HJ, Wang FT, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. *Soil Biol Biochem* 40:238–246
- Liu XY, Shuang W, Fang W, James EK, Gua XY, Zagar C, Xia LG, Dong X, Yi PW (2012) *Burkholderia* and *Cupriavidus* spp. are the preferred symbionts of *Mimosa* spp. in Southern China. *FEMS Microbiol Ecol* 80:417–426
- Long SM (2001) Genes and signals in the *Rhizobium*-legume symbiosis. *Plant Physiol* 75:69–72
- Maroti G, Kondorosi E (2014) Nitrogen-fixing *Rhizobium*-legume symbiosis: are polyploidy and host peptide-governed symbiont differentiation general principles of endosymbiosis? *Front Microbiol* 5:326. <https://doi.org/10.3389/fmicb.2014.00326>
- Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001) Nodulation of legumes by members of the β -subclass of Proteobacteria. *Lett Nat* 411:948–950
- Muofhe ML (1997) N₂ fixation and rhizosphere ecology of *Aspalathus linearis* subsp. *linearis* (Rooibos tea). MSc Thesis, Cape University of Peninsula
- Mutch LA, Young JP (2004) Diversity and specificity of *Rhizobium leguminosarum* biovar viciae on wild and cultivated legumes. *Mol Ecol* 13:2435–2444
- Ngom A, Nakagawa Y, Sawada H (2004) A novel symbiotic nitrogen fixing member of the Ochrobactrum clade isolated from root nodules of *Acacia mangium*. *J Gen Appl Microbiol* 50:17–27
- Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume rhizobial symbiosis. *Annu Rev Genet* 45:119–144
- Orgambide GG, Lee JI, Holingsworth RI, Dazo FB (1995) Structurally diverse chitolipooligosaccharide Nod factors accumulate primarily in membranes of wild type *Rhizobium leguminosarum* biovar trifolii. *Biochemistry* 34:3832–3840
- Perret X, Staehelin BWJ, Broughton WJ (2000) Molecular basis of symbiotic specificity. *Microbiol Mol Biol Rev* 64:180–201

- Phalane FL (2008) The diversity of root nodule bacteria associated with *Lebeckia* species in South Africa. MSc Thesis, University of Pretoria
- Platero R, James KE, Rios C, Iriarte A, Sandes L, Zabaleta M, Battistoni F, Fabiano E (2016) Novel Cupriavidus strains isolated from root nodules of native Uruguayan species. *Appl Environ Microbiol* 82:3150–3164
- Pueppke SG, Broughton WJ (1999) *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host range. *Mol Plant Microbe Interact* 12:293–318
- Ramírez MDA, España M, Aguirre C, Kojima K, Ohkama-Ohtsu N, Sekimoto H, Yokoyama T (2019) *Burkholderia* and *Paraburkholderia* are predominant soybean Rhizobial genera in Venezuelan soils in different climatic and topographical regions. *Microbes Environ* 34:43–58
- Richardson AE, Viccars LA, Watson JM, Gibson AH (1995) Differentiation of *Rhizobium* strains using the polymerase chain reaction with random and directed primers. *Soil Biol Biochem* 27:515–524
- Rivas R, Velazquez E, Willems A, Vizcaino M, Subba-Rao NS, Mateos PF, Gills M, Dazzo FB, Martinez Molina EA (2002) New species of *Devosia* that forms a unique nitrogen fixing root nodule symbiosis with the aquatic legume *Neputina natans* (L. f) Druce. *Appl Environ Microbiol* 68:5217–5222
- Roche P, Fabienne M, Claire P, Debele F, Ferro M, Georges T, Prome J-C, Denarie J (1996) The common *nodABC* genes of *Rhizobium meliloti* are host range determinants. *Proc Natl Acad Sci USA* 93:15305–15310
- Spaink HP, Wijfjes AHM, Lugtenberg BJJ (1995) *Rhizobium* NodI and NodJ proteins play a role in the efficiency of secretion of lipochitin oligosaccharides. *J Bacteriol* 177:6276–6281
- Sprent JI, Odee DW, Dakora FD (2009) African legume: a vital but underutilized resource. *J Exp Bot* 25:1–9. <https://doi.org/10.1093/jxb/erp342>
- Sprent JI, Ardley J, James EK (2017) Biogeography of nodulated legumes and their nitrogen fixing symbionts. *New Phytol* 215:40–56
- Staphorst JL, Strijdom BW (1975) Specificity in the *Rhizobium* symbiosis of *Aspalathus linearis* (Burm (f.)) Dahlgr. spp. *linearis*. *Phytophylactica* 7:95–96
- Sy A, Giraud E, Jourand P, Garcia N, Willems A, de Lajudie P, Prim Y, Neyra M, Gills M, Bovin-Dryefus B (2001) Methylophilic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. *J Bacteriol* 183:214–220
- Vandamme P, Coenye T (2004) Taxonomy of the genus *Cupriavidus*: a tale of lost and found. *Int J Syst Evol Microbiol* 54:2285–2289
- Vandamme P, Goris J, Chen W-M, de Vos P, Willems A (2002) *Burkholderia tuberum* sp. nov. and *Burkholderia phymatum* sp. nov. nodulate the roots of tropical legumes. *System Appl Microbiol* 25:507–512
- Velazquez E, Garcia-Fraile P, Ramirez-Bahena MH, Peix A, Rivas R (2010) Proteobacteria forming nitrogen-fixing symbiosis with higher plants. In: Sezena ML (ed) *Proteobacteria: phylogeny, metabolic diversity and ecological effects*. Nova Science, New York
- Wang D, Shengming Y, Fang T, Hongyan Z (2012) Symbiosis specificity in the legume-rhizobial mutualism. *Cell Microbiol* 14:334–342
- Wang Q, Liu J, Zhu H (2018) Genetic and molecular mechanisms underlying symbiotic specificity in legume-rhizobium interactions. *Front Plant Sci* 9:313. <https://doi.org/10.3389/fpls.2018.00313>
- Willems A (2006) The taxonomy of rhizobia: an overview. *Plant Soil* 287:3–14
- Young JPW, Haukka K (1996) Diversity and phylogeny of rhizobia. *New Phytol* 133:87–94
- Zhang JJ, Liu TY, Chen WF, Wang ET, Sui XH, Zhang XX, Li Y, Li Y, Chen WX (2012) *Mesorhizobium muleiense* sp. nov., nodulating with *Cicer arietinum* L. *Int J Syst Evol Microbiol* 62(11):2737–2742

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.