MPMI Vol. 24, No. 11, 2011, pp. 1276–1288. doi:10.1094/MPMI-06-11-0172. © 2011 The American Phytopathological Society

CURRENT REVIEW

Legume-Nodulating Betaproteobacteria: Diversity, Host Range, and Future Prospects

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Submitted 23 June 2011. Accepted 31 July 2011.

Rhizobia form specialized nodules on the roots of legumes (family Fabaceae) and fix nitrogen in exchange for carbon from the host plant. Although the majority of legumes form symbioses with members of genus *Rhizobium* and its relatives in class Alphaproteobacteria, some legumes, such as those in the large genus *Mimosa*, are nodulated predominantly by betaproteobacteria in the genera *Burkholderia* and *Cupriavidus*. The principal centers of diversity of these bacteria are in central Brazil and South Africa. Molecular phylogenetic studies have shown that betaproteobacteria have existed as legume symbionts for approximately 50 million years, and that, although they have a common origin, the symbiosis genes in both subclasses have evolved separately since then. Additionally, some species of genus *Burkholderia*, such as *B. phymatum*, are highly promiscuous, effectively nodulating several important legumes, including common bean (*Phaseolus vulgaris*). In contrast to genus *Burkholderia*, only one species of genus *Cupriavidus* (*C. taiwanensis*) has so far been shown to nodulate legumes. The recent availability of the genome sequences of *C. taiwanensis*, *B. phymatum*, and *B. tuberum* has paved the way for a more detailed analysis of the evolutionary and mechanistic differences between nodulating strains of alpha- and betaproteobacteria. Initial analyses of genome sequences have suggested that plant-associated *Burkholderia* spp. have lower G+C contents than *Burkholderia* spp. that are opportunistic human pathogens, thus supporting previous suggestions that the plant- and human-associated groups of *Burkholderia* actually belong in separate genera.

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History and taxonomy of legume-nodulating bacteria.

The Fabaceae (Leguminosae) family consists of over 19,000 species that are divided among three subfamilies, the Papilionoideae, the Mimosoideae, and the polyphyletic Caesalpinioideae (Lewis et al. 2005). There have been intensive efforts over the last 50 years to determine the full range of legumes that can nodulate, especially in the tropics, where legume diversity is very high (Doyle 2011; Sprent 2001, 2009). It is now known that the vast majority of species (assuming that nodulation is a generic character) in the Papilionoideae (96%) and the Mimosoideae (96%) form nodules, but relatively few of the Caesalpinioideae (22%) that have been examined are nodulated (Sprent 2009; J. I. Sprent *unpublished*). However, until 20 years ago, with the exception of a few crop species, we knew relatively little about the microorganisms that were involved in nodulating the majority of these legumes, with most being classified as types of genus *Rhizobium*, a bacterial genus that had been first described in the nineteenth century by Frank (1889). This situation changed after a revolution in bacterial phylogenetics based on sequences of the conserved small subunit ribosomal 16S rRNA gene (Young and Haukka 1996), which led to the division of rhizobia into the currently accepted genera *Azorhizobium*, *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, and *Ensifer* (syn. *Sinorhizobium*) (Graham 2008; MacLean et al. 2007; Velázquez et al. 2010; Willems 2006), which so far comprise approximately 100 defined species, with many more remaining to be classified to species level (Velázquez et al. 2010).

Interestingly, the legume-nodulating genera are not monophyletic but are widely dispersed within four families in class Alphaproteobacteria, in which they are intermingled among genera not regarded as nodulating, such as *Afipia*, *Brucella*, and *Zoogloea* (Velázquez et al. 2010; Willems 2006; Young and Haukka 1996). Another consequence of the molecular phylogenetical revolution combined with the intensification of interest in native legumes from many parts of the world is an

increasing number of reports of nodulation by bacteria belonging to "nonrhizobial" genera. For example, nodulation has so far been confirmed in *Neptunia natans* by *Devosia* spp. (Rivas et al. 2002), *Lupinus albus* by *Ochrobactrum lupini* (Trujillo et al. 2005), *L. albus* and *Trifolium repens* by *Phyllobacterium trifolii* (Valverde et al. 2005), members of genera *Crotalaria* and *Lotononis* by *Methylobacterium* spp. (Sy et al. 2001; Yates et al. 2007), and members of genus *Lotononis* by *Microvirga* spp. (Ardley et al. in press). This list continues to grow annually, and the reader is encouraged to consult the websites maintained by B. S. Weir (Auckland, New Zealand) and the Center for Genomic Sciences (CCG, Cuernavaca, Mexico) for comprehensive and updated lists. However, although these bacteria appear to be quite "exotic" in rhizobial terms, all of them belong to the class Alphaproteobacteria, and so their ability to harbor nodulation genes is not actually as problematic as it might first appear. Indeed, some, such as *Phyllobacterium trifolii* (Valverde et al. 2005) and *Ochrobactrum lupini* (Trujillo et al. 2005), are actually more closely related to *Rhizobium* spp. than are the "traditional" rhizobial genera *Bradyrhizobium* and *Azorhizobium* (Willems 2006), and given the plasticity of the nodulation trait, which, in genera *Rhizobium* and *Sinorhizobium*, is largely based upon easily transferred plasmid-borne genes (Barnett et al. 2001; Cummings et al. 2009; González et al. 2006; Young et al. 2006) and, in genus *Mesorhizobium*, by relatively mobile "symbiosis islands" in the chromosome (Sullivan and Ronson 1998), it is, thus, not surprising that the known diversity of legume-nodulating strains has increased greatly beyond genus *Rhizobium* and its immediate relatives in the family *Rhizobiaceae* (Velázquez et al. 2010).

The discovery of legume-nodulating betaproteobacteria.

Originally it was doubted that legumes could be nodulated by bacteria in other classes of the proteobacteria, such as the betaproteobacteria (β-rhizobia) or gammaproteobacteria. This was because, although N_2 fixation is common in these classes, the bacteria within them had long been considered to be exclusively free-living or loosely associated with plants, or both, and not as nodulating symbionts. Indeed, in his famous manual, Vincent (1970) cautioned against the possibility of isolating nonrhizobial "contaminants" from nodules (particularly if they were fast-growing). Nodules, with their ample supply of nutrients and their enclosed protective environment, are an attractive niche for a wide variety of nonsymbiotic bacteria that have the capability to colonize plants opportunistically (Sprent 2009). Recent published examples of nonnodulating contaminants (which are more correctly termed endophytes) that have been isolated from nodules include *Agrobacterium* strains (alphaproteobacteria) from various legumes in China (Wang et al. 2006), *Labrys neptuniae* (alphaproteobacteria) from *Neptunia* (Chou et al. 2007), *Herbaspirillum lusitanum* (betaproteobacteria) from *Phaseolus vulgaris* (Valverde et al. 2003), and various *Enterobacter* isolates (gammaproteobacteria) from genus *Hedysarum* (Muresu et al. 2008). In all of these examples, attempts to fulfill Koch's postulates (i.e., to see if they can renodulate their hosts) have failed. Therefore, it is not surprising that reports of symbiotic bacteria in so-called nonrhizobial genera being isolated from nodules were generally greeted with some degree of skepticism. Indeed, when the first claims of legume-nodulating β-rhizobia emerged in 2001 (Chen et al. 2001; Moulin et al. 2001), they were not unconditionally and universally accepted, especially as they were based largely on 16S rRNA sequences (van Berkum et al. 2003). Nevertheless, both studies contained intriguing results suggesting that legume nodulation may not be restricted to alphaproteobacteria. For example, Moulin and associates (2001) presented evidence that two *Burkholderia* strains, STM678 and STM815, which had been respectively isolated from nodules on the papilionoid legumes *Aspalathus carnosa* (South Africa) and *Machaerium lunatum* (French Guiana), possessed nodulation genes (*nodA* in this case) and, thus, might be nodulating symbionts. However, neither strain was tested for symbiosis on its original host, although it was demonstrated that they could form ineffective (i.e., non-N_2-fixing) nodules on the promiscuous host siratro (*Macroptilium atropurpureum*) and that STM678 required common nodulation genes to form these nodules. In parallel to this study, Chen and associates (2001) reported that *Ralstonia taiwanensis* (later renamed *Cupriavidus taiwanensis*), isolated from the nodules of *Mimosa pudica* and *M. diplotricha* in Taiwan, could form nodules on these legumes, but no nodulation data were presented.

Conclusive evidence for nodule formation and effective nitrogen fixation by a betaproteobacterium came from the study by Chen and associates (2003a) in which they used a green fluorescent protein (GFP)-marked strain of *C. taiwanensis* to perform a detailed microscopic analysis of the nodulation process on its *Mimosa* hosts. This study showed that the infection of *Mimosa* spp. was through root hairs, and that the development of classically indeterminate N_2-fixing nodules was essentially the same as that described for many other legumes (Sprent 2009). Effective nodulation by *Burkholderia* was finally demonstrated in two studies published in 2005, in which GFP-marked strains of the Brazilian isolates *B. nodosa* Br3461 and *B. mimosarum* MAP3-5 (Chen et al. 2005a), and the Taiwanese isolate *B. mimosarum* PAS44^T (Chen et al. 2005b), were used to confirm nodulation of various Brazilian native and invasive *Mimosa* spp.

The two *Burkholderia* strains in the study of Moulin and associates (2001), which were subsequently described as two new species, *B. tuberum* STM678^T and *B. phymatum* STM815^T (Vandamme et al. 2002), were also later shown to be effective nodulators of legumes, but surprisingly, not of their original hosts. Elliott and associates (2007a) examined the *nodA* gene sequences of these two strains, and observing that the sequence from *B. phymatum* STM815 was very similar to that of *Mimosa*-nodulating β-rhizobia, such as *B. mimosarum* and *C. taiwanensis*, inoculated *B. phymatum* STM815 onto 30 different *Mimosa* species, and obtained nodules on 29 of them, including 20 that could fix N_2 effectively. Elliott and associates (2007a) thus demonstrated for the first time that *B. phymatum* is, indeed, a symbiotic bacterium, at least with *Mimosa* spp. They failed to nodulate a *Machaerium* species with this strain, so there is no evidence that *B. phymatum* actually nodulates legumes in this genus. *Burkholderia tuberum* STM678 has a very different *nodA* sequence from all the other (all *Mimosa*-) nodulating *Burkholderia* spp. so-far described (Chen et al. 2005a), and thus, it was predicted that it would not nodulate *Mimosa* spp. This was, indeed, found to be the case by Elliott and associates (2007b), who showed that it could, however, nodulate members of the genus *Cyclopia*, which is a papilionoid genus native to South Africa.

The aforementioned studies have shown beyond doubt that betaproteobacteria in the genera *Burkholderia* and *Cupriavidus* can both nodulate legumes and fix N_2 within the nodules to the benefit of their plant hosts. These nodulating betaproteobacteria can thus be considered essentially rhizobial in nature and have been termed β-rhizobia to distinguish them from *Rhizobium* and relatives in the Alphaproteobacteria subgroup, which, accordingly, are termed α-rhizobia. This terminology, which was first used by Moulin and associates (2002), is not universally accepted by all researchers, some of whom prefer 'legume-nodulating bacteria' or 'root-nodulating bacteria' (Lima et al. 2009; Yates et al. 2007), but as they are simple in concept, and now in general use (Graham 2008; Lee and

Hirsch 2006; Sprent 2009), we shall use the terms α- and β-rhizobia for the remainder of this review.

Nodulation of *Mimosa* by β-proteobacteria.

Nodulation by *Burkholderia* and *Cupriavidus* has been further confirmed by several other studies, but it is found most particularly associated with species of *Mimosa*, and all the newly described β-rhizobial species, with the exception of South African strains of *B. tuberum* (Elliott et al. 2007b; Kock 2004), either come from or are capable of nodulating *Mimosa* (Fig. 1; Table 1). For example, *C. taiwanensis* is frequently isolated from nodules on the pan-tropical weeds *M. pudica* and *M. diplotricha* in Taiwan (Chen et al. 2001, 2003b), India (H. S. Gehlot and P. Gyaneshwar *unpublished*; Verma et al. 2004) and China (Liu et al. 2011), and *Burkholderia* spp. such as *B. mimosarum* and *B. phymatum* are also often isolated from these two *Mimosa* species, as well as from a third species, *M. pigra*, which is an extremely aggressive invader in South East Asia (Chen et al. 2005b; Liu et al. 2011) and Australia (Parker et al. 2007), and is rated in the top 10 world's worst weeds (Lowe et al. 2000). With further regard to β-rhizobia from southeast Asia, it is interesting to note that Trinick (1980) isolated many strains of so-called *Rhizobium* from invasive *Mimosa* spp. in Papua New Guinea in the early 1960s, and many of these isolates were later identified as *Burkholderia* and *Cupriavidus* spp. (Elliott et al. 2007a, 2009).

But what nodulates *Mimosa* spp. in its native environments, and what is the nature of the relationship between *Mimosa* spp. and β-rhizobia? Genus *Mimosa* is a large genus of more than 500 species, most of which are native to the New World (Simon et al. 2011). Its principal centers of radiation are in Brazil (about 300 species) and Mexico (about 100 species), where there are many endemic species, particularly in highland regions (Simon and Proença 2000; Simon et al. 2011). There are also several widespread species that are found throughout South and Central America, and these include the three invasive species *M. diplotricha*, *M. pigra*, and *M. pudica*, which are known to be nodulated by β-rhizobia in southeast Asia and Australia. So, are invasive *Mimosa* spp. also nodulated by β-rhizobia in their native ranges? The answer is very much affirmative, as shown by studies in Brazil and Venezuela (Bontemps et al. 2010; Chen et al. 2005a), Panama (Barrett and Parker 2005), Costa Rica (Barrett and Parker 2006), and Texas (Andam et al. 2007). Indeed, molecular analysis based on 16S rRNA, *nifH*, and *nodA* sequences has suggested that β-rhizobial strains isolated from the nodules of plants growing in nonnative environments are very similar, if not identical, to the ones isolated from the native regions, e.g., genetically very similar strains of *B. mimosarum* nodulate *M. pigra* in both Taiwan and South America (Chen et al. 2005a and b), and this also appears to be the case in Australia, where *M. pigra Burkholderia* isolates are also very similar to those from plants in their native range (Parker et al. 2007). This suggests that these invasive species have somehow taken their symbionts with them when they were transported (accidentally or deliberately) from their native environments to nonnative parts of the tropics.

The relationship between β-rhizobia and *Mimosa* spp. was investigated in more depth in a large-scale study reported by Bontemps and associates (2010) and dos Reis and associates (2010) in the Cerrado and Caatinga biomes of central and northeast Brazil, where the genus *Mimosa* has evolved and diversified into more than 200 native and endemic species (Fig. 2A and B). Bontemps and associates (2010) isolated rhizobia from 47 *Mimosa* spp. and all were found to be nodulated by *Burkholderia* spp., with one widespread species, *M. xanthocentra*, also nodulated by *Rhizobium* spp. The phylogenies obtained from the concatenated 16S rDNA and *recA* sequences of the isolates showed that, although they were related to the known legume-nodulating *Burkholderia* species, particularly to *B. nodosa* and *B. tuberum*, they were in seven deep and distinct clades that were sufficiently distant from the established species that they could probably be new species. Indeed, three of these clades (Fig. 1) are currently in the process of being formally described as new species using a polyphasic approach (W.-M. Chen et al. *unpublished*).

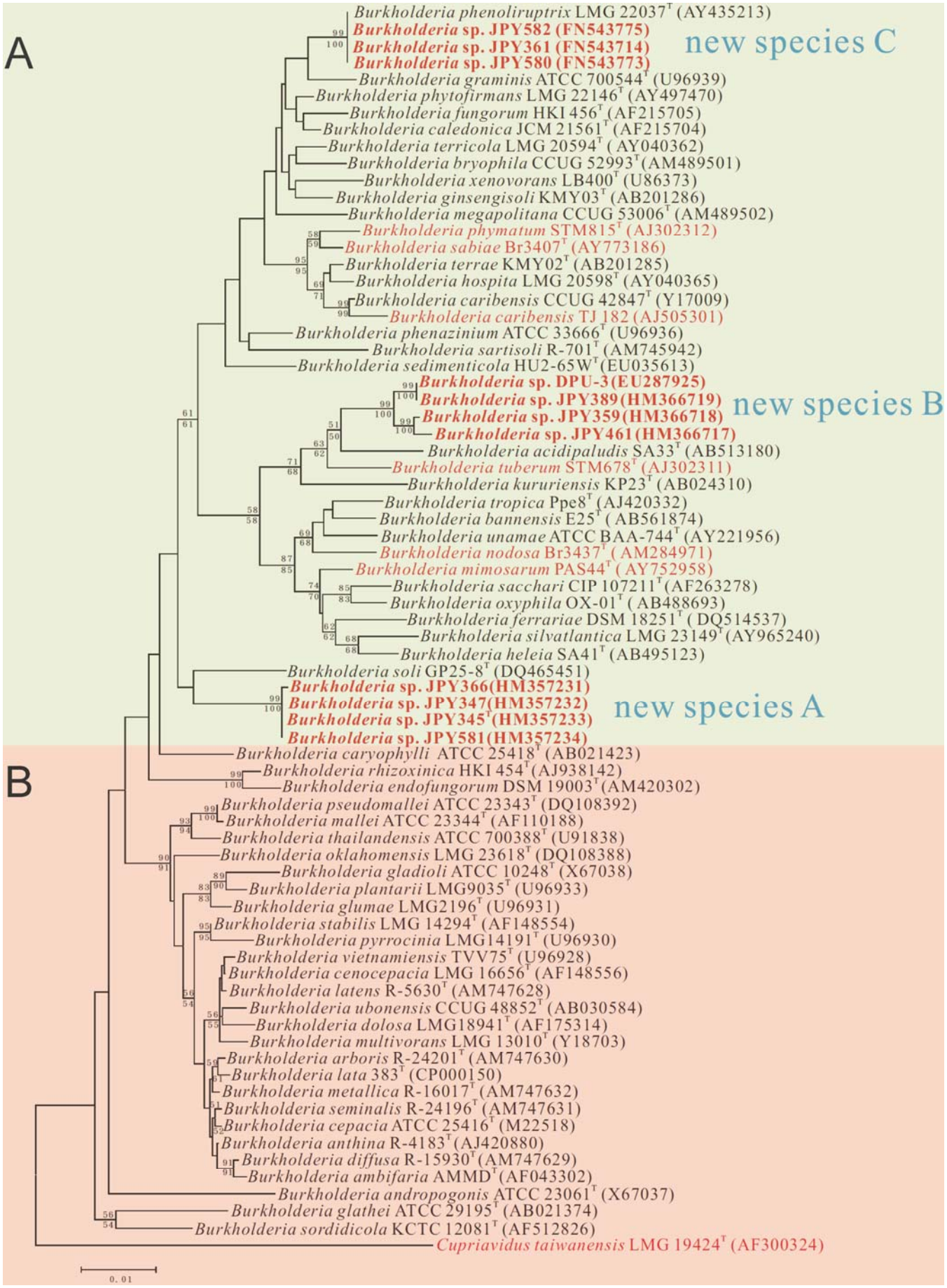
In parallel to the study of Bontemps and associates (2010), dos Reis and associates (2010) performed in situ immunolocalization on the nodules of 67 *Mimosa* species growing in the same biomes, using antibodies specific to *Burkholderia* spp., *C. taiwanensis*, and the *nifH* protein of nitrogenase, and confirmed the overwhelming predominance of *Burkholderia* spp. as symbionts of *Mimosa* spp. in Brazil (Fig. 2C and D). Additionally, dos Reis and associates (2010) showed, using the ^{15}N natural abundance technique, that the *Burkholderia* symbionts were capable of providing at least 60% of the total plant N in *Mimosa* spp. endemic to Brazil.

Conditions favoring nodulation of *Mimosa* spp. by β-rhizobia.

Given how frequently it is isolated from *Mimosa* spp. (both native and nonnative) in other regions, an unexpected finding by both studies was the absence of *C. taiwanensis* in *Mimosa* nodules from central Brazil (Bontemps et al. 2010; dos Reis et al. 2010). This curious observation might be partly explained by the results of inoculation studies that have shown that most Brazilian *Mimosa* spp. appear incapable of being nodulated effectively (if at all) by *C. taiwanensis*, whereas many of the same species are nodulated effectively by the promiscuous *Mimosa*-nodulating *Burkholderia* strain *B. phymatum* STM815 (dos Reis et al. 2010) (Fig. 2E). It would, thus, appear that most *Mimosa* spp. are inherently incapable of being nodulated by *C. taiwanensis* and that this bacterium is largely confined to nodulating either a few, widespread and invasive *Mimosa* spp. at the edges of the native range of the genus (Andam et al. 2007), invasive *Mimosa* spp. outside their native range (Chen et al. 2001, 2003a and b, 2005b; Elliott et al. 2007a, 2009), or both.

Although most *Mimosa* spp. are nodulated by *Burkholderia* spp. and some by *C. taiwanensis*, some of them can also form effective symbioses with α-rhizobia (Chen et al. 2003b; Elliott et al. 2009; Wang et al. 1999), which suggests the existence of genetic and physiological factors that determine their apparent preference for nodulation by β-rhizobia. To determine the conditions that favor nodulation by β-rhizobia, Elliott and associ-

Fig. 1. 16S rRNA gene phylogeny of all known *Burkholderia* spp. (betaproteobacteria species marked in red), including three proposed new *Burkholderia* species (in bold red) consisting of strains isolated from *Mimosa* nodules collected in central and northeast Brazil by Bontemps and associates (2010). Neighbor-joining bootstrap percentages are given above nodes and those for maximum likelihood below the nodes. The genus can be divided into two groups, group A (in the green box), which contains most of the plant-associated species and all of the currently known legume-nodulating species, and group B (in the pink box), which contains all the phytopathogenic species as well as the species that may cause disease in humans and animals. P. Estrada-de los Santos, P. Vinuesa, L. Martinez-Aguilar, A.M., Hirsch, and J. Caballero-Mellado (*unpublished*) propose that group A should be moved to the new genus *Caballeronia.*


A
B
new species C
new species B
new species A
Burkholderia phenoliruptrix LMG 22037T (AY435213)
Burkholderia sp. JPY582 (FN543775)
Burkholderia sp. JPY361 (FN543714)
Burkholderia sp. JPY580 (FN543773)
Burkholderia graminis ATCC 700544T (U96939)
Burkholderia phytofirmans LMG 22146T (AY497470)
Burkholderia fungorum HKI 456T (AF215705)
Burkholderia caledonica JCM 21561T (AF215704)
Burkholderia terricola LMG 20594T (AY040362)
Burkholderia bryophila CCUG 52993T (AM489501)
Burkholderia xenovorans LB400T (U86373)
Burkholderia ginsengisoli KMY03T (AB201286)
Burkholderia megapolitana CCUG 53006T (AM489502)
Burkholderia phymatum STM815T (AJ302312)
Burkholderia sabiae Br3407T (AY773186)
Burkholderia terrae KMY02T (AB201285)
Burkholderia hospita LMG 20598T (AY040365)
Burkholderia caribensis CCUG 42847T (Y17009)
Burkholderia caribensis TJ 182 (AJ505301)
Burkholderia phenazinium ATCC 33666T (U96936)
Burkholderia sartisoli R-701T (AM745942)
Burkholderia sedimenticola HU2-65WT (EU035613)
Burkholderia sp. DPU-3 (EU287925)
Burkholderia sp. JPY389 (HM366719)
Burkholderia sp. JPY359 (HM366718)
Burkholderia sp. JPY461 (HM366717)
Burkholderia acidipaludis SA33T (AB513180)
Burkholderia tuberum STM678T (AJ302311)
Burkholderia kururiensis KP23T (AB024310)
Burkholderia tropica Ppe8T (AJ420332)
Burkholderia bannensis E25T (AB561874)
Burkholderia unamae ATCC BAA-744T (AY221956)
Burkholderia nodosa Br3437T (AM284971)
Burkholderia mimosarum PAS44T (AY752958)
Burkholderia sacchari CIP 107211T (AF263278)
Burkholderia oxyphila OX-01T (AB488693)
Burkholderia ferrariae DSM 18251T (DQ514537)
Burkholderia silvatlantica LMG 23149T (AY965240)
Burkholderia heleia SA41T (AB495123)
Burkholderia soli GP25-8T (DQ465451)
Burkholderia sp. JPY366 (HM357231)
Burkholderia sp. JPY347 (HM357232)
Burkholderia sp. JPY345T (HM357233)
Burkholderia sp. JPY581 (HM357234)
Burkholderia caryophylli ATCC 25418T (AB021423)
Burkholderia rhizoxinica HKI 454T (AJ938142)
Burkholderia endofungorum DSM 19003T (AM420302)
Burkholderia pseudomallei ATCC 23343T (DQ108392)
Burkholderia mallei ATCC 23344T (AF110188)
Burkholderia thailandensis ATCC 700388T (U91838)
Burkholderia oklahomensis LMG 23618T (DQ108388)
Burkholderia gladioli ATCC 10248T (X67038)
Burkholderia plantarii LMG9035T (U96933)
Burkholderia glumae LMG2196T (U96931)
Burkholderia stabilis LMG 14294T (AF148554)
Burkholderia pyrrocinia LMG14191T (U96930)
Burkholderia vietnamiensis TVV75T (U96928)
Burkholderia cenocepacia LMG 16656T (AF148556)
Burkholderia latens R-5630T (AM747628)
Burkholderia ubonensis CCUG 48852T (AB030584)
Burkholderia dolosa LMG18941T (AF175314)
Burkholderia multivorans LMG 13010T (Y18703)
Burkholderia arboris R-24201T (AM747630)
Burkholderia lata 383T (CP000150)
Burkholderia metallica R-16017T (AM747632)
Burkholderia seminalis R-24196T (AM747631)
Burkholderia cepacia ATCC 25416T (M22518)
Burkholderia anthina R-4183T (AJ420880)
Burkholderia diffusa R-15930T (AM747629)
Burkholderia ambifaria AMMDT (AF043302)
Burkholderia andropogonis ATCC 23061T (X67037)
Burkholderia glathei ATCC 29195T (AB021374)
Burkholderia sordidicola KCTC 12081T (AF512826)
Cupriavidus taiwanensis LMG 19424T (AF300324)
0. 01

ates (2009) set up paired competition experiments between defined strains of *Burkholderia*, *C. taiwanensis*, and *Rhizobium* spp. and showed that the *Burkholderia* strains (*B. mimosarum* PAS44, *B. phymatum* STM815) outcompeted both *C. taiwanensis* LMG19424 and all the *Mimosa*-nodulating α-rhizobial strains (*R. etli* TJ167, *R. tropici* NGR181, and *R. tropici* UPRM8021) for nodulation of three invasive *Mimosa* species (*M. diplotricha*, *M. pigra*, *M. pudica*) in all the conditions tested. However, the competitive domination of *B. mimosarum* over *C. taiwanensis* was reduced in the presence of nitrate for the three plant hosts, with the largest significant effect on *M. pudica*, in which *C. taiwanensis* formed 57% of the nodules in the presence of 0.5 mM KNO_3. Further study of Brazilian legumes suggests that physical environment, rather than the host species, largely determines the distribution of *Burkholderia* species (Bontemps et al 2010; dos Reis et al. 2010). These data indicate that, in acidic soils containing very low levels of inorganic nitrogen, *Burkholderia* spp. will most likely be the preferred symbionts of endemic *Mimosa* spp. (and possibly other legumes that have evolved to live in low-N, acidic soils [Garau et al. 2009]).

Host range of β-rhizobia.

Although published data at the time of writing this review indicate that nodulation by *C. taiwanensis* is confined to the genus *Mimosa*, *Burkholderia* spp. appear to be able to nodulate a much wider range of legumes. This was indicated by Barrett and Parker (2005), who, in addition to isolating several *Burkholderia* strains from *Mimosa* spp. in Panama, also isolated strains from the mimosoid legume *Abarema macademia* (tribe Ingaeae). However, although these strains were capable of nodulating *Mimosa* spp., they were not tested on their original host, owing to lack of available seeds. More direct evidence comes from host-range studies with *B. phymatum* STM815, a strain that nodulates more than 40 *Mimosa* species from various parts of the world (dos Reis et al. 2010; Elliott et al. 2007a). These studies have shown this strain to be highly promiscuous outside the genus *Mimosa*, nodulating several legumes in the subfamily Mimosoideae (E. Gross, C. Bontemps, and E. K. James *unpublished*). *Burkholderia phymatum* STM815 has a particular affinity for species in the tribe Mimoseae, such as those in the "sister" group to *Mimosa*, *Piptadenia* (*sensu stricto*), and both of the other two clades in the polyphyletic genus *Piptadenia* (Jobson and Luckow 2007) (Fig. 2F and G). It also nodulates some species of genera *Anadenanthera*, *Leucaena*, and *Prosopis* (all in tribe Mimoseae) as well as several *Acacia* spp., but only those in the subgenus *Acacia*, which is phylogenetically close to the Mimoseae (Fig. 2H). A clue to the ability of *B. phymatum* STM815 to nodulate legumes in this tribe lies in its nodulation genes, as *Burkholderia* strains isolated from *Piptadenia* spp. in Brazil have very similar *nodC* sequences to *B. phymatum* and they are capable of nodulating invasive *Mimosa* spp. (e.g., *M. pudica*) (E. Gross, C. Bontemps, and E. K. James *unpublished*). It, thus, appears likely that there has been some coevolution between the plants in tribe Mimoseae and their *Burkholderia* symbionts, at least in a Brazilian context, and that this coevolution is not confined to the genus *Mimosa* (Bontemps et al. 2010).

Recent studies have extended the nodulation of legumes by *Burkholderia* spp. to the subfamily Papilionoideae, but there are distinct preferences in terms of the host range of Papilionoideae-nodulating *Burkholderia* spp., and these are based upon the nodulation genes. For example, the *B. tuberum* STM678 *nodA* gene sequence is distant from those of the *Mimosa*-nodulating *Burkholderia* spp. (Chen et al. 2003b, 2005a), and it does not nodulate *Mimosa* spp. or other members of the Mimosoideae (Elliott et al. 2007b). Interestingly, as well as *Cyclopia* (Elliott et al. 2007b; Kock, 2004), *B. tuberum* nodulates other South African genera in the same tribe, the Podalyrieae, such as *Podalyria* and *Virgilia* (Fig. 2I; E. K. James, J. I. Sprent, and W.-M. Chen *unpublished*). However, *B. tuberum* has not so far been demonstrated to nodulate species in the genus *Aspalathus* (tribe Crotalarieae) (Elliott et al. 2007b), which, although not closely related to the Podalyrieae, grow in the same acidic soils of the South African Cape Fynbos biome, and thus will be exposed to the same nodulating microflora, including burkholderias. *Burkholderia tuberum* STM678 also effectively nodulates siratro (Elliott et al. 2007b) and *Phaseolus vulgaris* (A. Angus and A. M. Hirsch *unpublished*), which are in the tribe Phaseoleae. Garau and associates (2009), in their study of *Rhynchosia ferufolia*, also in the tribe Phaseoleae but native to the acidic soils of the South African Cape, have shown that this plant is nodulated by *Burkholderia* strains with *nodA* genes similar to that of *B. tuberum* STM678. Indeed, the South African Cape Fynbos biome would appear to be a major reservoir of Papilionoideae-nodulating burkhold-

Table 1. Species of β-proteobacteria that have been confirmed to nodulate and fix N_2 in symbiosis with legumes

Species	Original host	Host-range	Geographical location	References
Burkholderia caribensis	*M. pudica*, *M. diplotricha*	*M. pudica*, *M. diplotricha*	Taiwan	Chen et al. 2003a; G. N. Elliott (*unpublished*)
B. sabiae	*M. caesalpiniifolia*	*M. caesalpiniifolia*, *M. pudica*	Brazil	Chen et al. 2005a, 2008
B. mimosarum	*M. pigra*, *M.pudica*	*M.. pigra*, *M pudica*	Taiwan, Brazil, Venezuela, China	Chen et al. 2005a and b, 2006; Liu et al. 2010
B. nodosa	*M. bimucronata*, *M. scabrella*	*M. bimucronata*, *M. scabrella*, *M pudica*	Brazil	Chen et al. 2005a, 2007
B. phymatum	Not known[a]	*Mimosa* spp., and other members of the tribe Mimoseae, *Phaseolus vulgaris*.	French Guiana, Papua New Guinea, Morocco, China	Trinick 1980; Moulin et al. 2001; Elliott et al 2007a; Talbi et al. 2010; Liu et al. 2011; E. Gross, C. Bontemps, and E. K. James, (*unpublished*)
B. tuberum	*Aspalathus carnosa* (Crotalarieae, Papilionoideae) but not yet shown to nodulate it; *Cyclopia* (Podalyrieae, Papilionoideae).	*Cyclopia*, and other members of the tribe Podalyrieae, *Macroptilium atropurpureum*, *Phaseolus vulgaris*	South Africa	Moulin et al. 2001; Elliott et al. 2007b; E. K. James (*unpublished*)
C. taiwanensis	*M. pudica*, *M. diplotricha*, *M. pigra*	*Mimosa* spp.	Taiwan, India, Panama, Costa Rica	Chen et al. 2003a and b, 2005b; Verma et al. 2004; Barrett and Parker 2005, 2006; Elliott et al. 2007a, 2009

[a] Reported to be isolated from the nodules of *Machaerium lunatum* but not confirmed to nodulate this legume (Moulin et al. 2001; Elliott et al. 2007a).

erias, as another native legume, *Lebeckia* (Crotalarieae), is also nodulated by *Burkholderia* spp. (Ardley et al. in press).

It would thus appear that there are at least two distinct groups of legume-nodulating burkholderias defined and separated neither by their core genomes nor by their *nif* genes (which are closely related [Bontemps et al. 2010]) but by geography and by their nodulation genes. These are the mimosoid-nodulating burkholderias centered on South America (e.g., *B. mimosarum*, *B. nodosa*, *B. phymatum*, *B. sabiae*) and the papilionoid-nodulating burkholderias centered on South Africa (e.g., *B. tuberum* STM678). Interestingly, however, although the papilionoid-nodulating *B. tuberum*-type symbionts appear incapable of nodulating mimosoids (Elliott et al. 2007b; Garau et al. 2009), the opposite is not the case. For example, Martinez-Romero (2009) has described partially effective nodulation of common bean (*Phaseolus vulgaris*) by the highly promiscuous strain, *B. phymatum* STM815. In addition, other *B. phymatum* strains have recently been isolated from nodules on common bean in Morocco by Talbi and associates (2010). These strains have a *nodC* gene almost identical to that of *B. phymatum* strains STM815 and NGR195A and, besides nodulating *Mimosa* spp., they are also apparently capable of fully effective nodulation of common bean.

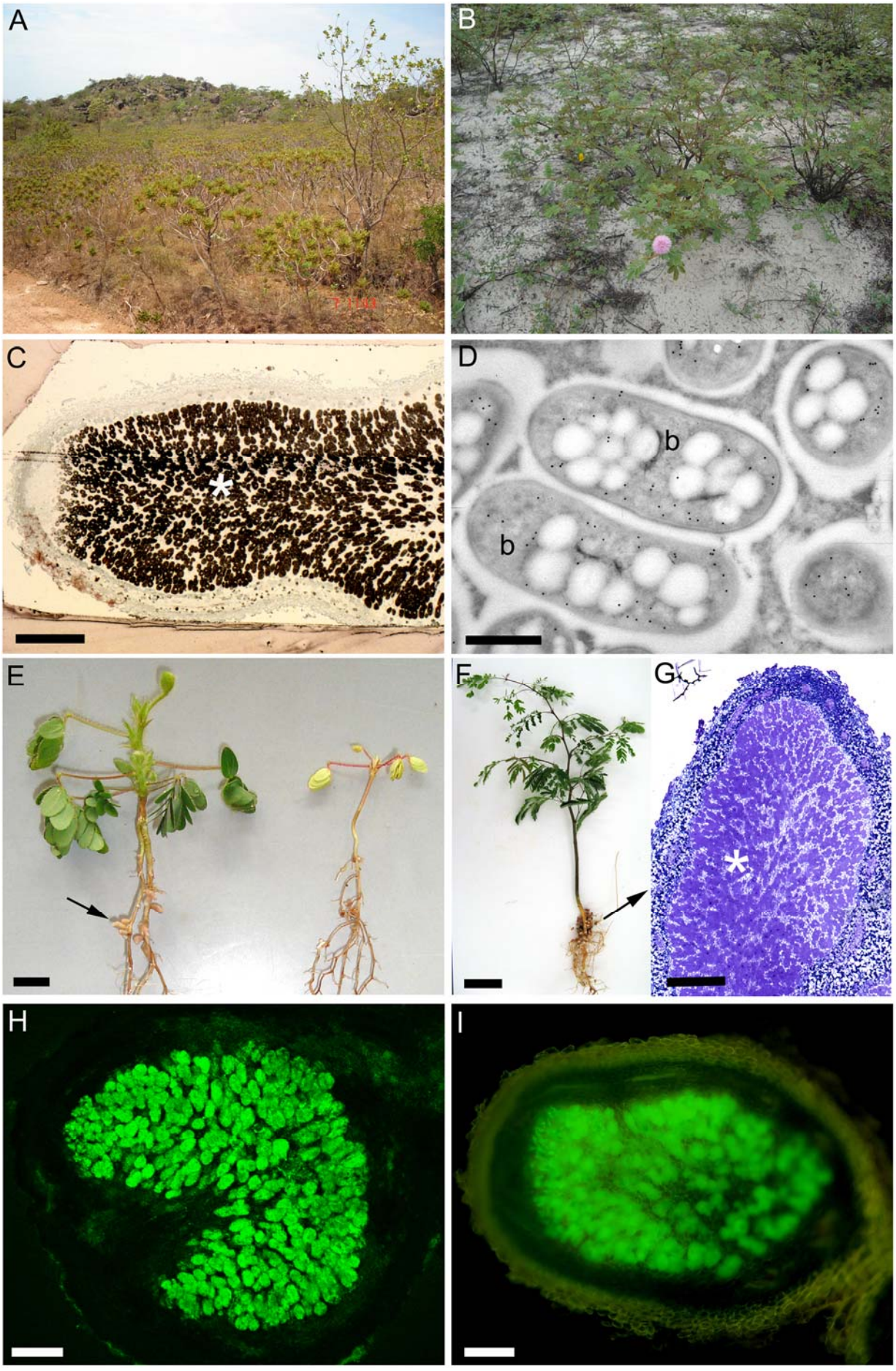
Data emerging from Australia suggest that it may house a third center for β-rhizobia, because two *Burkholderia* strains (WSM2230 and WSM2232) have been demonstrated to nodulate and fix nitrogen with the Australian native papilionoid legumes *Kennedia coccinea* (Phaseoleae) and *Gastrolobium capitatum* (Mirbelieae). Strains WSM2230 and WSM2232 were isolated from *K. coccinea* and *G. capitatum* trap plants grown in soil taken from Karijini National Park in western Australia, which has a semiarid climate and nutrient-poor soils. However, these soils differ from the South African Fynbos and Brazilian Cerrado soils in that they are alkaline (pH 8.0). Initial phylogenetic analyses suggest that the *Burkholderia* strains isolated from indigenous Australian legumes form a group separate from the *Burkholderia* strains that are microsymbionts of the invasive *M. pigra* in Australia (Ardley et al. in press).

Evolution of symbiosis in β-proteobacteria.

The recent availability of whole-genome sequences of both α- and β-rhizobia can provide a better understanding of the mechanisms and evolution of nodulation and nitrogen fixation by members of genera *Burkholderia* and *Cupriavidus*. Analysis of the DNA sequences of genes essential for nitrogen fixation (*nifH*) has shown that *nif* genes of the nodulating strains are closely related to *nifH* of nonnodulating *Burkholderia* strains and show some homology to *nifH* genes from genera *Bradyrhizobium* and *Azorhizobium* but are only distantly related to other alphaproteobacterial rhizobial strains (Bontemps et al. 2010; Chen et al. 2003b, 2005a and b). Indeed, genera *Bradyrhizobium* and *Azorhizobium* are postulated to have acquired *nif* genes from betaproteobacteria (Young 2005). It has been suggested that symbiotic *Burkholderia* strains may have contained *nif* genes before acquiring the essential nodulation genes (Bontemps et al. 2010). Unlike most α-rhizobial strains, which do not exhibit free-living diazotrophy, both *B. phymatum* STM815 and *B. tuberum* STM678 have significant nitrogenase (acetylene reduction) activity in free-living conditions (Elliott et al. 2007a; Wong-Villarreal and Caballero-Mellado 2010), but this activity is dependent upon the presence of some fixed nitrogen in the growth medium (e.g., yeast extract) and is significantly lower than acetylene reduction by nonsymbiotic *Burkholderia* strains (Elliott et al. 2007a). In contrast to *B. phymatum* and *B. tuberum*, *C. taiwanensis* strains did not reduce acetylene in free-living conditions (Elliott et al. 2007a), although Verma and associates (2004) have induced expression of the *nifH* protein of nitrogenase by *C. taiwanensis* growing in a semisolid N-free medium that is usually used to detect diazotrophic *Azospirillum* strains. These results indicate physiological differences for the regulation of nitrogen fixation between nonsymbiotic and symbiotic betaproteobacteria, as well as between β- and α-rhizobia, but the mechanisms underlying these differences remain to be determined.

In addition to *nif* genes, all legume-nodulating bacteria, with the exception of some photosynthetic stem-nodulating bradyrhizobia (Giraud et al. 2007), contain nodulation (*nod*) genes involved in the synthesis and transport of Nod factors. These are signaling molecules that are recognized by the plant hosts, and they confer various degrees of specificity in terms of which host the bacterium can nodulate (Sprent 2009). The *nod* genes are sufficiently conserved to use for determining the evolution of rhizobial-legume interactions and nodulation. Recent analysis using *nodC* sequences of 143 *Burkholderia* strains isolated from 47 native *Mimosa* species in central Brazil showed a monophyletic origin, suggesting a single acquisition of these genes (Bontemps et al. 2010). The almost complete congruence of the *nodC* sequence tree with those of other phylogenetic markers (16S rRNA, *recA*, and *nifH*) suggests that they have evolved together with little horizontal gene transfer. Additionally, the branch depth of *nodC* groups in genus *Burkholderia* was found to be similar to that observed in α-rhizobial groups. These data indicate that nodulation in *Burkholderia* spp. is an ancient and stable ecological trait, with a possible age of at least 50 million years (Angus and Hirsch 2010; Bontemps et al. 2010). Legumes evolved about 60 million years ago, and nodulation in some groups shortly after that (Doyle 2011; Sprent 2009). The tribes and genera that are nodulated by *Burkholderia* spp. are more recent, for example, *Mimosa* first appeared 28 million years ago (Simon et al. 2011).

So far, with the exception of the *Rhynchosia*-nodulating strains from the South African Cape Fynbos environment (Garau et al. 2009), no β-rhizobia have been reported to have *nod* gene sequences closely related to those of *B. tuberum* STM678, and this most likely explains its very different host range compared with the *Mimosa*-nodulating strains (discussed above). The possible origins of the *nod* genes in *B. tuberum* STM678 are hinted at by Kock (2004), who described more than 120 authenticated nodulating rhizobial strains isolated from 14 *Cyclopia* spp. The vast majority of these, according to their 16S rRNA sequences, were *B. tuberum* and related *Burkholderia* strains. Just as Bontemps and associates (2010) found with Brazilian *Mimosa*-nodulating *Burkholderia* symbionts, a very strong relationship is apparent between the genus *Cyclopia* and *B. tuberum* STM678-like strains, and a long history of coevolution between them is suggested. Interestingly, a few of the strains nodulating *Cyclopia* spp. were *Bradyrhizobium* spp. or *R. tropici*, and these had very similar *nodA* sequences to the *B. tuberum* strains, so it is highly likely that there had been horizontal gene transfer between the α- and β-rhizobia (Kock 2004). Although the direction of this gene transfer is not clear, it seems most likely that *Burkholderia* was the donor, given its preponderance as a *Cyclopia* symbiont. Interestingly, this situation is very different from the *Mimosa* symbiosis, in which the nodulation genes of α-rhizobia are not closely related to those of β-rhizobia and the host specificity must have evolved independently (Bontemps et al. 2010). Certainly the fascinating data of Kock (2004) are worthy of much further analysis, because they hint that the Cape region of South Africa and the acidic soils of the Fynbos biome, in particular (discussed above), might be another center of β-rhizobial evolution and diversity to rival that of the Cerrado and Caatinga biomes of Brazil (Bontemps et al. 2010).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Genomic analysis of nodulation and nitrogen fixation by β-rhizobia.

The complete genome sequence of *C. taiwanensis* has been published (Amadou et al. 2008) and consists of a chromosome of 3.4 Mb, a chromid of 2.5 Mb (Harrison et al. 2010), and a large symbiosis plasmid of 0.56 Mb (pRalta). The genes required for nodulation and nitrogen fixation are present on a symbiosis island of only 38 kb, which is the most compact known so far for any rhizobial species. *Cupriavidus taiwanensis* has one copy of *nodD* and makes a Nod factor that is sulfated at its reducing end and acetylated at its nonreducing end. Interestingly, cytochrome cbb3, which has a high affinity for oxygen and is critical for nitrogen fixation in the nodules, is localized in the chromosome, in contrast to its presence in the symbiosis plasmids in α-rhizobia. In addition, the genome lacks homologs of the α-rhizobial FixLJ two-component regulatory system that is required for nitrogen fixation in the nodules. This indicates that, in *C. taiwanensis*, the expression of genes required for efficient nitrogen fixation (*nif* and *fix*) might be regulated in a different way from the α-rhizobia, and this needs to be explored further.

Like that of many *Burkholderia* species (Harrison et al. 2010), the genome of *B. phymatum* STM815 (National Center for Biotechnology Information Bioproject website) comprises a chromosome (3.5 Mb), two chromids ('chromosome 2', 2.7 Mb and pBPHY01, 1.9 Mb), and a plasmid (0.59 Mb). The genome is in the process of being published together with its symbiotic transcriptome (L. Moulin *unpublished*). The organization of the *nod* and *nif* gene operons in the pSym of *C. taiwanensis* LMG19424 and *B. phymatum* STM815 are very similar, indicating that these β-rhizobia have acquired the symbiotic functions either from each other or from a common donor. It is interesting that, even though both *C. taiwanensis* and *B. phymatum* contain the same *nod* genes and, thus, probably make similar Nod factors, *B. phymatum* forms an effective symbiosis with many mimosoid legumes, whereas *C. taiwanensis* forms mostly ineffective symbioses with them (Fig. 2E; discussed above; Elliott et al. 2007a). This suggests that the broad host range of *B. phymatum* might involve genes other than those on the symbiosis plasmid and that other components besides Nod factors are needed for effective nodulation. It is also possible that *C. taiwanensis* contains protein secretion systems that are recognized as pathogenic by the legume hosts. Deletion of the type III secretion system in a *Ralstonia solanacearum* strain containing the *C. taiwanensis* symbiosis plasmid (pRalta) led to nodule formation on *M. pudica* (Marchetti et al. 2010).

The genome of *B. tuberum* STM678 has been sequenced and is still in the process of being annotated (A. M. Hirsch *unpublished*). With regard to nitrogenase genes, *B. tuberum* STM678 exhibits similar gene organization to that of other nitrogen-fixing *Burkholderia* species, including *B. unamae* MTI641, *B. xenovorans* LB400 (Chain et al. 2006), and *B. vietnamiensis* G4. However, unlike these but similar to *B. phymatum* STM815, *nifA* and *nifB* are not next to one other but, rather, are separated by a large number of genes. Also, in *B. phymatum* STM815, the *nifEN* genes are located at some distance from *nifHDK*, whereas, for *B. tuberum* STM678, the *nifHDK* and *nifEN* genes show a similar organization to the comparable genes in other nitrogen-fixing strains (A. M. Hirsch *unpublished*). Although the *nif* genes are similar to other nodulating *Burkholderia* spp., such as *B. phymatum* STM815, the *nod* genes of *B. tuberum* STM678 are very different from those of the other β-rhizobia. This has already been shown for *nodA* and *nodC* (Bontemps et al. 2010; Chen et al. 2003b, 2005a and b; Kock 2004), and the genome has provided deeper insight into these differences. For example, a second *nodC* gene exists in the *B. tuberum* genome, but it is a partial copy, as previously reported by Moulin and associates (2001). In terms of Nod factors, it is likely that those purified by Boone and associates (1999) from one of three "*Bradyrhizobium aspalati*" strains were actually from the *Cyclopia*-nodulating strain *B. tuberum* STM678. Elliott and associates (2007b) provides a detailed history of the relationship between *Aspalathus* and *Cyclopia*-nodulating rhizobia. Analysis of the *nod* genes in the genome of *B. tuberum* STM678 shows that these match the Nod factor structure reported by Boone and associates (1999), who identified two major Nod factors, each with a backbone consisting of three to five glucosamine residues, which is typical of the Nod factors of α-rhizobial species. However, the *B. aspalati* Nod factors are substituted on the nonreducing end sugar with an *N*-methyl and two carbamoyl groups but have no substitutions at the reducing end. Nod factors of α-rhizobia typically have substitutions on the reducing end, making *B. aspalati* Nod factors significantly different.

Numerous β-rhizobial genome-sequencing projects are currently under way, as listed in Table 2. Future analyses of these genomes in comparison with those of α-rhizobia should shed light on the adaptations that β-rhizobia possess that predisposes them to form symbioses with particular legumes in certain environments.

Relationship between plant-associated *Burkholderia* and human pathogens.

Apart from the nodulating symbiotic strains, many species of genus *Burkholderia* have been described that associate with plants such as maize, tomato, rice, sorghum, sugarcane, pineapple, and coffee (Caballero-Mellado et al. 2004; Castro-González et al. 2011; Estrada de los Santos et al. 2001; Gillis et al. 1995; Martinez-Aguilar et al. 2008; Perin et al. 2006; Reis et al. 2004), and several of these fix nitrogen and increase plant growth. On the other hand, a number of other *Burkholderia* species are phytopathogens, such as *B. caryophylli*, *B. gladioli*, and *B. glumae*. These are related to *B. cepacia*, which was originally isolated from rotting onions, as the name '*cepacia*' implies (Burkholder 1950). *Burkholderia cepacia* and some close relatives, the *B. cepacia* complex

Fig. 2. Some legumes that are commonly nodulated by betaproteobacteria (β-rhizobia). **A,** *Mimosa setosissima*, a species endemic to the Pirenopolis municipality in the Cerrado biome in central Brazil. The Cerrado is a tropical savannah characterized by highly seasonal rainfall (500 to 1,000 mm per annum and very acidic soils (pH 3 to 5) with low fertility and is regularly subjected to burning during the dry season. **B,** *Mimosa cordistipula*, a Caatinga endemic, growing in sand close to the Rio São Francisco in northeast Brazil. The Caatinga is a xeric shrub land with a climate classed as semiarid (<500 mm per annum); it has Fe-rich soils, many of which are as acidic as those in the Cerrado. **C,** Light micrograph of a *Mimosa velloziana* nodule from the Brazilian Cerrado that has been immunogold-labeled with an antibody against *Burkholderia phymatum* (the N_2-fixing *Burkholderia*-infected zone in the nodule section is marked by an asterisk). **D,** Transmission electron micrograph of bacteroids (b) from a *Mimosa caesalpiniifolia* nodule collected in central Brazil that has been immunogold-labeled with an antibody against nitrogenase. **E,** *Mimosa ursina* nodulated by *B. phymatum* STM815 (left, nodules indicated by an arrow) and *C. taiwanensis* LMG19424 (right). **F,** *Piptadenia viridiflora* (Mimosoideae, tribe Mimoseae) nodulated by *B. phymatum* STM815. **G,** The inset shows a section through the nodules marked with an arrow in F. An asterisk marks the N_2-fixing infected zone in the nodule section. **H,** Section through a nodule on *Acacia pennatula* (Mimosoideae, tribe Mimoseae) infected with *B. phymatum* STM815GFP. **I,** Section through a nodule on *Podalyria canescens* (Papilionoideae, tribe Podalyrieae) infected with *B. tuberum* STM678-GFP. Scale bars: C and G = 200 μm; D = 500 nm; E = 1 cm, F = 2 cm; H and I = 100 μm.

Table 2. Beta-rhizobia genome sequencing projects (as of June 22, 2011).

Taxonomy	Strain name	Plant host	Country of origin	Sequencing center[a]	GOLD identity[b]
Finished					
Burkholderia phymatum	STM815	*Machaerium lunatum*[c]	French Guiana	JGI	Gc00775
Cupriavidus taiwanensis	LMG19424	*Mimosa pudica*	Taiwan	Genoscope	Gc00754
Current					
Burkholderia tuberum	STM678	*Aspalathus carnosa*[c]	South Africa	Washington University	Gi07505
Burkholderia tuberum	STM3649	*Mimosa pudica*	French Guiana	Genoscope, France	Gi09600
Burkholderia mimosarum	LMG23256T	*Mimosa pigra*	Taiwan	GEBA-RNB, JGI	Gi08823
	STM3621	*Mimosa pudica*	French Guiana	GEBA-RNB, JGI,	Gi08839
Burkholderia sp.	WSM3937	*Rhynchosia ferulifolia*	South Africa	GEBA-RNB, JGI	Gi08878
	JPY580	*Mimosa cordistipula*	Brazil, Bahia	GEBA-RNB, JGI	Gi08877
	CCGE1002	*Mimosa occidentalis*	Mexico, Tepic	DOE-JGI	Gc01354
	JPY366	*Mimosa misera*	Brazil, Bahia	GEBA-RNB, JGI	Gi08876
	JPY347	*Mimosa cordistipula*	Brazil, Bahia	GEBA-RNB, JGI	Gi08875
	JPY251	*Mimosa velloziana*	Brazil	GEBA-RNB, JGI	Gi08874
	WSM4176	*Lebeckia ambigua*	South Africa,	GEBA-RNB, JGI	Gi08873
	WSM3556	*Lebeckia ambigua*	South Africa	GEBA-RNB, JGI	Gi08872
	Mcas7.1	*Mimosa casta*	Panama	GEBA-RNB, JGI	Gi08846
	MP20	*Mimosa pudica*	India, Bokaro	GEBA-RNB, JGI	Gi08833
	WSM2232	*Nemcia capitata*	Australia	GEBA-RNB, JGI	Gi08832
	WSM2230	*Kennedia coccinea*	Australia	GEBA-RNB, JGI	Gi08831
	4.13	*Parapiptadenia rigida*	Uruguay	GEBA-RNB, JGI	Gi08829
Cupriavidus taiwanensis	STM6018	*Mimosa pudica*	French Guiana	GEBA-RNB, JGI	Gi08840
	STM6070	*Mimosa pudica*	N.Caledonia	GEBA-RNB, JGI	Gi08841
Cupriavidus sp.	Amp6	*Mimosa asperata*	U.S.A. (Texas)	GEBA-RNB, JGI	Gi08845
	5v12	*Parapiptadenia rigida*	Uruguay	GEBA-RNB, JGI	Gi08830

[a] JGI = United States Department of Energy (DOE) Joint Genome Institute; GEBA-RNB:Genome encyclopedia of bacteria and archaea on root nodulating bacteria, a specific program of JGI.
[b] GOLD = Genome On-Line database.
[c] Strain STM815 has a broad host range on many *Mimosa* species, but was never proved to nodulate its original host *Machaerium lunatum.* Strain STM678 nodulates *Cyclopia* species but was also not proven to nodulate *Aspalathus* spp. Only the *C. taiwanensis* LMG19424 genome sequence has been published so far (Amadou et al. 2008).

Table 3. Characteristics of genomes from several sequenced and annotated *Burkholderia* species of varying life styles[a]

			% Genes for Metabolism					
Species and strain	GC%	Size[b]	Xenobiotic	Secondary	Lipid	Signal transduction	Lifestyle	*nifH*[c]
B. ambifaria MC40-6	66	7.64	11.66	9.4	10.78	0.22	Biocontrol, human pathogen	No
B. cenocepacia HI2424	67	7.7	12.50	10.22	10.54	0.27	Human pathogen	No
B. glumae BGR1	68	7.28	10.68	9.35	9.6	0.19	Plant pathogen	No
B. graminis C4D1M	63	7.48	13.98	9.05	10.75	0.37	Biocontrol	No
B mallei ATCC 23344	68	5.84	10.68	9.35	10.08	0.27	Human pathogen	No
B. multivorans ATCC 17616	67	7.01	12.28	9.74	10.02	0.18	Human pathogen	No
B. phymatum STM815	62	8.68	13.72	9.95	10.45	0.30	Mutualist	Yes
B. phytofirmans PsJN	62	8.21	13.67	9.74	10.83	0.20	Biocontrol	No
B. pseudomallei 1106a	68	7.09	10.53	9.45	10.19	0.17	Human pathogen	No
B. tuberum STM678	63	8.10	14.73	10.49	12.04	0.31	Mutualist	Yes
B. unamae MT1-641	65	9.64	16.52	11.34	14.79	0.17	Mutualist	Yes
B. vietnamiensis G4	66	8.39	11.28	9.44	9.88	0.22	Mutualist, human pathogen	Yes
B. xenovorans LB400	63	9.73	16.97	10.94	13.35	0.16	Mutualist	Yes

[a] All the genomes listed are finished, except for *B. graminis* C4D1M, *B. tuberum* STM678, and *B. unamae* MTI-641, which are still in draft form. The information above comes from IMG/ER (Markowitz et al. 2009) and A. M. Hirsch *unpublished.*
[b] Genome size in Mbp.
[c] Evidence for *nifH* in the sequenced genome.

(Bcc), are found in hospital environments and are opportunistic human pathogens (Mahenthiralingam et al. 2008). Interestingly, strains belonging to the Bcc are also frequently isolated from legume nodules (Rasolomampianina et al. 2005; Vandamme et al. 2002), although none have yet been confirmed as effective symbionts (Vandamme et al. 2007). Nevertheless, their presence within nodules well illustrates the diversity of niches that can be occupied by Bcc strains that are outside the clinical and phytopathogenic environments.

Because of the potential use of these plant growth-promoting, biocontrol-affecting, N_2-fixing, and nodulating *Burkholderia* strains as agricultural inoculants (Compant et al. 2005) as well as the perception (based on their generic name) that they may cause disease, various studies have compared the phylogenetic relationships between beneficial and nodulating plant–associated *Burkholderia* strains and those in the Bcc. Two subclades were evident from early studies of 16S rRNA sequences (Caballero-Mellado et al. 2004, Reis et al. 2004), and deeper analysis resulted in a distinct separation between *Burkholderia* spp. that are plant-associated and generally beneficial and those species that are either plant pathogens or opportunistic mammalian pathogens (Perin et al. 2006). This phylogenetic separation into two separate lineages was also observed when 16S rRNA and *recA* gene sequences of many nodulating strains of *Burkholderia* were analyzed (Bontemps et al. 2010). The separation based on 16S rRNA sequences is depicted in Figure 1, showing the same dichotomy between the plant- and animal-associated sublineages of *Burkholderia* spp.

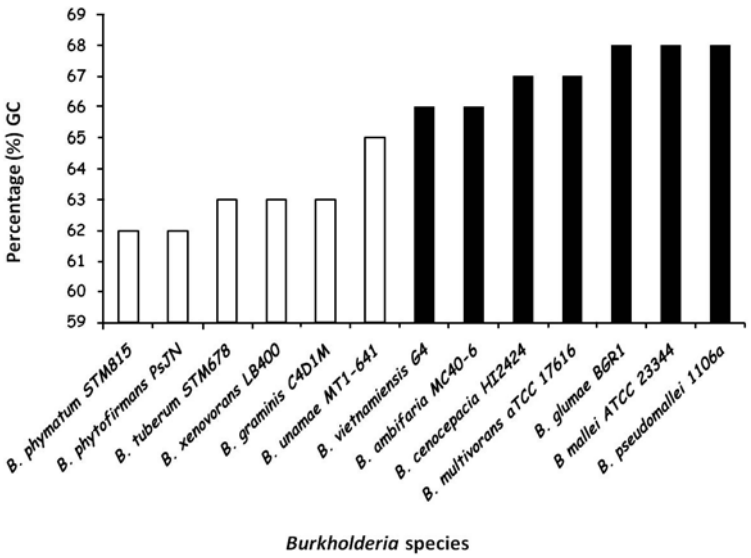

Fig. 3. The mean G+C content of the genomes of several *Burkholderia* species is displayed in graph form. The species with the lowest G+C content are members of the plant-associated clade (white bars) and are used for biocontrol, bioremediation, or to provide fixed nitrogen to plants. The species with the highest G+C content are phytopathogens or opportunistic mammalian pathogens (black bars).

as do phylogenetic schemes that are based upon the sequences of other genes, such as *acdS*, which encodes for 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Onofre-Lemus et al. 2009) and on the analysis of whole genomes (Vanlaere et al. 2009). A recent multilocus sequence analysis of five housekeeping genes from 67 type and reference *Burkholderia* strains confirmed the two distinct evolutionary lineages within the genus (P. Estrada-de los Santos, P. Vinuesa, L. Martinez-Aguilar, A.M., Hirsch, and J. Caballero-Mellado *unpublished*). These authors plan to propose that the plant- and animal-associated strains be split into two distinct genera, with *Caballeronia* being suggested as the name for a new genus containing the plant-associated but nonpathogenic strains, in honor of the late J. Caballero-Mellado, a Mexican microbiologist who pioneered many of the studies on the plant-associated strains.

Analysis of 22 *Burkholderia* species with completely or almost completely sequenced genomes available through The Integrated Microbial Genome IMG/ER system website (Markowitz et al. 2009) shows that the majority of plant-associated *Burkholderia* species have a lower G+C content than those in the Bcc (Table 3; Fig. 3). Expanding the analysis to 34 strains recapitulated the difference in G+C content in the two sublineages (A. M. Hirsch *unpublished*). Furthermore, the plant-associated species lack some genes that are important for the virulence of animal pathogens in the Bcc. Perin and associates (2006) could not detect the *Burkholderia* virulence genes *cblA* and *esmR* in nitrogen-fixing *Burkholderia* spp. associated with maize and sugarcane. By analyzing the entire genome sequence of four different plant-associated *Burkholderia* strains, including *B. tuberum* STM678, we have found that various secretion systems that are indicators of pathogenesis are missing (A. M. Hirsch *unpublished*), although additional studies are required to establish the effect of these *Burkholderia* strains on animal models.

Concluding remarks and future work.

β-Rhizobia are highly effective symbionts of legumes and have coevolved with their hosts for up to 50 million years, with their principal centers of diversity in South America and South Africa and, possibly, another center of diversity in Australia. It is only a decade since their existence was first recognized, and many more will undoubtedly be discovered in the coming years. There is much to learn about the genetic, taxonomic, and geographical factors underlying the ability (or preference) of particular legumes to nodulate with β-rhizobia and, after such a long period of separate evolution of the symbiosis genes in very different genomic backgrounds, there could be substantial differences from the way that α-rhizobia interact with their hosts. Although β-rhizobia are particularly associated with genus *Mimosa* and some related genera, they also nodulate several agriculturally important papilionoid legumes, including common bean (*Phaseolus vulgaris*) and honeybush tea (*Cyclopia* spp.), thus raising the possibility that they could be used as agricultural inoculants when their particular characteristics (e.g., tolerance to pH extremes, high salt tolerance) make them more suited to specific environments, such as in Morocco (Talbi et al. 2010) and the South African Cape (Elliott et al. 2007b). However, before developing these inocula, we need to know whether their relationship to human, animal, and plant pathogens would preclude their use in agriculture. Current fears about their use should be at least partly allayed by the evidence for divergence that underlies the proposed transfer of the plant-associated and nonpathogenic *Burkholderia* spp. to the new genus *Caballeronia*.

ACKNOWLEDGMENTS

We thank J. Ardley and J. Howieson for sharing papers that are in press or still in preparation, and A., C. Hughes, S. J. Kirchanski, A. Peix, I. Schroeder, and M. Simon for their insights and comments on this manuscript. P. Gyaneshwar was partially supported by the College of Letter and Sciences, University of Wisconsin Milwaukee. G. N. Elliott, C. Bontemps, J. I. Sprent, J. P. W. Young, and E. K. James were funded by the Natural Environment Research Council (NERC), U.K. E. Gross was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, L. Moulin's research on beta-rhizobial genomics is funded by Agence Nationale de la Recherche (ANR-09-JCJC-0046), and A. M. Hirsch's research on the genomes of four nitrogen-fixing *Burkholderia* species is funded by a U.S. National Science Foundation grant IOB-0537497 to George Weinstock and A. M. Hirsch.

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AUTHOR-RECOMMENDED INTERNET RESOURCES

ICSP–Subcommittee on the taxonomy of Rhizobium and Agrobacterium website: edzna.ccg.unam.mx/rhizobial-taxonomy
GOLD: Genomes Online database: www.genomesonline.org
The Integrated Microbial Genome (IMG) family systems website: genomebiology.jgi-psf.org/Content/IMG_system.htm
National Center for Biotechnology Information's Bioproject *B. phymatum* STM815 page: www.ncbi.nlm.nih.gov/bioproject/58699
B. S. Weir's taxonomy of rhizobia webpage: www.rhizobia.co.nz/taxonomy/rhizobia.html