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Characterization of the papilionoid–*Burkholderia* interaction in the Fynbos biome: The diversity and distribution of beta-rhizobia nodulating *Podalyria calyptrata* (Fabaceae, Podalyrieae)☆

Benny Lemaire[a,b,*], Jannick Van Cauwenberghe[b], Brecht Verstraete[c], Samson Chimphango[a], Charles Stirton[a], Olivier Honnay[b], Erik Smets[b,d], Janet Sprent[e], Euan K. James[f], A. Muthama Muasya[a]

[a] *Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, Cape Town, South Africa*
[b] *Plant Conservation and Population Biology, KU Leuven, Kasteelpark Arenberg 31, PO Box 2435, 3001 Heverlee, Belgium*
[c] *Botanic Garden Meise, Nieuwelaan 38, 1860 Meise, Belgium*
[d] *Naturalis Biodiversity Center, Leiden University, 2300 RA Leiden, The Netherlands*
[e] *Division of Plant Sciences, University of Dundee at JHI, Dundee DD2 5DA, UK*
[f] *The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK*

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ABSTRACT

The South African Fynbos soils are renowned for nitrogen-fixing *Burkholderia* associated with diverse papilionoid legumes of the tribes Crotalarieae, Hypocalypteae, Indigofereae, Phaseoleae and Podalyrieae. However, despite numerous rhizobial studies in the region, the symbiotic diversity of *Burkholderia* has not been investigated in relation to a specific host legume and its geographical provenance. This study analyzed the diversity of nodulating strains of *Burkholderia* from the legume species *Podalyria calyptrata*. Diverse lineages were detected that proved to be closely related to *Burkholderia* taxa, originating from hosts in other legume tribes. By analyzing the genetic variation of chromosomal (*recA*) and nodulation (*nodA*) sequence data in relation to the sampling sites we assessed the geographical distribution patterns of the *P. calyptrata* symbionts. Although we found a degree of genetically differentiated rhizobial populations, a correlation between genetic (*recA* and *nodA*) and geographic distances among populations was not observed, suggesting high rates of dispersal and rhizobial colonization within Fynbos soils.

Introduction

Over the last few decades of rhizobial exploration, various Leguminosae of the South African Fynbos biome have been reported to form symbioses with *Burkholderia* rhizobia (referred to as beta-rhizobia, beta-subclass of *Proteobacteria*; [19]), comprising diverse host associations with lineages from the tribes Crotalarieae [8,10,20], Hypocalypteae [2], Indigofereae [21], Phaseoleae [9,17] and Podalyrieae [2,12]. The abundance and success of *Burkholderia* in South African legumes, linked with the general ecological adaptation of these symbionts to oligotrophic acidic soil conditions [17,19,42], suggest that leguminous plants of the Fynbos biome have established various symbiotic interactions and have co-diversified with their rhizobial partners in one of the major symbiotic *Burkholderia* hotspots of the world [19].

In the tribe Podalyrieae, subendemic to the South African Fynbos biome, all host plant genera investigated to date (i.e. *Amphithalea* Eckl. & Zeyh, *Cyclopia* Vent., *Podalyria* Wild., *Stirtonanthus* B.-E. Van Wyk & A.L. Schutte and *Virgilia* Poir) are exclusively associated with *Burkholderia* [2,12,19,21]. Despite this degree of specificity within the tribe, the *Burkholderia*–legume interaction has never been investigated at a lower taxonomic level (among legume species and plant populations), although rhizobial screenings among selected Podalyrieae species showed preliminary evidence that root nodulating species of *Burkholderia* are associated with diverse host lineages, suggesting a relaxed interaction [2,21]. In addition, previous symbiotic *Burkholderia* studies on South American legumes of the tribe Mimoseae demonstrated a similar aspecificity between rhizobia and plants [5,6,27].

☆ Nucleotide sequence data reported are available in the GenBank database under the accession numbers: KM188254–KM188344 *(recA)*, KM188345–KM188434 *(nodA)*.

* Corresponding author at: Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa. Tel.: +27 21 650 3725; fax: +27 21 650 4041.
E-mail addresses: benny.lemaire@uct.ac.za, benny.lemaire@bio.kuleuven.be (B. Lemaire).

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The diversity and distribution of *Burkholderia* in the Fynbos, like those of other rhizobia and free-living micro-organisms [16,26], can vary over spatial scales [20]. In the latter study, *Burkholderia* symbionts of *Lebeckia ambigua* E. Mey (tribe Crotalarieae) showed distinct geographical distributions, probably as a result of physical differences in the environment. A strong influence of collection site on the taxonomy of the *Burkholderia* symbionts was also found among South American *Mimosa* species [4], indicating that strains of *Burkholderia* are not randomly distributed and may exhibit geographical distribution patterns. A spatial distribution pattern, attributed to dispersal barriers or different environmental conditions, is reflected by genetic differentiation among geographically isolated rhizobial populations within their genetic core and/or accessory genes. Because accessory genes (such as nodulation and nitrogen fixing genes) are rapidly evolving and encode for adaptive traits [18], they usually appear to retain more of the biogeographical information in rhizobial phylogenies than the conserved housekeeping genes [41]. Indeed, at a continental scale, various phylogenetic studies on *Burkholderia* revealed that plasmid-located nodulation genes are largely grouped according to whether they originate from either South Africa or South America, whereas chromosomal sequence data do not reflect the biogeographical variation of the legume symbionts [19,42]. Incongruence in the geographical structuring of chromosomal and symbiosis genes does occur as the latter accessory genes are located on mobile elements or plasmids as revealed within *Burkholderia* [7,24] and are prone to horizontal gene transfer (HGT) [2,6,27], although a similar pattern of HGT was not shown among burkholderias in *Mimosa* species from South America [4].

Despite numerous rhizobial screenings within Fynbos legumes [2,21] and many novel *Burkholderia* descriptions in the region [8–10], our knowledge about their geographical persistence is still very limited for one of the most speciose groups of rhizobia associated with legumes from diverse Fynbos groups (tribes Crotalarieae, Hypocalypteae, Indigofereae, Phaseoleae and Podalyrieae). A detailed biogeographical study at a lower taxonomic level within the South African region has never been carried out, and is needed to understand how these microbial dynamics operate in the Cape region. In this study we investigated the rhizobial diversity among different populations of *Podalyria calyptrata* (Retz.) Wild. (tribe Podalyrieae). *P. calyptrata* is ecologically widely adapted as a nonsprouter Fynbos element in wet and marshy habitats and as a resprouter in drier vegetations [37]. The ability of *P. calyptrata* to sprout from underground roots and stems (resprouter) or regenerate from seeds (nonsprouter) are two different fire-survival strategies, which are common in a fire-prone environment such as the Cape [37]. The specific objectives were to (i) examine the genetic diversity of *Burkholderia* symbionts within populations of *P. calyptrata*, using a phylogenetic analysis of chromosomal (*recA*) and nodulation (*nodA*) sequence data (ii) investigate genetic differentiation of *Burkholderia* symbionts among population of *P. calyptrata*.

Materials and methods

Legume and nodule sampling

All nodules were sampled from reseeder populations of *P. calyptrata* distributed from mountain Fynbos on the Cape Peninsula to other winter-wet habitats in the Paarl, Caledon, Stellenbosch and Hermanus districts, covering the whole natural distribution range of the species in the region, according to a recent revision of the genus [37].

Nodules from populations of *P. calyptrata* (30 host individuals, 11 localities) and other sympatrically distributed species, including *Bolusafra bituminosa* (2 individuals, 1 locality), *Hypocalyptus sophoroides* (1 individual, 1 locality), *Podalyria leipoldtii* (1 individual, 1 locality), *Podalyria sericea* (2 individuals, 2 localities), *Rhynchosia capensis* (1 individual, 1 locality) and *Virgilia divaricata* (4 individuals, 2 localities), were collected in this study (Table S1). The localities of the populations of *P. calyptrata* within the Western Cape Province are shown in Fig. S1. In total, 94 newly obtained rhizobial isolates were included representing 70 symbionts of *P. calyptrata* (Table S1: voucher information, origin and GenBank accession numbers).

Rhizobial cultivation, DNA extraction, amplification and sequencing

Root nodulating bacteria were isolated and cultivated on yeast extract mannitol agar (YEMA) following the protocol described by Vincent [46]. After an incubation period of 3 days at 28 °C, rhizobial isolates were purified by subculturing on YEMA plates. Slow growing rhizobia were given an additional period of 10 days incubation. Pure bacterial cultures were kept at −80 °C in glycerol stock solution (20% v/v) for long-term storage. Bacterial DNA was extracted and amplified as previously described [21]. Amplicons of *recA* and *nodA* were purified for sequencing by using a modification of the Exo/SAP enzyme cleaning protocol [47]. Purified PCR products were sent to Macrogen for sequencing (Macrogen Inc., Seoul, Korea) using the same sequencing primers as for the initial PCR (Table S2). Sequences were edited, assembled and aligned in Geneious Pro v.5.1.7 (www.geneious.com).

Phylogenetic inference

Nucleotide alignments of *recA* (462 bp) and *nodA* (568 bp) sequence data of all *Burkholderia* isolates and reference sequences from GenBank were analyzed, using Bayesian Inference (BI) and Maximum Likelihood (ML) criteria, both carried out on the CIPRES web portal (www.phylog.org). BI analyses were conducted with MrBayes v.3.2.2 [33], running four Markov chains (one cold and three heated) simultaneously for five million generations. MrModeltest v.3.06 [31] selected the general time reversible model of DNA substitutions with gamma-distributed rate variation and a proportion of invariant sites (GTR + I + G) for both *recA* and *nodA* datasets as the best fitting model of evolution under the Akaike information criterion. Conservatively, 25% of the first trees sampled were regarded as 'burnin' and discarded. Convergence of the chains was checked using Tracer v.1.4 [32]. ML analyses were done with RAxML-HPC2 v.8.0.24 using GTR-GAMMA as the most complex substitution model available [40]. A multi-parametric bootstrap resampling of 1000 pseudo-replicates was plotted onto the previously selected best-scored ML tree to test the robustness of the tree.

Authentication

Nodulation capabilities of new rhizobial isolates were verified through authentication experiments on the original host as previously described [21]. Briefly, seedlings from surface-sterilized seeds were grown in autoclaved plastic pots filled with a mixture of autoclaved sand:vermiculite (4:1). Plants of *P. calyptrata* (3 per pot) were watered with a sterile nitrogen-free nutrient solution through autoclaved PVC pipes. Sterile nylon beads (Lomold group HQ, South Africa) were placed on the sand/vermiculite surface to prevent cross-contamination. Root nodulation was assessed after 6 weeks and authentication was confirmed if isolates nodulated the roots of inoculated plants and uninoculated controls remained nodule free. For the *Mesorhizobium* and *Bradyrhizobium* isolates,

nodulation capabilities were also authenticated on siratro (*Macroptilium atropurpureum*) and *Psoralea pinnata*.

Statistical analyses of the population genetic data

We performed an analysis of molecular variance (AMOVA) [15] on the *recA* and *nodA* sequence data in GenAlEx 6.501 [29] to partition genetic diversity of rhizobia among *P. calyptrata* populations. The significance of the pairwise ϕ_{ST} values between populations was tested using 9999 permutations. A Mantel test based on 9999 permutations was conducted in GenAlEx 6.501 to examine an association between the matrices with genetic distances (ϕ_{ST} values) and geographic distances.

Results

Phylogeny of symbionts of P. calyptrata

Maximum Likelihood and Bayesian analyses of the *recA* and *nodA* datasets yielded fairly resolved topologies (Figs. 1 and 2) and classified all rhizobial isolates as *Burkholderia*, with the exception of one *Bradyrhizobium* isolate of *P. calyptrata* and two *Mesorhizobium*

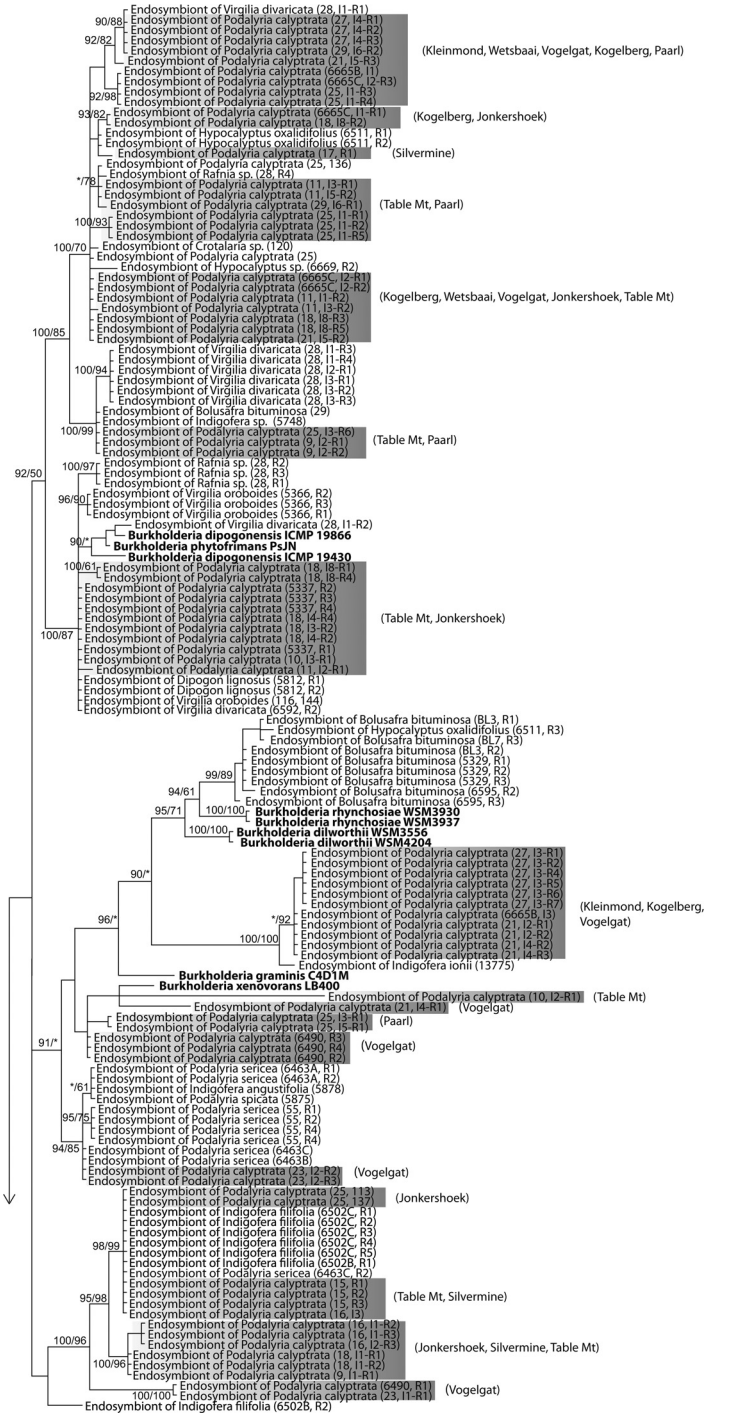

Fig. 1. Bayesian majority rule consensus tree of *Burkholderia* rhizobia based on *recA* sequence data. Support values of the Bayesian and Maximum Likelihood analyses are given at the nodes (Bayesian posterior probabilities – bootstrap values from the Maximum Likelihood analysis). The geographic localities of the *Podalyria calyptrata* isolates are highlighted for the major phylogenetic lineages. Reference *Burkholderia* strains are shown in bold. The scale bar represents 0.6 substitutions per site.

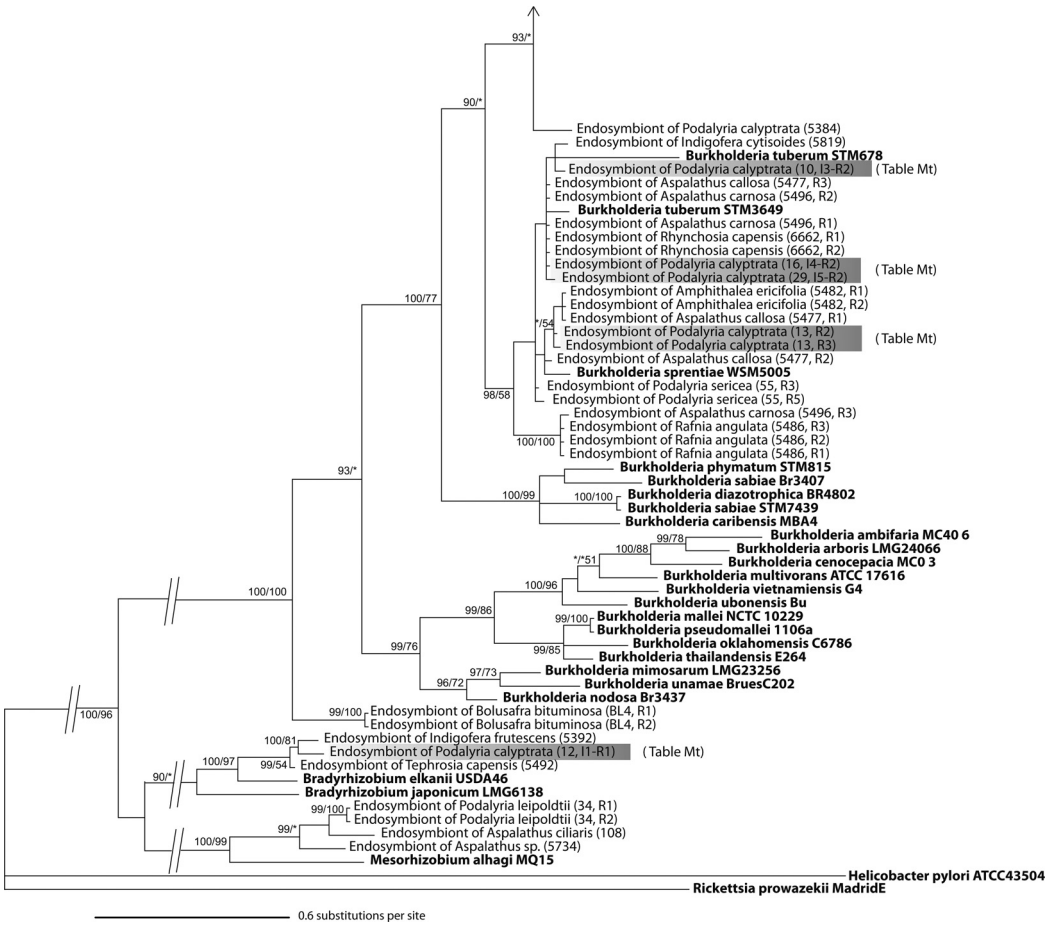

Fig. 1. (*Continued*).

isolates of *P. leipoldtii* (Lemaire 34). The isolates of *Bradyrhizobium* and *Mesorhizobium* were related to the reference strain *Bradyrhizobium elkanii* USDA46 (97% Bayesian posterior probability-BPP; 54% bootstrap support-BS) and *Mesorhizobium alhagi* (100% BPP; 99% BS), respectively (Fig. 1). Similar relationships among the alpha-rhizobia (*Mesorhizobium* and *Bradyrhizobium*) were found in the *nodA* phylogeny (Fig. 2).

All symbionts of *P. calyptrata* identified as *Burkholderia* were placed at distinct positions in the *recA* tree with relationships to the reference strains *Burkholderia dilworthii* WSM3556^T/WSM4204, *Burkholderia dipogonensis* ICMP19430^T/ICMP19866, *Burkholderia graminis* C4D1M^T, *Burkholderia phytofirmans* PsJN^T, *Burkholderia rhynchosiae* WSM3930/WSM3937^T, *Burkholderia sprentiae* WSM5005^T, *Burkholderia tuberum* STM3649/STM678^T and *Burkholderia xenovorans* LB400^T (Fig. 1). While a number of rhizobial isolates were closely related to known *Burkholderia* species (*B. phytofirmans* PsJN^T, *B. dipogonensis* ICMP19866, *B. xenovorans* LB400^T, *B. tuberum* STM678^T, *B. sprentiae* WSM5005^T), most groups did not include reference strains and might represent novel *Burkholderia* species. The *nodA* phylogeny (Fig. 2) revealed lineages with affinities to *B. tuberum* STM678^T, *B. sprentiae* WSM5005^T, *B. dilworthii* WSM3556^T/WSM4204, *B. dipogonensis* ICMP19430^T/ICMP19866, and *B. rhynchosiae* WSM3930/WSM3937^T. Similar to *recA*, most of the *nodA* sequences were not closely related to known reference strains and might represent novel *nodA* types in the Fynbos.

Isolates obtained from other legume groups (tribes Crotalarieae, Hypocalypteae, Indigofereae and Phaseoleae) were included in the *recA* and *nodA* analyses, and they were found to be genetically similar (≥99% sequence similarity) and closely related to the symbionts of *P. calyptrata* (Figs. 1 and 2). This pattern of symbiont heterogeneity among legume hosts was also observed at population level, showing different individuals of one population (e.g. *recA* accessions Lemaire 18–27) associated with different *Burkholderia* lineages (Fig. 1). Although phylogenetic relationships of the chromosomal *recA* and plasmid-located *nodA* genes were largely congruent (Figs. 1 and 2), different branching patterns among numerous isolates were observed (e.g. *P. calyptrata* nodule accessions 9 I1R1, 13N3, 21 I5R2, 21 I4R1), suggesting a degree of horizontal gene transfer of plasmid located nodulation genes.

The isolates from *P. calyptrata* were sampled from eight source populations. The geographical provenances of the populations of the *P. calyptrata* symbionts (Fig. S1, Table S3) are shown on the *recA* and *nodA* phylogenies (Figs. 1 and 2), indicating that most symbiont lineages are widely distributed among different host populations. While some symbiont clades are restricted to a single locality or host population, most rhizobial lineages were identified from different populations.

Authentication tests

Authentications were done for 15 isolates of *P. calyptrata*, representing one randomly chosen strain for each of the major *Burkholderia* clades, and for the isolates of *Mesorhizobium* and *Bradyrhizobium*, originally isolated from *P. calyptrata* (Lemaire 12) and *P. leipoldtii* (Lemaire 34), respectively. *P. calyptrata* nodulated with all symbionts with the exception of *Bradyrhizobium* and *Mesorhizobium* isolates. Although these harbored *nodA* genes, their inability to renodulate the host rejects these isolates as true *Podalyria* symbionts. Nodulation capabilities of isolates of

Mesorhizobium and *Bradyrhizobium* were also tested on siratro and *P. pinnata*. Siratro is known to be symbiotically promiscuous for a large number of alpha- and betaproteobacteria [1,27,44], whereas *P. pinnata* is naturally associated with mesorhizobia [21]. Only the *Bradyrhizobium* isolate formed nodules on siratro.

Population differentiation and isolation-by-distance

AMOVA tests detected significant ($p < 0.05$) population differentiation values (ϕ_{ST}) for the *recA* ($\phi_{ST} = 0.172$) and *nodA* ($\phi_{ST} = 0.094$) datasets, showing more populations differentiated for *recA* (14 significant ϕ_{ST} values) compared with *nodA* (10 significant ϕ_{ST} values) (Table 1). The highest pairwise ϕ_{ST} values (>0.400) were recorded among populations of *P. calyptrata* from Jonkershoek, Paarl, Kleinmond, Wetsbaai and Kogelberg (*recA*: C vs. F, E vs. F; *nodA*: E vs. F, F vs. G, F vs. H, G vs. H). The observed genetic differentiation across geographically distant locations suggests a degree of spatial genetic structuring among selected populations. A Mantel test, however, showed no correlation ($p = 0.004$) between genetic and geographic distances among populations for both *recA* and *nodA*. For the *nodA* dataset, however, the correlation was marginally significant ($p = 0.055$).

Discussion

Burkholderia-*philous Fynbos legumes*

The exclusiveness of *Burkholderia* symbionts associated with host genera of Podalyrieae (*Podalyria*, *Virgilia*) [2,21] and previous reports of *Burkholderia* in *Amphithalea, Stirtonanthus* [21] and

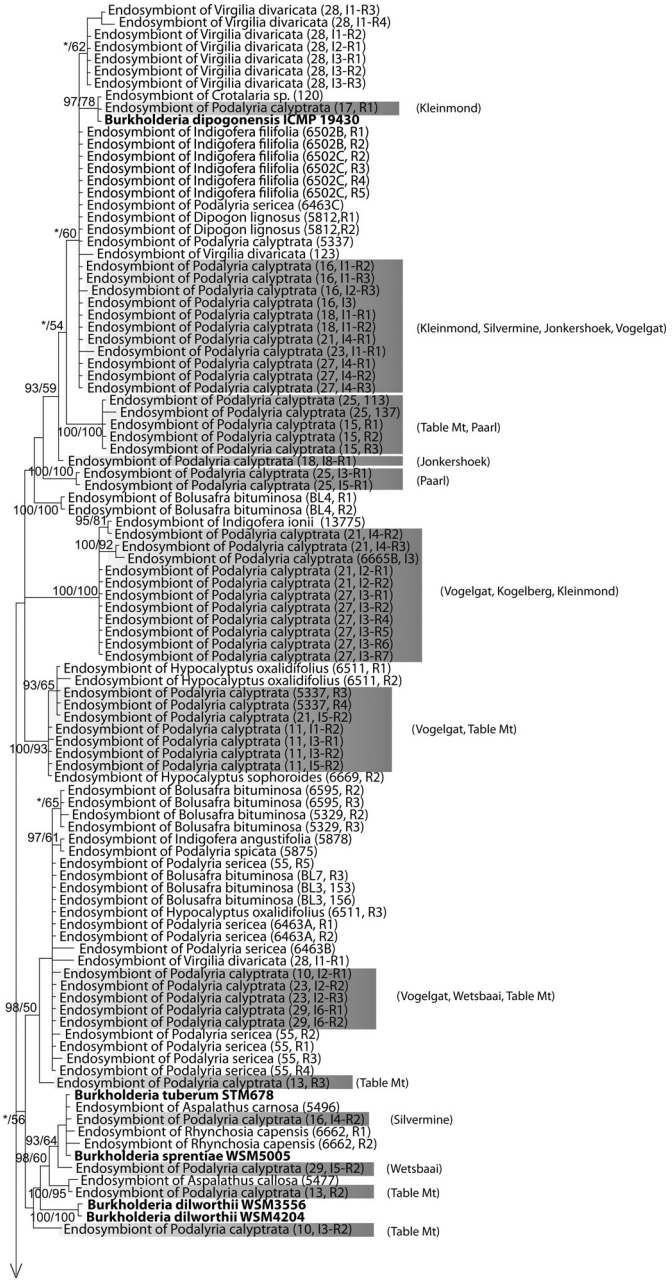

Fig. 2. Bayesian majority rule consensus tree of *Burkholderia* rhizobia based on *nodA* sequence data. Support values of Bayesian and Maximum Likelihood analyses are given at the nodes (Bayesian posterior probabilities – bootstrap values from the Maximum Likelihood analysis). The geographic localities of the *Podalyria calyptrata* isolates are highlighted for the major phylogenetic lineages. Reference *Burkholderia* strains are shown in bold. Reference *Burkholderia* strains are shown in bold. The scale bar represents 0.7 substitutions per site.

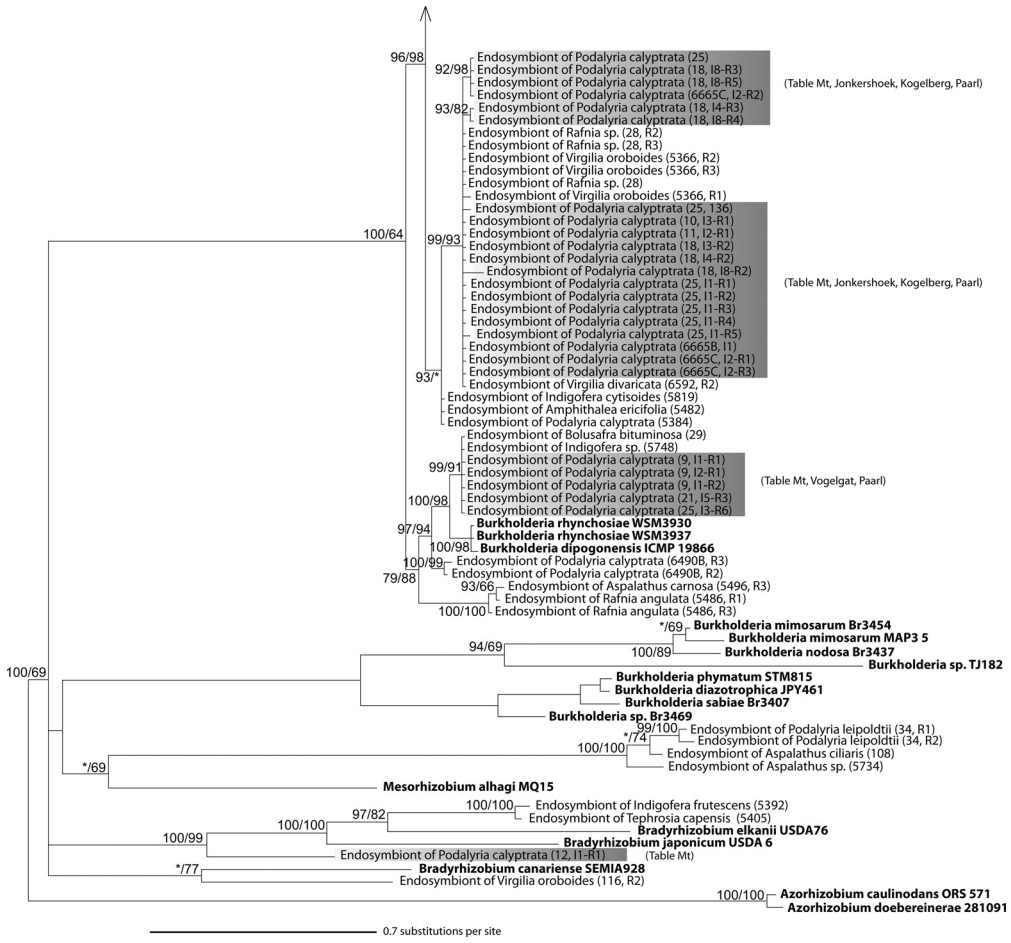

Fig. 2. (*Continued*).

Cyclopia [2,12] suggests that this tribe, largely endemic to the Core Cape Flora, has adapted to these below-ground mutualists since the origin of the tribe, estimated at about 30 million years ago (Mya) [3,34]. The other large Fynbos tribe Crotalarieae has a similar contemporaneous origin in the late Eocene [11], with root nodulated *Burkholderia* reported in the genera *Lebeckia* [8,10,20], *Crotalaria*, *Rafnia* [21] and *Aspalathus* [12,45]. The observation of *Burkholderia* within all Podalyrieae (except for *Calpurnia* [39]) and a

Table 1
Analyses of molecular variance (AMOVA) showing pairwise ϕ_{ST} values among populations for the of *recA* and *nodA* datasets.

recA

	A	B	C	D	E	F	G	H
A	–							
B	0.04	–						
C	0.018	**0.156***	–					
D	**0.104****	**0.197****	**0.196****	–				
E	0.057	**0.297****	**0.125***	**0.139***	–			
F	**0.303****	**0.396****	**0.429****	0.121	**0.4***	–		
G	0.000	0.156	0.099	0.072	0.000	0.294	–	
H	0.075	**0.303***	**0.141***	**0.134***	0.000	0.283	0.000	–

nodA

	A	B	C	D	E	F	G	H
A	–							
B	0.000	–						
C	0.054	0.128	–					
D	0.000	0.000	0.133	–				
E	0.054	**0.209****	0.000	**0.138****	–			
F	0.172	**0.220****	**0.337****	0.050	**0.453***	–		
G	0.000	0.063	0.155	0.000	**0.277***	**0.450***	–	
H	0.045	**0.189****	0.000	0.080	0.000	**0.422***	**0.401***	–

Significant ϕ_{ST} values (*$p < 0.05$, **$p < 0.01$) are indicated in bold. A = Table Mountain National Park, Rhodes Memorial, B = Table Mountain National Park, Silvermine Nature Reserve, C = Jonkershoek Nature Reserve, D = Vogelgat Nature Reserve, E = Paarl Mountain Nature Reserve, F = Kleinmond Nature Reserve, G = Wetsbaai, H = Kogelberg Nature Reserve.

wide range of crotalarioid lineages indicates that *Burkholderia* symbionts are prevalent within the Fynbos soils, and have probably co-diversified with their hosts, representing one of the oldest radiations of the Cape Flora. The genera *Rhynchosia* (tribe Phaseoleae) and *Hypocalyptus* (tribe Hypocalypteae), some species of *Indigofera* (tribe Indigofereae) and the less speciose legume groups in the Cape region such as the phaseoloid genera *Bolusafra* (one species) and *Dipogon* (one species) have also been shown to form interactions with *Burkholderia* ([2,17,21]; this study). The genus *Indigofera* has a large African Cape clade, endemic (ca. 70–75 out of 86 species) to the Greater Cape Floristic region, which has a crown clade age of less than 10 Mya [35,36].

In the South American Cerrado/Caatinga biomes, another major center of *Burkholderia* diversity has been reported with a separate origin of symbiont diversification adapted to nodulate the mimosoid legume flora [19]. The nodulation genes of these mimosoid burkholderias differ markedly to the South African representatives with observed sequence similarities ranging between 63% and 66% for *B. mimosarum* Br3454 and similarities between 66% and 70% for *B. phymatum* STM815^T. The South American and South African strains are clearly separated on the basis of their nodulation genes [19] and the high divergence of *nodA* genes examined in all South African species of *Burkholderia* supports a previous study, showing a distinct and separate origin of nodulation genes within indigenous Fynbos *Burkholderia* [2]. The origin of *Burkholderia*-philous Fynbos species during the Eocene overlaps with the origin of South American mimosoid species, estimated about 28 million years ago [38], suggesting a parallel historical time frame of *Burkholderia* diversifications within the Fynbos and Cerrado/Caatinga biomes of the African and South American continents, respectively.

Distribution of Burkholderia *among host populations*

The chromosomal and plasmid sequence data revealed that most rhizobial lineages were not restricted to a single host population but widely distributed among different host populations (Fig. 1), suggesting large-scale distribution ranges of *Burkholderia* types. Like many other microorganisms, *Burkholderia* symbionts seem not to be spatially restricted to specific localities, at least within the region. This is most likely a result of high dispersal capacities as revealed by the absence of an effect of genetic isolation-by-distance. Although *Burkholderia* lineages showed a widespread distribution pattern at a regional scale (Fig. 1), probably as a result of high migration rates and successful niche colonization, a degree of genetic differentiation was apparent among different *P. calyptrata* populations (Table 1, Fig. S1). Previous studies have revealed a site effect in the distribution of *Burkholderia* and a link between genetic differentiation and soil parameters (pH, phosphate, $CaCO_3$ and soil texture) [6,17,23,27] or geographical differences such as site elevation [4,21], suggesting that these rhizobial communities are shaped by different and most likely a mix of ecological variables.

Diverse Burkholderia *species and association with* P. calyptrata

Although *Burkholderia* is the prevalent symbiont of Podalyrieae and other Fynbos legume groups, a specific interaction among legume species and/or populations was not observed. A broad range of host interactions was demonstrated by an overlap of *P. calyptrata* symbionts with hosts from various distantly related legume taxa, belonging to different tribes (Fig. 1), rejecting a correlation between rhizobial and plant evolution.

Because *nodA* genes are involved in the synthesis of Nod factors (lipochitololigosaccharides LCOs), which are key signal molecules for host plant recognition and host range [30], they are expected to evolve under constraints imposed by the host plant. The intertwined phylogenetic pattern of *nodA* sequences of diverse hosts, however, suggests that Fynbos legumes may not influence the *nodA* gene evolution and are associated with a particular *nodA* genotype with a low degree of genetic variation among the *Burkholderia* strains (minimum pairwise sequence similarity ranging between 93% and 95%). Although data on genetic divergence of nodulation genes alone cannot be used to predict host range [30], the low genetic variation of *nodA* sequences and probably also minor variations in LCOs reflects the aspecificity of the *Burkholderia*-legume symbiosis in the Fynbos biome. Evidence to date indicates that Papilionoideae-nodulating *Burkholderia* (e.g. *B. tuberum* strain STM678^T and *B. dipogonensis* ICMP 19320^T) are able to form nodules on a broad range of species representing the tribes Podalyrieae [19,22,39]) and Phaseoleae [1], indicating that Fynbos burkholderias, which resemble each other in terms of nodulation genes, are able to nodulate various legume hosts. Furthermore, host range studies elsewhere have shown that *B. tuberum* STM678^T and *B. dipogonensis* ICMP 19320^T, although able to nodulate diverse papilionoids, have not so far been demonstrated to nodulate members of the mimosoid legume subfamily [12,19], which are nodulated by another group of burkholderias with distinct nodulation genes [4,6,13,14,27]. The division between papilionoid- and mimosoid-nodulating *Burkholderia* is well illustrated in *B. tuberum*, showing two distinct symbiotic interactions with *Mimosa* or *Cyclopia* species (referred to as *B. tuberum* symbiovar papilionoideae and symbiovar mimosae, respectively) defined by genetically different nodulation genes [27]. However, the link between papilionoid- and mimosoid-nodulating *Burkholderia* and their nodulation genes has been rejected for some promiscuous *Mimosa*-type strains (e.g. *B. phymatum* STM815^T; [28]), which are also able to effectively nodulate the papilionoids (both in tribe Phaseoleae) *Phaseolus vulgaris* [25,43] and *Dipogon lignosus* [22].

Conclusions

This study investigated the diversity and distribution pattern of *Burkholderia* symbionts in populations of one host legume (*P. calyptrata*). We discovered a hyper-diverse *Burkholderia* interaction, with rhizobial lineages assigned to known South African type strains and others being putatively new species. Most of these symbiont lineages were isolated from different *P. calyptrata* populations, suggesting a widespread distribution range of *Burkholderia* species in the Fynbos region. Furthermore, a 'relaxed' interaction was observed, as the rhizobial phylogeny did not group according to the hosts.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2015.09.006.

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