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Similar strains of *Burkholderia* spp. nodulate the South African invasive legume *Dipogon lignosus* in New Zealand and Australian soils

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Key Words
β-Rhizobia, rhizobia-legume symbiosis

Introduction
Brazil and South Africa are centres of diversity of *Burkholderia* spp. that nodulate legumes (Gyaneschwar *et al*. 2011; Beukes *et al*. 2013). The *nod* gene sequences of *Burkholderia* spp. capable of nodulating South African plants are clearly separated from those of *Burkholderia* spp. shown to nodulate South American plants. Where tested, the South African strains did not nodulate South American plants nodulated by *Burkholderia* spp. (Gyaneschwar *et al*. 2011).

Dipogon lignosus is an herbaceous legume (tribe Phaseoleae) native to the Fynbos biome of the Cape of South Africa which has become invasive in the Australian-Pacific region (Lewis *et al*. 2005; Popay *et al*. 2010). Eight bacterial isolates which produced functional nodules on *D. lignosus* sampled at two field sites in NZ, were identified as *Burkholderia* sp. (Liu *et al*. 2014). Both 16S rRNA and *recA* gene sequences placed the eight *Burkholderia* isolates separate from previously described *Burkholderia* rhizobial species. *Burkholderia* isolates obtained from *D. lignosus* sampled in southwest Australia had identical 16S rRNA sequences (930 bp) to the New Zealand *D. lignosus* strain ICMP 19430. Here, we present the *nodC* gene sequences of the eight NZ isolates and argue that evidence is strong that these *Burkholderia* isolates originated in South Africa and were somehow transported with the plants from their native habitat to NZ and Australia.

Methods
DNA was extracted from the bacterial cultures using the Gentra Puregene DNA Purification Kit (Qiagen) following the protocol for gram-negative bacteria and the N-acetylglucosaminyl transferase nodulation protein C (*nodC*) gene sequenced (Liu *et al*. 2014). DNA sequences were aligned, and a maximum likelihood tree was constructed with 1000 bootstrap replications with partial deletion and an 80% coverage cut-off using MEGA5 software. All closely related strains and selected type strains were used in the trees. *Azorhizobium caulinodans* ORS 571 was used as an out-group. The tree was constructed using the MEGA5 software using the 'best' model (lowest Bayesian information score). This was the Tamura three parameter (T92) gamma distribution (+G) invariant sites (+I) model.

Results and Discussion
The eight *Burkholderia* isolates were separated into two groups on the basis of their *nodC* sequences, one of five isolates from one field site and the other 3 isolates from another field site (Figure 1). The *nodC* sequences for the two groups showed 96.06% similarity (507 bp) to each other and clustered with *B. tuberum* STM678^T, *B. rhynchosiae* WSM3937^T, *B. sprentiae* WSM5005^T, *B. dilworthii* WSM3556^T and several other strains isolated from different plants and sites in the Cape Floristic Region (CFR) of South Africa (Figure 1; Liu *et al*. 2014). Strain ICMP 19430 was tested and shown to produce N_2-fixing nodules on *Cyclopia subternata, Hypocalyptus sophoroides, Podalyria calyptrata* and *Virgilia oroboides* previously reported to be nodulated by *Burkholderia* spp. isolated from legumes in the CFR (Liu *et al*. 2014). It did not nodulate *Mimosa pudica*, which is nodulated by *Burkholderia* spp. isolated from *Mimosa* and *Piptadenia* spp. in South America (Liu *et al*. 2014).

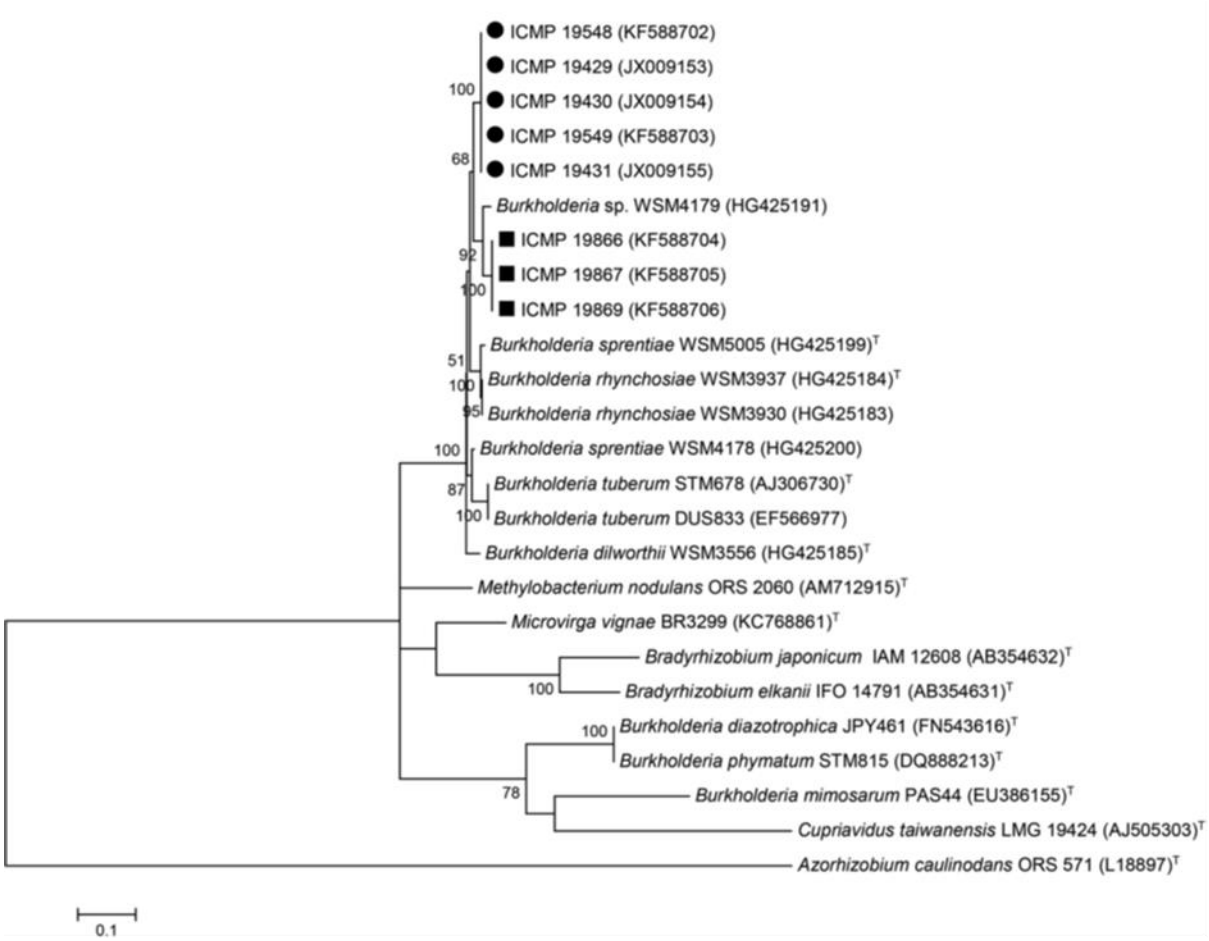

Figure 1. Phylogenetic tree of nodC gene sequences (ca. 507 bp) of eight bacterial isolates from *Dipogon lignosus* sampled in NZ soils (●, ■). Genbank accession numbers are in parentheses. Numbers on branches are bootstrap per cent from 1000 replicates (shown only when > 50 %). Scale bar = 10% sequence divergence (one substitution per 10 replicates).

Conclusion

Burkholderia isolates from *D. lignosus* growing in Australia and NZ are a novel lineage of rhizobia closely related to *Burkholderia* spp. isolated from South African plants: it is likely that these novel *Burkholderia* originated in South Africa in association with *D. lignosus*.

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