The taxonomic status of selected *Paraburkholderia* isolates based on comparative genomic and evolutionary approaches

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Introduction

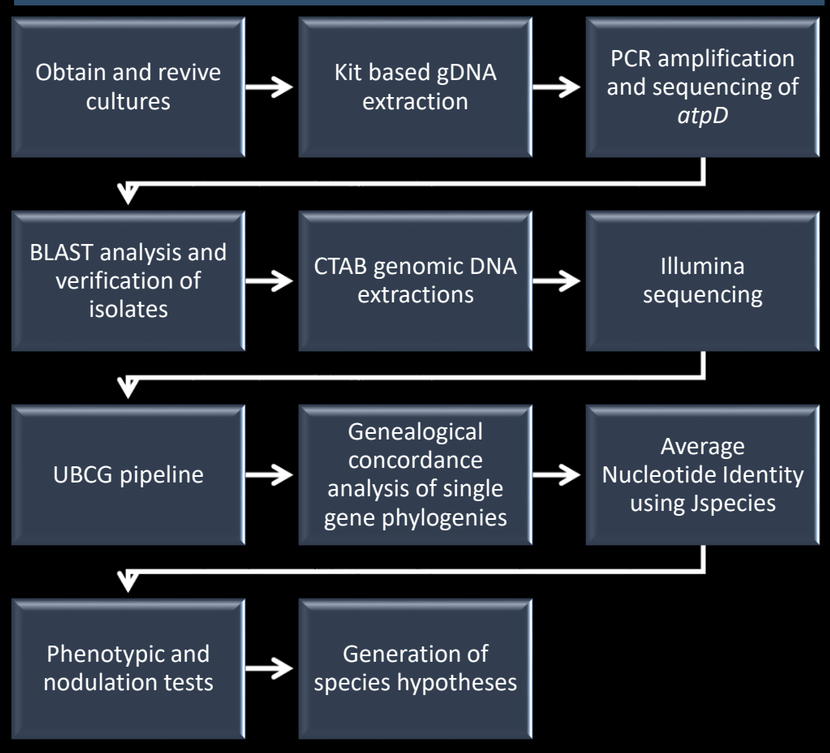
Root nodulating *Paraburkholderia* are of great importance and interest in agriculture and ecology (Mulder *et al.,* 2005) due to their ability to thrive in diverse environments and form symbiotic interactions with members of the Leguminosae. These rhizobia can fix atmospheric dinitrogen, in the root nodules, converting it into soluble forms for use by the plant (Markmann, Giczey and Parniske 2008; Mulder *et al.,* 2005; Platero *et al.,* 2016). Rhizobial innocula could therefore be used as alternatives to artificial fertilizers. These bacteria are phylogenetically diverse and form part of the α-Proteobacteria (α-rhizobia) and the β-Proteobacteria (β-rhizobia) (Lardi *et al.,* 2017).

The diversity of these bacteria has been largely understudied (Moulin *et al.,* 2001), particularly in the Core Cape Subregion (CCR) of South Africa, which is believed to be a centre of rhizobial diversity (Dludlu *et al.,* 2018; Steenkamp *et al.,* 2015), given the large diversity of indigenous legumes within this biodiversity hotspot (Gyaneshwar *et al.,* 2011; Lemaire *et al.,* 2016). The four predominant legume tribes within the CCR are Crotalarieae, Psoraleeae, Podalyrieae and Indigofereae (Manning and Goldblatt 2012). Isolations from members of these tribes suggests a large diversity of *Paraburkholderia* that are genetically diverse but closely related that still remain to be described and characterized (Beukes *et al.,* 2013).

Aim

The aim of this study was to resolve the relationship between 15 *Paraburkholderia* strains isolated from hosts in the tibes Hypocalypteae and Podalyrieae found in the Core Cape Subregion of South Africa resulting from previous studies. This was achieved using a genealogical concordance approach and by comparing genomic metrics.

Methods and materials



Results

Based on the 92 single gene phylogenies and Multi-locus Sequence Analysis (MLSA) three distinct groups were recovered namely; *Paraburkholderia* spp. 1, 2 and 3 (Figure 2). These groups were supported by 100% bootstrap support. None of these putative species grouped closely to any of the known *Paraburkholderia* species except *P.* sp. 2, which grouped closely to *P. phytofirmans* PsJN^T. ANI values supported three distinct groups. The intraspecies ANI values for *Paraburkholderia* sp. 1 and 2 were above the suggested threshold of 95%. Interspecies ANI values for *Paraburkholdera* sp. 2 and *P. phytofirmans* PsJN^T were below 95%, indicating that *Paraburkholderia* sp. 2 is not conspecific to *P. phytofirmans* PsJN^T. Additionally, phenotypic tests indicated that all isolates were Gram negative, motile rods and catalase and oxidase positive. All isolates grew at salt concentrations of 0% to 1% (w/v), with no growth occurring at higher concentrations except for isolates 25.3.6 and CI1. All the isolates grew between pH 8 and 10 and none grew at pH 3 or 11. Furthermore, isolates could nodulate *Macroptilium atropurpureum* (Siratro).

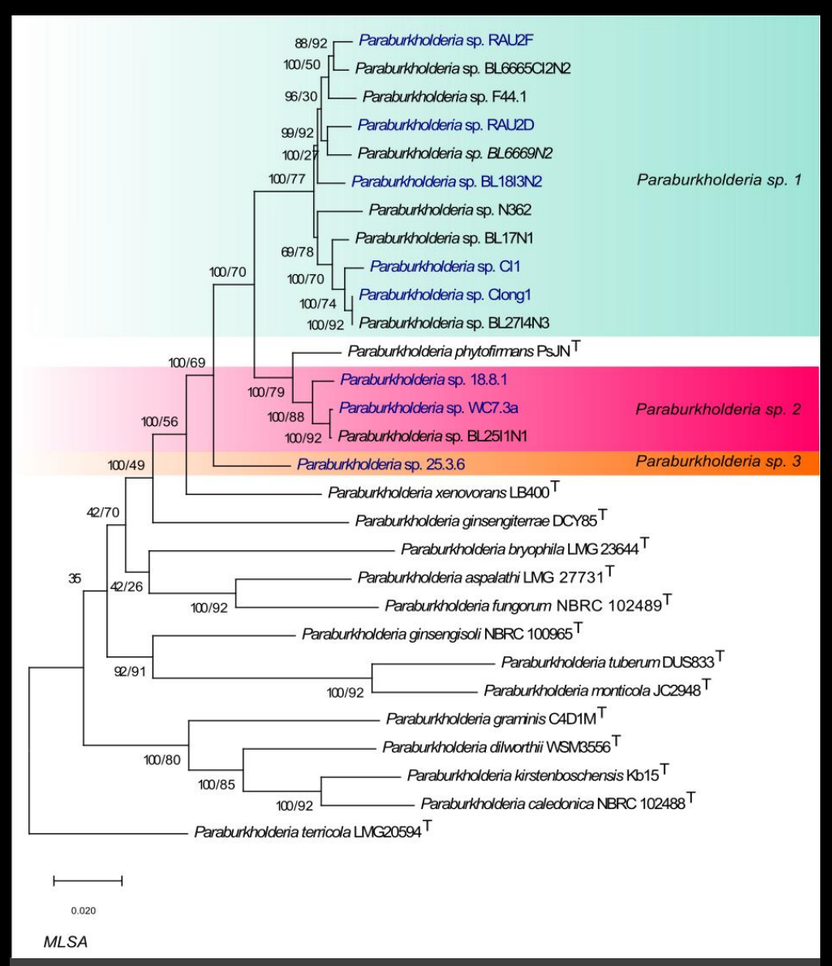


Figure 1: A maximum-likelihood (ML) phylogeny of *Paraburkholderia* species based on 92 protein-coding genes. Phylogeny generated through the UBCG pipeline and RAxML (Na *et al.,* 2018; Stamatakis 2014). Putative species are indicated in colour and isolates that underwent genome sequencing appear in blue. The bootstrap support values (%) and number of trees supporting the branch appear on the phylogeny respectively. The scale bar indicates substitutions per site.

Discussion

An evolutionary and genealogical concordance approach for species delineation as per Venter *et al.* (2017) was used to obtain a species hypothesis for 15 *Paraburkholderia* isolates. Single gene phylogenies and MLSA of 92 individual genes recovered three recurring groups (Figure 1), namely *Paraburkholderia* sp. 1, *Paraburkholderia* sp. 2, and *Paraburkholderia* sp. 3. These groups are phylogenetically distinct.

The putative new species hypotheses drawn were further supported by metrics such as Average Nucleotide Identity (ANI) (Table 1). The ANI values of putative species 1 and 2 were highly supportive of the grouping as values were above the suggested threshold, whereas *Paraburkholderia* sp. 25.3.6 at the moment appears to be single strain species, exhibiting ANI values lower when compared to all the other isolates as well as *P. phytofirmans* PsJN^T.

Additionally, *P. phytofirmans* which appears to be closely related to putative *Paraburkholderia* spp. 1, 2 and 3 has not been observed as a nodulating species, however, nodules were observed on all Siratro plants when inoculated with putative species 1, 2 and 3. This supports the separation of *P. phytofirmans* from these novel rhizobial species.

Conclusion

The relationships between 15 *Paraburkholderia* strains isolated form the CCR was resolved through an evolutionary and genealogical concordance approach. Three novel *Paraburkholderia* species have been identified that are not conspecific to the closely related *P. phytofirmans* PsJN^T.

Table 1: Average Nucleotide Identity values of selected *Paraburkholderia* species

	Paraburkholderia sp. F44.1	*Paraburkholderia* sp. BL6665CI2N2	*Paraburkholderia* sp. RAU2F	*Paraburkholderia* sp. BL6669N2	*Paraburkholderia* sp. RAU2D	*Paraburkholderia* sp. BL18I3N2	*Paraburkholderia* sp. BL17N1	*Paraburkholderia* sp. CI1	*Paraburkholderia* sp. Clong1	*Paraburkholderia* sp. BL27I4N3	*Paraburkholderia* sp. N362	*Paraburkholderia phytofirmans* PsJN^T	*Paraburkholderia* sp. 18.8.1	*Paraburkholderia* sp. BL25I1N1	*Paraburkholderia* sp. WC7.3a	*Paraburkholderia* sp. 25.3.6	*Paraburkholderia xenovorans* LB400^T	*Paraburkholderia ginsengiterrae* DCY85^T	*Paraburkholderia bryophila* LMG 23644^T	*Paraburkholderia graminis* C4D1M^T	*Paraburkholderia dilworthii* WSM3556^T	*Paraburkholderia caledonica* NBRC 102488^T	*Paraburkholderia kirstenboschensis* KB15^T	*Paraburkholderia monticola* JC2948^T	*Paraburkholderia tuberum* DUS833^T	*Paraburkholderia ginsengisoli* NBRC 100965^T	*Paraburkholderia fungorum* NBRC 102489^T	*Paraburkholderia aspalathi* LMG 27731^T	*Paraburkholderia terricola* LMG 20594^T
Paraburkholderia sp. F44.1	100	97.6	97.6	95.7	97	97	95.2	96.2	95.8	96.1	95.4	91.6	92	92.6	92.2	90.4	87.4	85.4	84.7	82.9	83.2	82.5	83.4	82.2	82.7	84.9	83.9	84.7	84.6
Paraburkholderia sp. BL6665CI2N2	97.9	100	98	95.9	97.4	97	95.5	96.5	96.2	96.4	95.6	92	92.1	92.3	92.3	90.5	87.6	85.7	85	83	83.3	82.7	83.6	82.5	82.7	85.1	84.3	84.8	84.9
Paraburkholderia sp. RAU2F	97.4	97.7	100	95.3	97.2	96.5	95	96	95.6	96	95.4	91.5	91.7	91.9	92	90	87.1	85.3	84.4	82.6	83	82.3	83.3	82.1	82.5	84.6	84.1	84.4	84.4
Paraburkholderia sp. BL6669N2	97.4	97.4	97.3	100	97.3	97.2	97.1	96.8	96.6	96.9	96.2	92.8	92.8	93.3	93.7	91.5	88.8	86.9	86.3	84.6	84.8	84.1	84.6	83.7	83.7	86.2	85.6	86.1	86
Paraburkholderia sp. RAU2D	97	97.2	97.2	95.5	100	96.7	95.1	96.4	96.2	96.2	95.6	91.8	92	92.1	92.2	90.4	87.3	85.4	84.6	82.8	83.2	82.4	83.6	82.1	82.6	84.7	84	84.6	84.7
Paraburkholderia sp. BL18I3N2	97.2	96.9	96.8	95.6	96.9	100	95.4	96.5	96	96.3	95.7	91.9	92.1	93	92.3	90.5	87.5	85.5	84.7	83	83.2	82.6	83.2	82.3	82.6	84.9	84	84.7	84.7
Paraburkholderia sp. BL17N1	97.2	97.2	97.2	97.3	97.2	97.2	100	98.2	98.2	98.3	96.6	93.2	93.4	93.5	93.4	91.9	89.1	87.2	86.5	84.8	84.9	84.3	84.9	84.2	84.2	86.5	85.9	86.4	86.4
Paraburkholderia sp. CI1	96.5	96.6	96.5	95.3	96.7	96.6	96.4	100	98.1	98.5	95.8	92	92.3	92.3	92.3	90.6	87.5	85.6	84.8	82.9	83.1	82.6	83.3	82.3	82.7	85	84.2	84.7	84.7
Paraburkholderia sp. Clong1	96.3	96.6	96.3	95.3	96.6	96.3	96.6	98.4	100	99.3	95.7	92	92.3	92.4	92.2	90.7	87.5	85.5	84.8	83.1	83.3	82.6	83.3	82.5	82.6	85.1	84.2	84.8	84.9
Paraburkholderia sp. BL27I4N3	96.5	96.7	96.4	95.3	96.6	96.5	96.7	98.6	99.1	100	95.9	92.2	92.8	92.5	92.4	91.1	87.6	85.7	85	83	83.5	82.9	83.5	82.6	82.7	85.2	84.4	85	84.8
Paraburkholderia sp. N362	95.8	95.9	95.9	94.7	96	95.9	95.1	96	95.7	96	100	91.9	92.2	92.5	92.3	90.6	87.5	85.7	84.8	83.1	83.4	82.6	83.3	82.4	82.6	85	84.1	84.8	84.8
Paraburkholderia phytofirmans PsJN^T	91.9	92.1	92	91.5	92.1	92	91.6	92	91.9	92.1	91.8	100	95	95.2	95.1	90.8	87.6	85.8	84.9	83	83.3	82.8	83.2	82.6	82.5	85.2	84.5	85	84.9
Paraburkholderia sp. 18.8.1	91.6	91.6	91.5	90.6	91.5	91.6	91.3	91.7	91.6	92.1	91.6	94.4	100	97	96.6	90.8	87.1	85.1	84.5	82.8	82.9	82.3	83.1	81.9	82.2	84.6	83.9	84.7	84.5
Paraburkholderia sp. BL25I1N1	92.7	92.2	92.2	91.7	92.2	92.9	91.7	92.2	92	92.2	92.1	95	97.5	100	99.1	90.9	87.7	85.4	84.7	83.1	83.2	82.6	83.3	82.3	82.5	84.9	84.1	84.9	84.7
Paraburkholderia sp. WC7.3a	92.2	92.1	92.3	92	92.1	92.1	91.6	92.1	91.8	92.1	92	94.8	97	98.9	100	90.7	87.5	85.5	84.6	82.9	83.2	82.4	83.2	82.2	82.5	84.8	83.9	84.6	84.5
Paraburkholderia sp. 25.3.6	90	90	89.9	89.5	90.1	90	89.8	90.1	89.9	90.5	89.9	90.2	90.7	90.5	90.4	100	87.9	85.6	84.8	82.6	83.2	82.4	83	82.1	82.3	85	84.3	85	84.7
Paraburkholderia xenovorans LB400^T	87.1	87.4	87.2	87.3	87.3	87.3	87.4	87.1	87.1	87.1	86.9	87.1	87.2	87.3	87.2	87.9	100	85.4	84.4	82.6	82.9	82.4	82.7	82.1	82.1	84.6	83.8	84.3	84.6
Paraburkholderia ginsengiterrae DCY85^T	85.4	85.5	85.4	85.6	85.4	85.4	85.5	85.4	85.3	85.5	85.3	85.6	85.4	85.4	85.4	86.1	85.5	100	84.1	82.5	82.6	82	82.3	82.1	82	84.3	83.6	83.9	84.3
Paraburkholderia bryophila LMG 23644^T	85.2	85.1	85	85.2	85.1	85	85.3	85	84.9	85	84.9	85.1	85.1	85.2	85.2	85.5	85.1	84.6	100	82.9	82.9	82.3	82.8	82.6	82.6	85	84.1	84.3	84.5
Paraburkholderia graminis C4D1M^T	83.4	83.4	83.3	83.7	83.3	83.3	83.8	83.3	83.3	83.3	83.2	83.4	83.5	83.4	83.4	83.5	83.4	82.9	82.9	100	86.3	85.9	86.1	82.1	82	83.6	82.3	82.6	83.9
Paraburkholderia dilworthii WSM3556^T	83.8	83.7	83.6	84.1	83.8	83.6	84	83.5	83.4	83.6	83.6	83.5	83.6	83.9	83.8	83.6	83.1	83.1	86.4	100	88.1	89.2	81.8	81.7	83.6	82.6	82.9	83.8	
Paraburkholderia caledonica NBRC 102488^T	83	83.1	83.1	83.5	83	83	83.7	83	83	83.1	82.9	83.1	83	83	83	83.2	83	82.5	82.5	85.9	88.1	100	92.7	81.3	81.3	83.1	82.1	82.4	83.3
Paraburkholderia kirstenboschensis KB15^T	83.6	83.6	83.6	83.6	83.7	83.3	83.5	83.3	83.2	83.3	83.1	83.1	83.5	83.3	83.4	83.5	83.1	82.5	82.6	85.9	88.9	92.1	100	81.3	81.7	83.2	82.3	82.6	83.4
Paraburkholderia monticola JC2948^T	82.6	82.6	82.6	82.8	82.5	82.5	82.9	82.5	82.5	82.5	82.4	82.6	82.5	82.5	82.5	82.7	82.6	82.3	82.5	81.9	81.6	81.2	81.5	100	89.8	84.2	81.8	81.9	82.8
Paraburkholderia tuberum DUS833^T	82.9	82.6	82.8	82.8	83	82.7	82.9	82.9	82.7	82.7	82.6	82.4	82.7	82.7	82.9	82.8	82.4	82.1	82.3	81.8	81.6	81	81.7	89.6	100	83.8	81.6	81.9	82.3
Paraburkholderia ginsengisoli NBRC 100965^T	85.7	85.7	85.6	85.8	85.6	85.5	85.9	85.7	85.5	85.7	85.5	85.7	85.6	85.7	85.7	86	85.6	85.2	85.4	84	83.9	83.4	83.9	84.6	84.6	100	84.5	84.9	85.6
Paraburkholderia fungorum NBRC 102489^T	84	84.1	84.1	84.3	84.1	83.9	84.5	84.1	83.9	84.1	84	84.3	84.1	84.2	84.1	84.7	83.9	83.7	83.7	82	82.1	81.7	82.2	81.6	81.4	83.7	100	87.8	84.1
Paraburkholderia aspalathi LMG 27731^T	84.5	84.4	84.4	84.6	84.4	84.3	84.8	84.4	84.3	84.5	84.3	84.4	84.7	84.6	84.5	85.1	84.2	83.7	83.8	82	82.2	81.7	82.3	81.4	81.4	83.9	87.4	100	84.5
Paraburkholderia terricola LMG 20594^T	85.3	85.4	85.4	85.3	85.3	85.3	85.4	85.2	85.2	85.2	85.1	85.4	85.5	85.4	85.3	85.7	85.5	85	84.8	84.1	83.9	83.5	83.7	83.1	83	85.4	84.8	85.3	100

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