Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species

Amandeep Sawana, Mobolaji Adeolu and Radhey S. Gupta*

Department of Biochemistry and Biomedical Sciences, Health Sciences Center, McMaster University, Hamilton, ON, Canada

The genus *Burkholderia* contains large number of diverse species which include many clinically important organisms, phytopathogens, as well as environmental species. However, currently, there is a paucity of biochemical or molecular characteristics which can reliably distinguish different groups of *Burkholderia* species. We report here the results of detailed phylogenetic and comparative genomic analyses of 45 sequenced species of the genus *Burkholderia*. In phylogenetic trees based upon concatenated sequences for 21 conserved proteins as well as 16S rRNA gene sequence based trees, members of the genus *Burkholderia* grouped into two major clades. Within these main clades a number of smaller clades including those corresponding to the clinically important *Burkholderia cepacia* complex (BCC) and the *Burkholderia pseudomallei* groups were also clearly distinguished. Our comparative analysis of protein sequences from *Burkholderia* spp. has identified 42 highly specific molecular markers in the form of conserved sequence indels (CSIs) that are uniquely found in a number of well-defined groups of *Burkholderia* spp. Six of these CSIs are specific for a group of *Burkholderia* spp. (referred to as Clade I in this work) which contains all clinically relevant members of the genus (viz. the BCC and the *B. pseudomallei* group) as well as the phytopathogenic *Burkholderia* spp. The second main clade (Clade II), which is composed of environmental *Burkholderia* species, is also distinguished by 2 identified CSIs that are specific for this group. Additionally, our work has also identified multiple CSIs that serve to clearly demarcate a number of smaller groups of *Burkholderia* spp. including 3 CSIs that are specific for the *B. cepacia* complex, 4 CSIs that are uniquely found in the *B. pseudomallei* group, 5 CSIs that are specific for the phytopathogenic *Burkholderia* spp. and 22 other CSI that distinguish two groups within Clade II. The described molecular markers provide highly specific means for the demarcation of different groups of *Burkholderia* spp. and they also offer novel and useful targets for the development of diagnostic assays for the clinically important members of the BCC or the *pseudomallei* groups. Based upon the results of phylogenetic analyses, the identified CSIs and the pathogenicity profile of *Burkholderia* species, we are proposing a division of the genus *Burkholderia* into two genera. In this new proposal, the emended genus *Burkholderia* will correspond to the Clade I and it will contain only the clinically relevant and phytopathogenic *Burkholderia* species. All other *Burkholderia* spp., which are primarily environmental, will be transferred to a new genus *Paraburkholderia* gen. nov.

Keywords: *Burkholderia*, *Burkholderia cepacia* complex, conserved signature indels, phylogenetic trees, molecular signatures

INTRODUCTION

The genus *Burkholderia* is a morphologically, metabolically, and ecologically diverse group of gram-negative bacteria (Yabuuchi et al., 1992; Coenye and Vandamme, 2003; Mahenthiralingam et al., 2005; Palleroni, 2005; Compan et al., 2008). *Burkholderia* species are ubiquitous in the environment (Coenye and Vandamme, 2003). They inhabit a wide range of ecological niches, ranging from soil to the human respiratory tract (Coenye and Vandamme, 2003). A group of 17 closely related *Burkholderia* species, the *Burkholderia cepacia* complex (BCC), are responsible for prevalent and potentially lethal pulmonary infections in immunocompromised individuals, such as individuals with cystic
fibrosis (Mahenthiralingam et al., 2002, 2005; Biddick et al., 2003; Hauser et al., 2011). *Burkholderia pseudomallei*, a *Burkholderia* species related to the BCC, is the causative agent for the disease melioidosis, a potentially lethal septic infection which accounts for up to 20% of all community-acquired septicemias in some regions (White, 2003; Limmathurotsakul and Peacock, 2011). Other species related to the BCC are the causative agents of major infections in both animals (*Burkholderia mallei*) and plants (*Burkholderia glumae* and *Burkholderia gladioli*) (Whitlock et al., 2007; Nandakumar et al., 2009).

In spite of the large diversity and varied pathogenicity among the >70 members of the group, all *Burkholderia* species are currently placed within one genus (Coenye and Vandamme, 2003; Palleroni, 2005). The phylogeny and taxonomy of the genus *Burkholderia* is primarily defined on the basis of 16S rRNA sequence analysis (Yabuuchi et al., 1992; Palleroni, 2005; Yarza et al., 2008). The inferences obtained from 16S rRNA analysis have been further substantiated by other phylogenetic methods, including *recA* gene based analysis (Payne et al., 2005), *acdS* gene based analysis (Onofre-Lemus et al., 2009), DNA–DNA hybridization (Gillis et al., 1995), whole cell fatty acid analysis (Stead, 1992), multilocus sequence analysis (Tayeb et al., 2008; Spilker et al., 2009; Estrada-de los Santos et al., 2013), gene gain/loss analysis (Zhu et al., 2011), and whole genome phylogenetic analysis (Ussery et al., 2009; Segata et al., 2013). In many of these phylogenetic studies, the members of the genus *Burkholderia* can be divided into two or more distinct phylogenetic groups, with one group consisting of members of the BCC and related species (Payne et al., 2005; Tayeb et al., 2008; Yarza et al., 2008; Spilker et al., 2009; Ussery et al., 2009; Gyaneshwar et al., 2011; Vandamme and Dawyndt, 2011; Zhu et al., 2011; Estrada-de los Santos et al., 2013; Segata et al., 2013). Although there are some commonly shared features among closely related groups of *Burkholderia* species, there is no known morphological, biochemical, or molecular characteristic specific to the larger phylogenetic groups within the genus (ex. the BCC and related species).

The advent of next generation sequencing methods has led to a rapid increase in the number of genome sequences available for bacterial species (Mardis, 2008). The availability of these sequences for members of the genus *Burkholderia* provides us better means to evaluate the phylogenetic relationships among different species (Ciccarelli et al., 2006; Wu et al., 2009). Importantly, the large data sets of sequences allows for the use of comparative genomic techniques to discover novel molecular markers that can provide independent evidence for different phylogenetic groups within the genus *Burkholderia* (Gupta, 1998, 2014; Gao and Gupta, 2012). In this work, we describe one type of molecular marker, conserved sequence insertions or deletions (CSIs), which are uniquely present in protein sequences from a defined group of organisms, that can be used to delineate different phylogenetic groups of *Burkholderia* species independently of traditional phylogenetic methods (Gupta, 1998, 2001; Gao and Gupta, 2012). Our comparative analysis of *Burkholderia* genomes has led to the identification of 42 unique CSIs that delineate different phylogenetic groups within the genus in clear molecular terms. A clade of *Burkholderia* containing the BCC and related organisms (Clade I) was supported by both phylogenetic evidence and 6 identified CSIs. We have also identified 3 CSIs specific for the BCC, 4 CSIs specific for the *B. pseudomallei* group, and 5 CSIs specific for the plant pathogenic *Burkholderia* spp. The remaining members of the genus *Burkholderia* formed another monophyletic clade (Clade II) in our phylogenetic trees which was supported by 2 CSIs. Within Clade II, we identified two smaller clades of *Burkholderia* that were supported by 16 and 6 CSIs. The grouping of members of the genus *Burkholderia* into at least two large, monophyletic groups has also been observed in a large body of prior phylogenetic research (Payne et al., 2005; Tayeb et al., 2008; Yarza et al., 2008; Spilker et al., 2009; Ussery et al., 2009; Gyaneshwar et al., 2011; Zhu et al., 2011; Estrada-de los Santos et al., 2013; Segata et al., 2013). Based on the phylogenetic evidence and our identified CSIs, we propose division of the genus *Burkholderia* into two genera: an emended genus *Burkholderia* containing clinically important and phytopathogenic members of the genus and a new genus *Paraburkholderia* gen. nov. harboring the environmental species.

**MATERIALS AND METHODS**

**PHYLOGENETIC ANALYSIS**

A concatenated sequence alignment of 21 highly conserved proteins (viz. ArgRS, EF-G, GyrA, GyrB, Hsp60, Hsp70, IleRS, RecA, RpoB, RpoC, SecY, ThrRS, TrpS, UvrD, ValRS, 50S ribosomal proteins L1, L5 and L6, and 30S ribosomal proteins S2, S8 and S11) was used to perform phylogenetic analysis. Due to their presence in most bacteria, these proteins have been extensively utilized for phylogenetic studies (Gupta, 1998, 2009; Kyrpides et al., 1999; Harris et al., 2003; Charlebois and Doolittle, 2004; Ciccarelli et al., 2006). The amino acid sequences for these conserved proteins were obtained from NCBI database for all of the species/stains listed in Table 1, which includes 45 sequenced species of the genus *Burkholderia*. Furthermore, three genomes from other members of class Betaproteobacteria (viz. *Cupriavidus necator* N-1, *Bordetella pertussis* Tohama I, and *Neisseria meningitidis* MC58), serving as outgroups in our analysis, were also retrieved from NCBI database. Depending on genome availability, type strains were selected for most of the species. Multiple sequence alignments for these proteins were created using Clustal_X 1.83 and concatenated into a single alignment file (Jeanmougin et al., 1998). Poorly aligned regions from the alignment file were removed using Gblocks 0.91b and the resulting alignment, which contained 7688 aligned characters, was ultimately utilized for phylogenetic analysis (Castresana, 2000). A maximum likelihood (ML) tree based on 100 bootstrap replicates of this alignment was constructed using MEGA 6.0 while employing Jones-Taylor–Thornton substitution model (Jones et al., 1992; Tamura et al., 2013).

A maximum likelihood 16S rRNA gene sequence consensu-
Table 1 | Genome characteristics of the sequenced members of the genus *Burkholderia*.

| Organism                        | BioProject       | Size (Mb) | GC%  | Chromosomes | Proteins | References         |
|---------------------------------|------------------|-----------|------|-------------|----------|--------------------|
| *Burkholderia cenocepacia* J2315| PRJN57953        | 8.06      | 66.9 | 3           | 7116     | Holden et al., 2009|
| *Burkholderia pseudomallei* K96243 | PRJN57733       | 7.25      | 68.1 | 2           | 5727     | Holden et al., 2004|
| *Burkholderia mallei* ATCC 23344 | PRJN57725        | 5.84      | 68.5 | 2           | 5022     | Nierman et al., 2004|
| *Burkholderia thailandensis* E264 | PRJN58081        | 6.72      | 67.6 | 2           | 5632     | Kim et al., 2005   |
| *Burkholderia oklahomensis* C6786 | PRJN54789        | 6.99      | 67.0 | –           | 6954     | NMRCb              |
| *Burkholderia multivorans* ATCC 17616 | PRJN58909       | 7.01      | 66.7 | 3           | 6111     | DOEd               |
| *Burkholderia ambifaria* AMMD   | PRJN58303        | 7.53      | 66.8 | 3           | 6610     | Coenye et al., 2001b|
| *Burkholderia glumae* BGR1     | PRJN59397        | 7.28      | 67.9 | 2           | 5773     | Lim et al., 2009   |
| *Burkholderia xenovorans* LB400 | PRJN57823        | 9.73      | 62.6 | 3           | 8702     | Chain et al., 2006 |
| *Burkholderia sp.* CCGE1002    | PRJN54789        | 7.04      | 63.2 | 2           | 5988     | DOEd               |
| *Burkholderia sp.* Ch1-1       | PRJN46253        | 7.04      | 63.2 | 2           | 5988     | DOEd               |
| *Burkholderia sp.* J2315       | PRJN46975        | 7.84      | 62.4 | –           | 7742     | DOEd               |
| *Burkholderia sp.* H160        | PRJN45101        | 7.89      | 62.9 | –           | 7460     | Ormeno-Orrillo et al., 2012|
| *Burkholderia sp.* JI49        | PRJN458073       | 8.68      | 66.3 | 3           | 7716     | DOEd               |
| *Burkholderia sprentiae* WSM5005 | PRJN46661        | 7.76      | 63.2 | –           | –        | DOEd               |
| *Burkholderia sp.* YI23        | PRJN481081       | 8.90      | 63.3 | 3           | 7804     | Lim et al., 2012   |
| *Burkholderia sp.* SJ98        | PRJN160003       | 7.88      | 61.4 | –           | 7268     | Kumar et al., 2012 |
| *Burkholderia sp.* WSM2230     | PRJN165309       | 6.31      | 63.1 | –           | –        | DOEd               |
| *Burkholderia sp.* KJ006       | PRJN165871       | 6.63      | 67.2 | 3           | 6024     | Kwak et al., 2012  |
| *Burkholderia sp.* TI49        | PRJN179699       | 7.38      | 66.9 | –           | 8940     | Khan et al., 2013  |
| *Burkholderia sp.* BT03        | PRJN180532       | 10.64     | 61.9 | –           | 10126    | Oak Ridgec         |
| *Burkholderia sp.* WSM2232     | PRJN182741       | 7.21      | 63.1 | –           | –        | DOEd               |
| *Burkholderia sp.* WSM3556     | PRJN182743       | 7.68      | 61.8 | –           | –        | DOEd               |
| *Burkholderia sp.* URHA0054    | PRJN190816       | 7.24      | 62.8 | –           | –        | DOEd               |
| *Burkholderia sp.* WSM4176     | PRJN199219       | 9.07      | 62.9 | –           | 8336     | DOEd               |
| *Burkholderia sp.* JPY251      | PRJN199221       | 8.61      | 63.1 | –           | 7873     | DOEd               |
| *Burkholderia sp.* JPY347      | PRJN199222       | 6.39      | 63.1 | –           | 5963     | DOEd               |
| *Burkholderia sp.* RPE64       | PRJN200541       | 6.96      | 63.1 | 3           | 6498     | Shibata et al., 2013|
| *Burkholderia vietnamiensis* G4 | PRJN58075        | 8.39      | 65.7 | 3           | 7617     | DOEd               |
| *Burkholderia dolosa* AUO158   | PRJN54351        | 6.42      | 66.8 | –           | 4795     | Broad Institutea   |
| *Burkholderia phytophylum* STM815 | PRJN56999       | 8.68      | 62.3 | 2           | 7496     | Vandamme et al., 2002b|
| *Burkholderia phytofirmans* PsJN | PRJN56729        | 8.21      | 62.3 | 2           | 7241     | Weiharter et al., 2011|
| *Burkholderia ubonensis* Bu    | PRJN54793        | 6.93      | 67.3 | –           | 7181     | NMRCb              |
| *Burkholderia graminis* C4D1M  | PRJN54887        | 7.48      | 62.9 | –           | 6747     | DOEd               |
| *Burkholderia rhizoxinica* HKI 454 | PRJN60487     | 3.75      | 60.7 | 1           | 3870     | Lackner et al., 2011|
| *Burkholderia gladioli* BSR3   | PRJN66301        | 9.05      | 67.4 | 2           | 7411     | Seo et al., 2011   |
| *Burkholderia cepacia* GG4     | PRJN173858       | 6.47      | 66.7 | 2           | 5825     | Hong et al., 2012  |
| *Candidatus Burkholderia* kirkii UZHbot1 | PRJN74017   | 4.01      | 62.9 | –           | 2069     | Van Develen et al., 2002b|
| *Burkholderia mimosarum* LMG 23256 | PRJN163559     | 8.41      | 63.9 | –           | –        | DOEd               |
| *Burkholderia terrae* B5001   | PRJN168186       | 11.29     | 61.8 | –           | 10234    | Nazir et al., 2012 |
| *Burkholderia pyrrocinia* CH-67 | PRJN199595      | 8.05      | 67.4 | –           | 7324     | Song et al., 2012  |
| *Burkholderia kururiensis* M130 | PRJN199910      | 7.13      | 65.0 | –           | 6311     | Coutinho et al., 2013|
| *Burkholderia phenoliruptrix* BR3459a | PRJN176370  | 7.65      | 63.1 | 2           | 6496     | Oliveira Cunha et al., 2012|
| *Burkholderia bryophila* 376MFSHa3.1 | PRJN201182    | 7.38      | 61.9 | –           | 6722     | DOEd               |

aThe Broad Institute Genome Sequencing Platform (Broad Institute).
bNaval Medical Research Center/Biological Defense Research Directorate (NMRC).
cOak Ridge National Lab (Oak Ridge).
dDOE Joint Genome Institute (DOE).
this alignment was constructed using the General Time Reversible Model (Tavaré, 1986) in MEGA 6.0 (Tamura et al., 2013).

IDENTIFICATION OF MOLECULAR MARKERS (CSIs)
BLASTp searches were conducted for all proteins from chromosomes 2 and 3 (accession numbers NC_008061 and NC_008061) of *Burkholderia cenocepacia* J2315 (Holden et al., 2009) to identify CSIs that are shared by different members of the genus *Burkholderia*. Species that appeared as top hits with high scoring homologs (*E* values < 1e−20) from the genus *Burkholderia* and other outgroups were selected. Multiple sequence alignments were created using the Clustal X 1.83 (Jeanmougin et al., 1998). These alignments were visually inspected for the presence of insertions or deletions (indels) restricted to either some or all members of the genus *Burkholderia* and flanked by at least 5–6 conserved amino acid residues on both sides in the neighboring 30–40 amino acids. Indel queries that were not flanked by conserved regions were not further evaluated. The species specificity of the indel queries meeting the above criterion was further evaluated by performing BLASTp searches on short sequence segments containing the insertions or deletions, and their flanking conserved regions (60–100 amino acids long). The searches were conducted against the NCBI non-redundant (nr) database and a minimum of 250 BLAST hits were examined for the presence or absence of CSIs. The results of these analyses were evaluated as described in detail in our recent work (Gupta, 2014). Signature files for the CSIs that were specific for members of the genus *Burkholderia* were created and formatted using the programs SIG_CREATE and SIG_STYLE (accessible from Gleans.net) as described by Gupta (2014). The sequence alignment files presented here contain information for all detected insertions or deletions from the *Burkholderia* group of interest, but only a limited number from species that are serving as outgroups. Sequence information for different strains of various species is not shown, but they all exhibited similar pattern. Lastly, unless otherwise indicated, the CSIs shown here are specifically found in the indicated groups and similar CSIs were not detected in the 250 Blast hits with the query sequences.

RESULTS
BRANCHING PATTERN OF BURKHOLDERIA SPECIES IN CONCATENATED PROTEIN AND 16S rRNA TREES

Genome sequences of 45 species of *Burkholderia* were available from the NCBI genome database at the time of this work (NCBI, 2014). Some characteristics of these genomes are listed in Table 1. The genome sizes of the sequenced *Burkholderia* species show large variation (from 3.75–11.29 Mb) and the numbers of proteins in them also varied in a similar proportion. In this work we have produced a ML phylogenetic tree based on the concatenated amino acid sequences of 21 conserved housekeeping and ribosomal proteins obtained from 45 sequenced *Burkholderia* species (Figure 1). The *Burkholderia* species formed two large clades in the protein based ML tree: One consisting of the BCC and related organisms (Clade I) and another comprised mainly of environmental or poorly characterized *Burkholderia* species (Clade II). Within Clade I, three smaller, distinct clades are also observed. The first of these clades (Clade Ia) is wholly comprised of the sequenced BCC species, the second clade (Clade Ib) groups *B. pseudomallei* and closely related species, and the third clade (Clade Ic) consists of the plant pathogenic species, *B. glumae* and *B. gladioli*. Clade II could also be divided into two smaller clades, Clade IIa and Clade IIb. Clade IIa is separated from Clade IIb by a long branch, suggesting that a large amount of genetic divergence has occurred between the two groups. In addition to the two main clades of *Burkholderia*, two species, *Burkholderia sp*. JPY347 and *Burkholderia rhizoxinica*, branched early in the tree and did not associate with either Clade I or II.

We have also constructed a 16S rRNA based ML phylogenetic tree for 97 *Burkholderia* strains and candidate species (Figure 2). In this 16S rRNA based phylogenetic tree we observed broadly similar patterns to our protein based phylogeny. A clade consisting of the BCC and related organisms (Clade I) was clearly resolved. The three subclades within Clade I, the BCC (Clade Ia), the *B. pseudomallei* group (Clade Ib), and the plant pathogenic species (Clade Ic) were well resolved, though some species exhibited aberrant branching (ex. *B. oklahomensis* and *B. pseudomultivorans*). A large assemblage of the remaining *Burkholderia* species, roughly corresponding to Clade II in our concatenated protein based phylogenetic tree, was also observed in the 16S rRNA tree. However, due to significant number of unsequenced *Burkholderia* species which are present in the 16S rRNA database it is difficult to accurately identify the groups within Clade II of the 16S rRNA tree which correspond to Clades IIa and IIb in our concatenated protein based phylogenetic tree. Bootstrap support for branches in the 16S rRNA based tree were also significantly lower than they were in the concatenated protein tree indicating that some of the observed branching patterns may not be reliable. However, the clade consisting of the BCC and related organisms (Clade I) has strong bootstrap support and has been identified in a large number of previous 16S rRNA based phylogenetic studies (Yabuuchi et al., 1992; Palleroni, 2005; Yarza et al., 2008; Suarez-Moreno et al., 2012).

MOLECULAR SIGNATURES DISTINGUISHING THE CLADE I AND CLADE II BURKHOLDERIA
Rare genetic changes, such as insertions and deletions in essential genes/proteins, which occur in a common ancestor can be inherited by the various decedent species related to this common ancestor (Gupta, 1998; Rokas and Holland, 2000; Gogarten et al., 2002; Gupta and Griffiths, 2002). Due to the rarity and the specific presence of these rare genetic changes to a related group of organisms, they can serve as important molecular markers and provide a novel means to understand the evolutionary interrelationships between different closely related species (Gupta, 1998; Gupta and Griffiths, 2002; Gao and Gupta, 2012).

The comparative analysis of protein sequences from *Burkholderia* species that was carried out in the present work has identified a number of CSIs that serve to clearly distinguish a number of different clades within the genus *Burkholderia*. These studies have led to identification of 6 CSIs that are specific for the Clade I *Burkholderia*, consisting of the BCC and related organisms, enabling clear distinction of this group from all other *Burkholderia*. This clade, which contains all well characterized pathogens within the genus, represents the most clinically...
A maximum likelihood phylogenetic tree of the genome sequenced members of the genus *Burkholderia* based upon concatenated sequences of 21 conserved proteins. The tree was rooted using *Cupriavidus necator* N-1, *Bordetella pertussis* Tohama I, and *Neisseria meningitides* MC58. Bootstrap analysis scores are indicated for each node. The major *Burkholderia* clades (Clades I and II) and their main sub-clades are indicated by brackets.

relevant group within the *Burkholderia*. All species within this clade are potentially pathogenic to human, animals, or plants and most have been isolated from clinical human samples (Simpson et al., 1994; Mahenthiralingam et al., 2002, 2005; Biddick et al., 2003; O’Carroll et al., 2003). One example of a CSI that is specific to the Clade I *Burkholderia* is shown in Figure 3A. In this case, a one amino acid deletion is present in a highly conserved region of a periplasmic amino acid-binding protein. The indel is flanked on both sides by highly conserved regions indicating that it is not the result of alignment artifacts and that it is a reliable genetic characteristic. This CSI is present in all of the sequenced members of the Clade I *Burkholderia*, but absent in all other bacterial homologs of this protein. Our work has identified 5 additional CSIs in other widely distributed proteins that are
FIGURE 2 | A maximum likelihood tree based on the 16S rRNA gene sequences of 97 members of the genus *Burkholderia*. Accession numbers for the 16S rRNA sequenced used for each organism are provided in the brackets following the name of the organism. The tree was rooted using four species from the genera *Cupriavidus* and *Ralstonia*. Bootstrap analysis scores are indicated for each node. The major *Burkholderia* clades (Clades I and II) and the subclades within Clade I are indicated by brackets.
FIGURE 3 | Partial sequence alignments of (A) a periplasmic amino acid-binding protein showing a 1 amino acid deletion identified in all members of Clade I of the genus *Burkholderia* (B) a dehydrogenase showing a 1 amino acid insertion (boxed) identified only in members of Clade II of the genus *Burkholderia*. These CSIs were not found in the sequence homologs of these proteins from any other sequenced bacteria. In each case, sequence information for a *Burkholderia* species and a limited number other bacteria are shown, but unless otherwise indicated, similar CSIs were detected in all members of the indicated group and not detected in any other bacterial species in the top 250 BLAST hits. The dashes (–) in the alignments indicate identity with the residue in the top sequence. GenBank identification (GI) numbers for each sequence are indicated in the second column. Sequence information for other CSIs specific to the members of Clade I and Clade II of the genus *Burkholderia* are presented in Supplemental Figures 1–5 and Supplemental Figure 6, respectively, and their characteristics are summarized in Table 2.
specific for the Clade I *Burkholderia* and sequence alignments for these CSIs are shown in Supplemental Figures 1–5 and a summary of their characteristics is provided in Table 2.

Two additional CSIs identified in this work are specific for the Clade II *Burkholderia* species which is made up of mainly environmental organisms. One of these CSIs, shown in Figure 3B, consists of a one amino acid insertion in a dehydrogenase protein that is uniquely found in members of the Clade II *Burkholderia* and absent in all other *Burkholderia* species as well all other bacterial groups. A sequence alignment for another CSI that is specific for the Clade II *Burkholderia* (a 2 aa deletion in a LysR family of transcription regulator protein) is shown in Supplemental Figure 6 and its characteristics are summarized in Table 2.

**CSIs distinguishing different main groups within the clade I Burholderia**

The species within Clade I of the genus *Burkholderia* are responsible for a range of human, animal, and plant diseases (Biddick et al., 2003; Mahenthiralingam et al., 2005). The members of Clade I (i.e., the BCC and related *Burkholderia*) are commonly separated into 3 main groups which correspond to clades identified in our phylogenetic trees. The first group, the members of the BCC (Clade 1a), are prevalent pathogens in cystic fibrosis patients, the second group, the *B. pseudomallei* group (Clade 1b), contains the causative agents of melioidosis and glanders, while the third group contains the plant pathogenic *Burkholderia* species (Clade 1c) (White, 2003; Mahenthiralingam et al., 2005; Whitlock et al., 2007; Nandakumar et al., 2009). Our analysis has identified 3 CSIs that are specific for all members of the BCC clade (Clade 1a). One example of a BCC clade specific CSI is shown in Figure 4A. This CSI consists of a 2 amino acid insertion in a conserved region of a histidine utilization repressor which is only found in members of the BCC. Sequence alignments for two other BCC clade specific CSIs are shown in Supplemental Figures 7, 8 and their characteristics are summarized in Table 3.

Our work has also identified 4 CSIs that are specific for the *B. pseudomallei* group (Clade Ib) which contains the most prevalent human pathogen within the genus, *B. pseudomallei* (Wiersinga et al., 2006). One example of a CSI specific to the *B. pseudomallei* group, which consists of a 1 amino acid insertion in a conserved region of a periplasmic oligopeptide-binding protein, is shown in Figure 4B. Sequence alignments for three other CSIs in three different proteins that are specific for the *B. pseudomallei* group are shown in Supplemental Figures 9–11 and their characteristics are summarized in Table 3.

We have also identified 5 CSIs that are specific for the major plant pathogenic group within the genus *Burkholderia* (Clade 1c) which contains the species *B. glumae* and *B. gladioli*. An example of a CSI representing this group is shown in Figure 4C. This CSI consists of a 1 amino acid insertion in a conserved region of a SMP-30/gluconolactonase/LRE-like region-containing protein that is found in the members of Clade 1c of the genus *Burkholderia* but absent in all other *Burkholderia* and all other bacterial groups. Sequence alignments for the other 4 CSIs are shown in Supplemental Figures 12–15 and their key features are highlighted in Table 3.

### Table 2 | Conserved signature indels specific for the two major clades within the genus *Burkholderia*.

| Protein Name                                | GI Number   | Figures                  | Indel size | Indel position | Specificity |
|---------------------------------------------|-------------|--------------------------|------------|----------------|-------------|
| Periplasmic amino acid-binding protein      | 385357135   | Figure 3A                | 1 aa del   | 135–195        | Clade I     |
| Putative lyase                              | 167724527   | Supplemental Figure 1    | 1 aa del   | 70–121         | Clade I     |
| 4-hydroxybenzoate 3-monoxygenase            | 238023559   | Supplemental Figure 2    | 1 aa ins   | 101–171        | Clade I     |
| 6-phosphogluconate dehydrogenase, decarboxylating | 330620932 | Supplemental Figure 3    | 1 aa ins   | 137–202        | Clade I     |
| Putative lipoprotein                        | 121598811   | Supplemental Figure 4    | 1 aa del   | 363–393        | Clade I     |
| Sarcosine oxidase subunit alpha             | 493818877   | Supplemental Figure 5    | 3 aa ins   | 904–965        | Clade I     |
| Dehydrogenase                              | 497456569   | Figure 3B                | 1 aa ins   | 279–333        | Clade II    |
| LysR family transcriptional regulator       | 187919777   | Supplemental Figure 6    | 2 aa del   | 260–294        | Clade II    |

*The region of the specified protein that contains the indel.*
## FIGURE 4 | Continued

### A

| Species                                      | Accession Number | Molecular Signatures |
|----------------------------------------------|------------------|----------------------|
| *Burkholderia ambifaria* MC40-6              | 172064454        |                      |
| *Burkholderia cepacia* GQ4                   | 402570387        |                      |
| *Burkholderia cenocepa* AU 10                | 107027579        |                      |
| *Burkholderia sp.* 383                       | 78060928         |                      |
| *Burkholderia sp.* 7J49                      | 497760287        |                      |
| *Burkholderia vietnamiensis* G4              | 134292445        |                      |
| *Burkholderia ubonensis*                     | 497780720        |                      |
| *Burkholderia multivorans* ATCC              | 161519776        |                      |
| *Burkholderia sp.* KJ006                     | 367904119        |                      |
| *Burkholderia pyrrocina*                     | 515903949        |                      |
| *Burkholderia sp.* 1C1-1                     | 494315769        |                      |
| *Burkholderia xenovorans* LB400              | 91778287         |                      |
| *Burkholderia graminis*                      | 492398493        |                      |
| *Burkholderia phytofirmans* PsJN             | 157919544        |                      |
| *Burkholderia sp.* COGE1001                  | 323526957        |                      |
| *Burkholderia phenoliruptrix* BR             | 407710689        |                      |
| *Burkholderia terrae*                        | 484863638        |                      |
| *Burkholderia sp.* BT03                      | 495019334        |                      |
| *Burkholderia sp.* CCGE1003                  | 307776262        |                      |
| *Burkholderia phymatum* STU15                | 180473897        |                      |
| *Burkholderia sp.* CCGE1002                  | 295693099        |                      |
| *Burkholderia sp.* H160                      | 496198692        |                      |
| *Burkholderia thailandensis*                 | 492999232        |                      |
| *Comamonas testosteroni* C2                 | 264672622        |                      |
| *Allicyclophilus denitrificans* B            | 319763461        |                      |
| *Acidovorax cyclitluri* AAC00-1              | 120611829        |                      |
| *Hyphomonas gracilis*                        | 493342257        |                      |
| *Verrminephrobacter eiseniae* EF0            | 131615071        |                      |
| *Delftia acidovorans*                        | 512560547        |                      |
| *Polaromonas sp.* JS666                      | 91786943         |                      |
| *Cupriavidus sp.* HM-1                       | 495920195        |                      |
| *Ralstoniaeutropha* JMP34                     | 73542402         |                      |

### B

| Species                                      | Accession Number | Molecular Signatures |
|----------------------------------------------|------------------|----------------------|
| *Burkholderia thailandensis* MSM            | 488606492        |                      |
| *Burkholderia mallei* ATCC 23344             | 5376164         |                      |
| *Burkholderia pseudomallei*                  | 497801103        |                      |
| *Burkholderia oklahomensis*                 | 497809584        |                      |
| *Burkholderia gladioli* BS80                | 330382176        |                      |
| *Burkholderia sp.* 383                       | 78060968        |                      |
| *Burkholderia dolosa*                        | 49391092        |                      |
| *Burkholderia multivorans*                   | 49345093        |                      |
| *Burkholderia ubonensis*                     | 49775972        |                      |
| *Burkholderia sp.* TJ49                      | 497378269        |                      |
| *Burkholderia ambifaria* AMMD                | 115350611        |                      |
| *Burkholderia sp.* KJ006                     | 367904089        |                      |
| *Burkholderia cenocepa* MC0-3                | 17073090         |                      |
| *Burkholderia vietnamiensis* G4              | 134292420        |                      |
| *Burkholderia cepacia* GQ4                   | 402570412        |                      |
| *Burkholderia phymatum* CCGE1003             | 307972711        |                      |
| *Burkholderia phytofirmans* PsJN             | 187919153        |                      |
| *Burkholderia graminis*                      | 492927968        |                      |
| *Burkholderia sp.* BT03                      | 495017718        |                      |
| *Burkholderia terrae*                        | 494757322        |                      |
| *Burkholderia sp.* CH1-1                     | 494314859        |                      |
| *Burkholderia sp.* CGGE1001                  | 323526511        |                      |
| *Burkholderia phymatum* STU15                | 18473232         |                      |
| *Burkholderia xenovorans* LB400              | 91779339         |                      |
| *Burkholderia sp.* RPE64                     | 507526505        |                      |
| *Burkholderia sp.* SJ98                      | 495628597        |                      |
| *Burkholderia sp.* Y123                      | 377812739        |                      |
| *Kingella kingae*                             | 489807671        |                      |
| *Polaromonas sp.* CF318                      | 495145811        |                      |
| *Yersinia enterocolitica* (type)             | 51043109         |                      |
| *Neisseria weaveri*                          | 49041119         |                      |
Sawana et al. Molecular signatures distinguishing Burkholderia species

FIGURE 4 | Partial sequence alignments of (A) a histidine utilization repressor showing a 2 amino acid insertion (boxed) identified in all members of the Burkholderia cepacia complex (Clade Ia) within the genus Burkholderia (B) a periplasmic oligopeptide-binding protein showing a 1 amino acid insertion (boxed) identified in all members of the Burkholderia pseudomallei group (Clade Ib) within the genus Burkholderia (C) a SMP-30/gluconolaconase/LRE-like region-containing protein showing a 1 amino acid insertion (boxed) identified in all members of the phytopathogenic Burkholderia clade (Clade Ic). These CSIs were not found in the sequence homologs of these proteins from any other sequenced bacteria in the top 250 BLAST hits. Sequence information for other CSIs specific to subclades within Clade I of the genus Burkholderia are presented in Supplemental Figures 7–15 and their characteristics are summarized in Table 3.

The second group within Clade II of the Burkholderia (Clade IIb), is comprised of a large variety of environmental Burkholderia species (Coenye and Vandamme, 2003; Suarez-Moreno et al., 2012). Our analysis has identified 6 CSIs that are specific to this large group of Burkholderia species. One example of a CSI specific to the members of Clade IIb of the genus Burkholderia is shown in Figure 5B. The CSI consists of a one amino acid insertion in 4-hydroxyacetophenone monooxygenase, which is only present in members of Clade IIb of the genus Burkholderia and not in protein homologs from any other sequenced bacterial group. Information for other 5 CSIs which are specific to members of Clade IIb of the genus Burkholderia are shown in Supplemental Figures 31–35 and their characteristics are summarized in Table 3.

DISCUSSION

The genus Burkholderia is one of the largest groups of species within the class Betaproteobacteria (Palleroni, 2005; Parte, 2013). The genus contains a variety of bacteria that inhabit a wide range of ecological niches including a number of bacteria that have pathogenic potential (Yabuuchi et al., 1992; Coenye and Vandamme, 2003; Mahenthiralingam et al., 2005; Palleroni, 2005; Compart et al., 2008). The phylogeny of the genus Burkholderia has been studied using a wide array of methodologies based on phenotypic, biochemical, genetic, and genomic characteristics (Stead, 1992; Gillis et al., 1995; Payne et al., 2005; Tayeb et al., 2008; Onofre-Lemus et al., 2009; Spilker et al., 2009; Ussery et al., 2009; Gyaneshwar et al., 2011; Vandamme and Dawyndt, 2011; Zhu et al., 2011; Estrada-de los Santos et al., 2013). These studies have provided novel insights into the evolutionary relationship of the species within the genus Burkholderia. However, no taxonomic changes have been made to date due to a lack of discrete, distinguishing characteristics identified for the different phylogenetic lineages within the genus (Estrada-de los Santos et al., 2013).

In the present work, we have outlined two major groups of species within the genus Burkholderia: Clade I, which contains all pathogenic members of the genus, and Clade II, which contains a large variety of environmental species. These two groups were found to branch distinctly in a highly resolved phylogenetic tree based on a large number of concatenated protein sequences produced in this work (Figure 1). Evidence for the distinctness of Clade 1 organisms from other Burkholderia species has been observed in a wide range of previous phylogenetic studies (Payne et al., 2005; Tayeb et al., 2008; Yarza et al., 2008; Spilker et al., 2009; Ussery et al., 2009; Gyaneshwar et al., 2011; Vandamme and Dawyndt, 2011; Zhu et al., 2011; Suarez-Moreno et al., 2012; Estrada-de los Santos et al., 2013; Segata et al., 2013). Importantly, we have also identified 6 and 2 CSIs that serve as discrete molecular characteristics of Clade I and Clade II, respectively (Figure 6 and Table 2). These CSIs are the...
first discrete features that have been identified that are unique to either Clade I or Clade II of the genus *Burkholderia*. These CSIs act as independent verification of the phylogenetic trends identified in this and other studies and provide clear evidence that the species from the Clade I are distinct from all other *Burkholderia* and that they are derived from a common ancestor exclusive of all other *Burkholderia*. Although sequence information for Clade II members is at present somewhat limited, based upon the shared presence of two CSIs by them, it is likely that they are also derived from a common ancestor exclusive of other bacteria.

Additionally, we have identified molecular evidence, in the form of large numbers of CSIs, which support the distinctiveness of several smaller groups within the genus *Burkholderia*. The most important of these groups, the *B. cepacia* complex (BCC; Clade Ia) and the *B. pseudomallei* group (Clade Ib), are supported by the 3 and 4 of the identified CSIs, respectively. The BCC are a group of opportunistic pathogens which colonize immunodeficient human hosts and are among the most prevalent and lethal infections in cystic fibrosis patients (Mahenthiralingam et al., 2002, 2005; Biddick et al., 2003; Hauser et al., 2011). The 17 species that make up the BCC are closely related and form a tight monophyletic cluster within the genus *Burkholderia* (Vandamme and Dawyndt, 2011). The identified CSIs are highly specific for Clade I and II of the genus *Burkholderia*.
Sawana et al. Molecular signatures distinguishing *Burkholderia* species

**FIGURE 5** Partial sequence alignments of (A) 3-phosphoglycerate dehydrogenase showing a 1 amino acid insertion (boxed) identified in all members of Clade IIa of the genus *Burkholderia* (B) 4-hydroxyacetophenone monoxygenase showing a 1 amino acid insertion (boxed) identified only in members of Clade IIb of the genus *Burkholderia*. These CSIs were not found in the sequence homologs of these proteins from any other sequenced bacteria in the top 250 BLAST hits. Sequence information for other CSIs specific to subclades within Clade II of the genus *Burkholderia* are presented in Supplemental Figures 16–35 and their characteristics are summarized in Table 3.
Sawana et al. Molecular signatures distinguishing \textit{Burkholderia} species

FIGURE 6 | A summary diagram depicting the distribution of identified CSIs and the proposed names of the two major groups (Clade I and II) within \textit{Burkholderia}. The major \textit{Burkholderia} clades are indicated by brackets and highlighting.

provide novel and useful targets for the development of diagnostic assays for either the BCC or the \textit{B. pseudomallei} group (Ahmod et al., 2011; Wong et al., 2014). We have identified CSIs for three other groups within the genus \textit{Burkholderia}: A group containing unnamed and candidate \textit{Burkholderia} species (Clade Ia), a group consisting of environmental \textit{Burkholderia} (Clade Iib). We have identified 6, 16, and 6 CSIs for these three groups, respectively. These CSIs provide important differentiating characteristics for these groups, particularly for Clades Iia and Iib which are related groups that have no other identified differentiating characteristics (Suarez-Moreno et al., 2012).

The phylogenetic analyses, identified CSIs, and the pathogenic characteristics of the different \textit{Burkholderia} species presented in this work strongly suggest that the genus \textit{Burkholderia} is made up of at least two distinct lineages. One lineage consisting of the BCC and related organisms (Clade I) and another consisting of a wide range of environmental organisms (Clade II). This latter clade is phylogenetically highly diverse and there is a paucity of sequence information available for its members. Thus, it is possible that in future this latter clade may be found to consist of more than one distinct bacterial lineage, however, it is currently clear that Clade I and Clade II represent distinct lineages. Evidence for the distinctness of the Clade I members from other \textit{Burkholderia} species has been identified in...
Table 4 | Descriptions of the new combinations in the genus *Paraburkholderia* gen. nov.

| New Combination                        | Basonym                  | Type Strain                                                                 | References              |
|----------------------------------------|--------------------------|----------------------------------------------------------------------------|-------------------------|
| *Paraburkholderia acidipaludis* comb. nov. | *Burkholderia acidipaludis* | SA33<br>NBRC 101816<br>VTCC-D6-6                                           | Aizawa et al., 2010b    |
| *Candidatus Paraburkholderia andongensis* comb. nov. | *Candidatus Burkholderia andongensis* | —                                                                          | Lemaire et al., 2011    |
| *Paraburkholderia andropogonis* comb. nov.    | *Burkholderia andropogonis* | ATCC 23061<br>CCUG 32772<br>CFBP 2421<br>CIP 105771<br>DSM 9511<br>ICMP 2807<br>JCM 10487<br>LMG 2129<br>NCPPB 934<br>NRRL B-14296 | Gillis et al., 1995         |
| *Paraburkholderia aspalathi* comb. nov.        | *Burkholderia aspalathi*     | VG1C<br>DSM 27239<br>LMG 27731                                              | Mavengere et al., 2014  |
| *Paraburkholderia bannensis* comb. nov.        | *Burkholderia bannensis*     | E25<br>BCC 36998<br>NBRC 103871                                            | Aizawa et al., 2011     |
| *Paraburkholderia bryophila* comb. nov.         | *Burkholderia bryophila*     | 1S18<br>CCUG 52993<br>LMG 23644                                            | Vandamme et al., 2007   |
| *Paraburkholderia caballeronis* comb. nov.       | *Burkholderia caballeronis*   | TNe-841<br>CIP 110324<br>LMG 26416                                          | Martinez-Aguilar et al., 2013 |
| *Paraburkholderia caledonica* comb. nov.        | *Burkholderia caledonica*     | W50D<br>CCUG 42236<br>CIP 107098<br>JCM 21561<br>LMG 19076<br>NBRC 102488 | Coenye et al., 2001a    |
| *Candidatus Paraburkholderia calva* comb. nov.   | *Candidatus Burkholderia calva* | —                                                                          | Van Oevelen et al., 2004 |
| *Paraburkholderia caribensis* comb. nov.        | *Burkholderia caribensis*     | MWAP64<br>CCUG 42847<br>CIP 106784<br>DSM 13236<br>LMG 18531              | Achouak et al., 1999    |
| *Paraburkholderia caryophylli* comb. nov.       | *Burkholderia caryophylli*    | ATCC 25418<br>CCUG 20834<br>CFBP 2429<br>CFBP 3818<br>CIP 105770<br>DSM 50341<br>HAMBI 2159<br>ICMP 512 | Yabuuchi et al., 1992    |

(Continued)
| New Combination                        | Basonym                  | Type Strain                  | References                  |
|----------------------------------------|--------------------------|------------------------------|------------------------------|
| **Paraburkholderia choica comb. nov.**  | *Burkholderia choica*    | LMG 22940                    | Vandamme et al., 2013        |
|                                        |                          | CCUG 63063                   |                              |
| **Paraburkholderia denitrificans comb. nov.** | *Burkholderia denitrificans* | KIS30-44                    | Lee et al., 2012              |
|                                        |                          | DSM 24336                    |                              |
|                                        |                          | KACC 12733                   |                              |
| **Paraburkholderia diazotrophica comb. nov.** | *Burkholderia diazotrophica* | JPY461                      | Sheu et al., 2013             |
|                                        |                          | NKMU-JPY461                  |                              |
|                                        |                          | BCRC 80259                   |                              |
|                                        |                          | KCTC 23308                   |                              |
|                                        |                          | LMG 26031                    |                              |
| **Paraburkholderia dilworthii comb. nov.** | *Burkholderia dilworthii* | WSM3556                     | De Meyer et al., 2014         |
|                                        |                          | LMG 27173                    |                              |
|                                        |                          | HAMBI 3353                   |                              |
| **Paraburkholderia eburne comb. nov.**  | *Burkholderia eburne*    | RR11                         | Kang et al., 2014             |
|                                        |                          | KEMC 7302-065                |                              |
|                                        |                          | JCM 18070                    |                              |
| **Paraburkholderia endofungorum comb. nov.** | *Burkholderia endofungorum* | HKI 456                     | Partida-Martinez et al., 2007|
|                                        |                          | CIP 109454                   |                              |
|                                        |                          | DSM 19003                    |                              |
| **Paraburkholderia ferrariae comb. nov.** | *Burkholderia ferrariae* | FeGl01                       | Valverde et al., 2006         |
|                                        |                          | CECT 7171                    |                              |
|                                        |                          | DSM 18251                    |                              |
|                                        |                          | LMG 23612                    |                              |
| **Paraburkholderia fungorum comb. nov.** | *Burkholderia fungorum*  | Croize P763-2                 | Coenye et al., 2001a          |
|                                        |                          | CCUG 31961                   |                              |
|                                        |                          | CIP 107096                   |                              |
|                                        |                          | JCM 21562                    |                              |
|                                        |                          | LMG 16225                    |                              |
|                                        |                          | NBRC 102489                  |                              |
| **Paraburkholderia ginsengisoli comb. nov.** | *Burkholderia ginsengisoli* | KMY03                       | Kim et al., 2006              |
|                                        |                          | KCTC 12389                   |                              |
|                                        |                          | NBRC 100965                  |                              |
| **Paraburkholderia glathei comb. nov.**  | *Burkholderia glathei*   | ATCC 29195                   | Vandamme et al., 1997         |
|                                        |                          | CFBP 4791                    |                              |
|                                        |                          | CIP 105421                   |                              |
|                                        |                          | DSM 50014                    |                              |
|                                        |                          | JCM 10563                    |                              |
|                                        |                          | LMG 14190                    |                              |
| **Paraburkholderia graminis comb. nov.** | *Burkholderia graminis*  | C4D1M                        | Viallard et al., 1998         |
|                                        |                          | ATCC 700544                  |                              |
|                                        |                          | CCUG 42231                   |                              |
|                                        |                          | CIP 106649                   |                              |
|                                        |                          | LMG 18924                    |                              |
Table 4 | Continued

| New Combination | Basonym | Type Strain | References |
|-----------------|---------|-------------|------------|
| Paraburkholderia grimmiae comb. nov. | Burkholderia grimmiae | R27 CGMCC 1.11013 DSM 25160 | Tian et al., 2013 |
| Paraburkholderia heleia comb. nov. | Burkholderia heleia | SA41 NBRC 101817 VTCC-D6-7 | Aizawa et al., 2010a |
| Candidatus Paraburkholderia hispidae comb. nov. | Candidatus Burkholderia hispidae | — | Lemaire et al., 2012 |
| Paraburkholderia hospita comb. nov. | Burkholderia hospita | LMG 20598 CCUG 43658 | Goris et al., 2002 |
| Paraburkholderia humi comb. nov. | Burkholderia humi | LMG 22934 CCUG 63059 | Vandamme et al., 2013 |
| Candidatus Paraburkholderia kirkii comb. nov. | Candidatus Burkholderia kirkii | — | Van Oevelen et al., 2002a |
| Paraburkholderia kururiensis comb. nov. | Burkholderia kururiensis | KP23 ATCC 700977 CCUG 43663 CIP 106643 DSM 13646 JCM 10599 LMG 19447 | Zhang et al., 2000 |
| Paraburkholderia megapolitana comb. nov. | Burkholderia megapolitana | A3 CCUG 53006 LMG 23650 | Vandamme et al., 2007 |
| Paraburkholderia mimosarum comb. nov. | Burkholderia mimosarum | PAS44 BCRC 17516 LMG 23256 | Chen et al., 2006 |
| Candidatus Paraburkholderia nigropunctata comb. nov. | Candidatus Burkholderia nigropunctata | — | Van Oevelen et al., 2004 |
| Paraburkholderia nodosa comb. nov. | Burkholderia nodosa | Br3437 BCRC 17575 LMG 23741 | Chen et al., 2007 |
| Paraburkholderia oxyphila comb. nov. | Burkholderia oxyphila | OX-01 DSM 22550 NBRC 105797 | Otsuka et al., 2011 |
| Candidatus Paraburkholderia petitii comb. nov. | Candidatus Burkholderia petitii | — | Lemaire et al., 2011 |
| Paraburkholderia phenazinium comb. nov. | Burkholderia phenazinium | ATCC 33666 CCUG 20836 CFBP 4793 CIP 106502 DSM 10684 JCM 10564 LMG 2247 NCIMB 11027 | Viaillard et al., 1998 |

(Continued)
Table 4 | Continued

| New Combination | Basonym | Type Strain | References |
|-----------------|---------|-------------|------------|
| Paraburkholderia phenoliruptrix comb. nov. | Burkholderia phenoliruptrix | AC1100 CCUG 48558 LMG 22037 | Coenye et al., 2004 |
| Paraburkholderia phymatum comb. nov. | Burkholderia phymatum | STM815 LMG 21445 CCUG 47179 | Vandamme et al., 2002a |
| Paraburkholderia phytofirmans comb. nov. | Burkholderia phytofirmans | PsJN CCUG 49060 LMG 22146 | Sessitsch et al., 2005 |
| Paraburkholderia rhizoxinica comb. nov. | Burkholderia rhizoxinica | HKI 454 CIP 109453 DSM 19002 | Partida-Martinez et al., 2007 |
| Paraburkholderia rhynchosiae comb. nov. | Burkholderia rhynchosiae | WSM3937 LMG 27174 HAMBI 3354 | De Meyer et al., 2013b |
| Candidatus Paraburkholderia rigidae comb. nov. | Candidatus Burkholderia rigidae | — | Lemaire et al., 2012 |
| Paraburkholderia sabiae comb. nov. | Burkholderia sabiae | Br3407 BCRC 17587 LMG 24235 | Chen et al., 2008 |
| Paraburkholderia sacchari comb. nov. | Burkholderia sacchari | CCT 6771 CCUG 46043 CIP 107211 IPT 101 LMG 19450 | Brämer et al., 2001 |
| Paraburkholderia sartisoli comb. nov. | Burkholderia sartisoli | RP007 CCUG 53604 ICMP 13529 LMG 24000 | Vanlaere et al., 2008 |
| Candidatus Paraburkholderia schumannianiae comb. nov. | Candidatus Burkholderia schumannianiae | — | Lemaire et al., 2012 |
| Paraburkholderia sediminicola comb. nov. | Burkholderia sediminicola | HU2-65W KCTC 22086 LMG 24239 | Lim et al., 2008 |
| Paraburkholderia silvatlantica comb. nov. | Burkholderia silvatlantica | SRMfh-20 ATCC BAA-1244 LMG 23149 | Perin et al., 2006 |
| Paraburkholderia soli comb. nov. | Burkholderia soli | GP25-8 DSM 18235 KACC 11589 | Yoo et al., 2007 |
| Paraburkholderia sordidicola comb. nov. | Burkholderia sordidicola | CCUG 49583 JCM 11778 KCTC 12081 | Lim et al., 2003 |
| Paraburkholderia sprentiae comb. nov. | Burkholderia sprentiae | WSM5005 LMG 27175 HAMBI 3357 | De Meyer et al., 2013a |

(Continued)
Table 4 | Continued

| New Combination                              | Basonym                  | Type Strain                | References         |
|----------------------------------------------|--------------------------|----------------------------|--------------------|
| Paraburkholderia symbiotica comb. nov.       | Burkholderia symbiotica  | JPY-345                    | Sheu et al., 2012  |
|                                              |                          | NKMU-JPY-345               |                    |
|                                              |                          | BCRC 80258                 |                    |
|                                              |                          | KCTC 23309                 |                    |
|                                              |                          | LMG 26032                  |                    |
| Paraburkholderia telluris comb. nov.         | Burkholderia telluris    | LMG 22936                  | Vandamme et al., 2013 |
|                                              |                          | CCUG 63060                 |                    |
| Paraburkholderia terrae comb. nov.           | Burkholderia terrae     | KMY02                      | Yang et al., 2006  |
|                                              |                          | KCTC 12388                 |                    |
|                                              |                          | NBRC 100964                |                    |
| Paraburkholderia terrestris comb. nov.       | Burkholderia terrestris  | LMG 22937                  | Vandamme et al., 2013 |
|                                              |                          | CCUG 63062                 |                    |
| Paraburkholderia terricola comb. nov.        | Burkholderia terricola   | CCUG 44527                 | Goris et al., 2002 |
|                                              |                          | LMG 20594                  |                    |
| Paraburkholderia tropica comb. nov.          | Burkholderia tropica     | Ppe8                       | Reis et al., 2004  |
|                                              |                          | ATCC BAA-831               |                    |
|                                              |                          | DSM 15359                  |                    |
|                                              |                          | LMG 22274                  |                    |
| Paraburkholderia tuberum comb. nov.          | Burkholderia tuberum     | STM678                     | Vandamme et al., 2002a |
|                                              |                          | CCUG 47178                 |                    |
|                                              |                          | LMG 21444                  |                    |
| Paraburkholderia udeis comb. nov.            | Burkholderia udeis       | LMG 27134                  | Vandamme et al., 2013 |
|                                              |                          | CCUG 63061                 |                    |
| Paraburkholderia unamae comb. nov.           | Burkholderia unamae      | MTI-641                    | Caballero-Meliado et al., 2004 |
|                                              |                          | ATCC BAA-744               |                    |
|                                              |                          | CIP 107921                 |                    |
| Paraburkholderia xenovorans comb. nov.       | Burkholderia xenovorans  | LB400                      | Goris et al., 2004 |
|                                              |                          | CCUG 46959                 |                    |
|                                              |                          | LMG 21483                  |                    |
|                                              |                          | NRRL B-18064               |                    |
| Paraburkholderia zhejiangensis comb. nov.    | Burkholderia zhejiangensis | OP-1                       | Lu et al., 2012    |
|                                              |                          | KCTC 23300                 |                    |

A number of previous phylogenetic studies (Payne et al., 2005; Tayeb et al., 2008; Yarza et al., 2008; Spilker et al., 2009; Ussery et al., 2009; Gyaneshwar et al., 2011; Vandamme and Dawyndt, 2011; Zhu et al., 2011; Suarez-Moreno et al., 2012; Estrada-de los Santos et al., 2013; Segata et al., 2013). Estrada-de los Santos et al. (2013) recently completed a phylogenetic analysis of the genus *Burkholderia* utilizing the multilocus sequence analysis of *atpD*, *gltB*, *lepA*, and *recA* genes in combination with the 16S rRNA gene, which provides compelling evidence for the presence of two distinct evolutionary lineages within the genus *Burkholderia*. However, these authors have refrained from formally proposing a division of the genus into two genera due to a paucity of differentiating characteristics for the two groups. Our comparative analysis of *Burkholderia* genomes has identified a set of distinctive molecular characteristics that clearly differentiate the two evolutionary lineages within the genus *Burkholderia* in addition to the phylogenetic evidence. In light of the abundance of phylogenetic and molecular evidence for the presence of two distinct evolutionary lineages within the genus *Burkholderia*, and the distinct pathogenicity profiles of the members of these two groups, we are proposing that genus *Burkholderia* should be divided into two separate genera. The first of these monophyletic genera, which comprises of all the clinically relevant species and clearly distinguished from all other *Burkholderia* species, will retain the name *Burkholderia* (Clade I). For the remainder of the *Burkholderia* species (Clade II), which include a wide range of environmental species, we propose the name *Paraburkholderia* gen. nov. An emended description of the genus *Burkholderia* and a description of *Paraburkholderia* gen. nov. are provided below. Brief descriptions of the new species
EMENDED DESCRIPTION OF THE GENUS BURKHOLDERIA (Yabuuchi et al., 1993 EMEND. Gillis et al., 1995)

The genus contains the type species B. cepacia (Yabuuchi et al., 1993). The species from this genus are gram-negative, straight or slightly curved rods, which exhibit motility mediated by one or more polar flagella. Only, B. mallei lacks flagella and is non-motile. The species do not produce sheaths or prosthecae and do not go through any resting stages. Most species are able to accumulate and utilize poly-β-hydroxybutyrate (PHB) for growth. The species are mostly aerobic chemoorganotrophs, but some species are capable of anaerobic respiration using nitrate as the terminal electron acceptor. The G+C content for the members of the genus ranges from 65.7 to 68.5%. The members of the genus form a distinct monophyletic clade in phylogenetic trees, and they are distinguished from all other bacteria by the conserved sequence indels reported in this work in the following proteins: Periplasmic amino acid-binding protein, 4-hydroxybenzoate 3-monoxygenase, 6-phosphogluconate dehydrogenase, Sarcosine oxidase subunit alpha, a putative lipoprotein, and a putative lyase (Table 2).

DESCRIPTION OF THE GENUS PARABURKHOLDERIA GEN. NOV.

The genus contains the type species Paraburkholderia graminis comb. nov. (Basonym: Burkholderia graminis, Viallard et al., 1998) The species from this genus are gram-negative straight or slightly curved rods with one or more polar flagella. Other morphological and metabolic characteristics are similar to genus Burkholderia. The G+C content for the members of the genus ranges from 61.4 to 65.0%. The species are not associated with humans. The members of this genus generally form a distinct clade in the neighborhood of genus Burkholderia in phylogenetic trees, and they lack the molecular signatures which are specific for Burkholderia. Most of the sequenced members from this genus contain the conserved sequence indels reported in this work in the protein sequences of an unnamed dehydrogenase and a LysR family transcriptional regulator (Table 2).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fgene.2014.00429/abstract

REFERENCES

Achouak, W., Christen, R., Barakat, M., Martel, M.-H., and Heulin, T. (1999). Burkholderia caribensis sp. nov., an exopolysaccharide-producing bacterium isolated from vertisol microaggregates in Martinique. Int. J. Syst. Bacteriol. 49, 787–794. doi: 10.1099/00207713-49-2-787

Ahmed, N. Z., Gupta, R. S., and Shah, H. N. (2011). Identification of a Bacillus anthracis specific indel in the recA gene and development of a rapid pyrosequencing assay for distinguishing B. anthracis from the B. cereus group. J. Microbiol. Methods 87, 279–285. doi: 10.1016/j.mimet.2011.08.015

Aizawa, T., Ve, N. B., Vijarnsorn, P., Nakajima, M., and Sunairi, M. (2010b). Burkholderia acidipaludis sp. nov., aluminium-tolerant bacterium isolated from Chinese water chestnut (Eleocharis dulcis) growing in highly acidic swamps in South-East Asia. Int. J. Syst. Evol. Microbiol. 60, 2036–2041. doi: 10.1099/ijsem.0.018283-0

Aizawa, T., Vijarnsorn, P., Nakajima, M., and Sunairi, M. (2011). Burkholderia bannensis sp. nov., an acid-neutralizing bacterium isolated from torred grass (Panicum repens) growing in highly acidic swamps. Int. J. Syst. Evol. Microbiol. 61, 1645–1650. doi: 10.1099/ijsem.0.026278-0

Biddick, R., Spiller, T., Martin, A., and LiPuma, J. J. (2003). Evidence of transmission of Burkholderia cepacia, Burkholderia multivorans and Burkholderia dolosa among persons with cystic fibrosis. FEMS Microbiol. Lett. 228, 57–62. doi: 10.1016/S0378-1097(03)00724-9

Brämer, C. O., Vandamme, P., da Silva, L. F., Gomez, J., and Steinbüchel, A. (2001). Polyhydroxyalkanoate-accumulating bacterium isolated from soil of a sugar-cane plantation in Brazil. Int. J. Syst. Evol. Microbiol. 51, 1709–1713. doi: 10.1099/00207713-51-5-1709

Caballero-Mellado, J., Martinez-Aguilar, L., Paredes-Valdez, G., and Estrada-de los Santos, P. (2004). Burkholderia unamae sp. nov., an N2-fixing rhizospheric and endophytic species. Int. J. Syst. Evol. Microbiol. 54, 1165–1172. doi: 10.1099/ijs.0.02951-0

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552. doi: 10.1093/oxfordjournals.molbev.a026334

Chai, P. S., Denef, V. J., Konstantinidis, K. T., Vergez, L. M., Aguilo, L., Reyes, V. L., et al. (2006). Burkholderia xenovorans LB400 harbors a multi-replicon, 9.73-Mbp genome shaped for versatility. Proc. Natl. Acad. Sci. U.S.A. 103, 15280–15287. doi: 10.1073/pnas.0606924103

Charlebois, R. L., and Doolittle, W. F. (2004). Computing prokaryotic gene ubiquity: rescuing the core from extinction. Genome Res. 14, 2469–2477. doi: 10.1101/gr.304274

Chen, W.-M., de Faria, S. M., Chou, J.-H., James, E. K., Elliott, G. N., Spret, J. I., et al. (2008). Burkholderia sabiae sp. nov., isolated from root nodules of Mimosa caesalpiniifolia. Int. J. Syst. Evol. Microbiol. 58, 2174–2179. doi: 10.1099/ijs.0.05816-0

Chen, W.-M., De Faria, S. M., James, E. K., Elliott, G. N., Lin, K.-Y., Chou, J.-H., et al. (2007). Burkholderia nodosa sp. nov., isolated from root nodules of the woody Brazilian legumes Mimosa bimucronata and Mimosa scabrella. Int. J. Syst. Evol. Microbiol. 57, 1055–1059. doi: 10.1099/ijs.0.64873-0

Chen, W.-M., James, E. K., Coenye, T., Chou, J.-H., Barrios, E., De Faria, S. M., et al. (2006). Burkholderia mimosarum sp. nov., isolated from root nodules of Mimosa spp. from Taiwan and South America. Int. J. Syst. Evol. Microbiol. 56, 1847–1851. doi: 10.1099/ijs.0.04325-0

Ciccariello, F., Doerk, T., Von Mering, C., Creevey, C. J., Sneb, B., and Bork, P. (2006). Toward automatic reconstruction of a highly resolved tree of life. Science 311, 1283–1287. doi: 10.1126/science.1123061

Coenye, T., Henry, D., Speert, D. P., and Vandamme, P. (2004). Burkholderia pheno-liruptrix sp. nov., to accommodate the 2, 4, 5-trichlorophenoxycetic acid and halophenol-degrading strain AC1100. Syst. Appl. Microbiol. 27, 623–627. doi: 10.1078/0723204238999

Coenye, T., Laevens, S., Willems, A., Ohlén, M., Hannant, W., Govan, J., et al. (2001a). Burkholderia fungorum sp. nov. and Burkholderia calemonica sp. nov., two new species isolated from the environment, animals and human clinical samples. Int. J. Syst. Evol. Microbiol. 51, 1099–1107. doi: 10.1099/00207713-51-3-1099

Coenye, T., Mahenthiralingam, E., Henry, D., LiPuma, J. J., Laevens, S., Gillis, M., et al. (2001b). Burkholderia ambifaria sp. nov., a novel member of the Burkholderia cepacia complex including biocontrol and cystic fibrosis-related isolates. Int. J. Syst. Evol. Microbiol. 51, 1481–1490. doi: 10.1099/00207713-51-4-1481

Coenye, T., and Vandamme, P. (2003). Diversity and significance of Burkholderia species occupying diverse ecological niches. Environ. Microbiol. 5, 719–729. doi: 10.1046/j.1462-2920.2003.00471.x

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., et al. (2009). The ribosomal database project: improved alignments and new tools for RNA analysis. Nucleic Acids Res. 37, D141–D145. doi: 10.1093/nar/gkn879

Compan, S., Nowak, J., Coenye, T., Clement, C., and Ait Barka, E. (2008). Diversity and occurrence of Burkholderia spp. in the natural environment. FEMS Microbiol. Rev. 32, 607–626. doi: 10.1111/j.1574-6976.2008.00113.x
Coutinho, B. G., Passos da Silva, D., Prevatio, J. O., Mendonca-Prevatio, L., and Venturi, V. (2013). Draft genome sequence of the rice endophyte Burkholderia kururiensis M130. Genome Announc. 1, e00251-12. 10.1128/genomeA.00251-12
De Meyer, S. E., Crookaert, M., Ardley, J. K., Maker, G., Yates, R., Howieson, J. G., et al. (2013a). Burkholderia sp. strain LB400T and relatives as N2-fixing isolates from rice in Vietnam. Int. J. Syst. Evol. Microbiol. 64(4 Pt 1), 1990–1995. 10.1099/ijs.0.058602-0
Deshazer, D. (2007). Virulence of clinical and environmental isolates of Burkholderia oklahomensis and Burkholderia thailandensis in hamsters and mice. FEMS Microbiol. Lett. 277, 64–69. doi: 10.1111/j.1574-6968.2007.00946.x
Estrada-de los Santos, P., Vinuesa, P., Martínez-Aguilar, L., Hirsch, A. M., and Deshazer, D. (2007). Identification of Conserved Indels that are Useful for Evolutionary Studies Methods in Microbiology, Vol. 41. Oxford: Academic Press.
Gupta, R. S. (2001). The branching order and phylogenetic placement of species in the genus Burkholderia leading to an emended description of the genus and proposition of Burkholderia vietnamiensis sp. nov. from N2-fixing isolates from rice in Vietnam. Int. J. Syst. Bacteriol. 45, 274–289. doi: 10.1099/0027131-4-2-274
Gogarten, J. P., Doolittle, W. F., and Lawrence, J. G. (2002). Prokaryotic evolution in light of gene transfer. Mol. Biol. Evol. 19, 2226–2238. doi: 10.1093/oxfordjournals.molbev.a004046
Hogen, M. T., Seth-Smith, H. M., Crossman, L. C., Sebastia, M., Bentley, S. D., Cerdeno-Tarraga, A. M., et al. (2009). The genome of Burkholderia ambigua J2315, an epidemic pathogen of cystic fibrosis patients. J. Bacteriol. 191, 261–277. doi: 10.1128/JB.01230-08
Holden, M. T., Tittball, R. W., Peacock, S. J., Cerdeno-Tarraga, A. M., Atkins, T., Crossman, L. C., et al. (2004). Genomic plasticity of the causative agent of meliodosis, Burkholderia pseudomallei. Proc. Natl. Acad. Sci. U.S.A. 101, 14240–14245. doi: 10.1073/pnas.030326-0
Hong, K. W., Koh, C. L., Sam, C. K., Yin, W. F., and Chan, K. G. (2012). Complete genome sequence of Burkholderia sp. strain GG4, a betaproteobacterium that reduces 3-oxo-N-acylhomoserine lactones and produces different N-acylhomoserine lactones. J. Bacteriol. 194, 6317–6312. doi: 10.1128/JB.01578-12
Jeanmougin, F., Thompson, J. D., Gouy, M., Higgins, D. G., and Gibson, T. J. (1998). Mafft multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. doi: 10.1093/molbev/msn010
Khan, A., Asif, H., Studholme, D. J., Khan, I. A., and Azim, M. K. (2013). Genome characterization of a novel Burkholderia cepacia complex genomovar isolated from diabase affected mango orchards. World J. Microbiol. Biotechnol. 29, 2033–2044. doi: 10.1007/s11274-013-1366-5
Kim, H.-B., Park, M.-I., Yang, H.-C., An, D.-S., Jin, H.-Z., and Yang, D.-C. (2006). Burkholderia ginsengisoli sp. nov., a β-glucosidase-producing bacterium isolated from soil of a ginseng field. Int. J. Syst. Evol. Microbiol. 56, 2529–2533. doi: 10.1099/ijs.0.063487-0
Kim, H. S., Schell, M. A., Yu, Y., Ulrich, R. L., Sarria, S. H., Nierman, W. C., et al. (2005). Bacterial genome adaptation to niches: divergence of the potential virulence genes in three Burkholderia species of different survival strategies. BMC Genomics 6:174. doi: 10.1186/1471-2164-6-174
Kumar, S., Vikram, S., and Raghava, G. P. (2012). Genome sequence of the nitroaromatic compound-degrading bacterium Burkholderia sp. strain SJ98. J. Bacteriol. 194, 3268–3212. doi: 10.1128/JB.00497-12
Kwak, M. J., Song, J. Y., Kim, S. Y., Jeong, H., Kang, S. G., Kim, B. K., et al. (2012). Complete genome sequence of the endophytic bacterium Burkholderia sp. strain KJ006. J. Bacteriol. 194, 4432–4433. doi: 10.1128/JB.00821-12
Kyrpides, N., Overbeek, R., and Ouzounis, C. (1999). Universal protein families and the functional content of the last universal common ancestor. J. Mol. Evol. 49, 413–423. doi: 10.1007/PL00006564
Lackner, G., Moebius, N., Partida-Martinez, L., and Hertweck, C. (2011). Complete genome sequence of Burkholderia rhizoxinica, an Endosymbiont of Rhizopus microsporus. J. Bacteriol. 193, 783–784. doi: 10.1128/JB.01318-10
Lee, C.-M., Weon, H.-Y., Yoon, S.-H., Kim, S.-I., Koo, B.-S., and Kwon, S.-W. (2012). Burkholderia dentrficircins sp. nov., isolated from the soil of Dokdo Island, Korea. J. Microbiol. 50, 855–859. doi: 10.1007/s12275-012-1554-2
Lemaire, B., Robrebrect, E., van Wyk, B., Van Oevelen, S., Verstreeta, D., Prinsen, E., et al. (2012). Identification, origin, and evolution of leaf nodulating symbionts of Sericinatae (Rubiaceae). J. Microbiol. 49, 935–941. doi: 10.1271/bj2011-011-1163-5
Lemaire, B., Van Oevelen, S., De Block, P., Verstreeta, B., Smets, E., Prinsen, E., et al. (2012). Identification of the bacterial endosymbionts in leaf nodules of Pavetta (Rubiaceae). Int. J. Syst. Evol. Microbiol. 62, 200–209. doi: 10.1099/ijs.0.00819-0
Lim, J. H., Baek, S.-H., and Lee, S.-T. (2008). Burkholderia sediminicola sp. nov., isolated from freshwater sediment. Int. J. Syst. Evol. Microbiol. 58, 565–569. doi: 10.1099/ijs.0.06550-0
Lim, J., Lee, T. H., Nahm, B. H., Cha, Y. D., Kim, M., and Hwang, I. (2009). Complete genome sequence of Burkholderia glumae BGR1. J. Bacteriol. 191, 3758–3759. doi: 10.1128/JB.00349-09
Lim, J. S., Choi, B. S., Choi, A. Y., Kim, K. D., Kim, D. L., Choi, I. Y., et al. (2012). Complete genome sequence of the fenitrothion-degrading Burkholderia sp. strain YJ123. J. Bacteriol. 194, 896–811. doi: 10.1128/JB.06479-11
Lim, Y. W., Baik, K. S., Han, S. K., Kim, S. B., and Bae, K. S. (2003). Burkholderia sordidicola sp. nov., isolated from the white-rot fungus Phanerochaete sordida. J. Int. J. Syst. Evol. Microbiol. 53, 1631–1636. doi: 10.1099/0027131-4-2-274
Mol. signatures distinguishing Burkholderia species

Frontiers in Genetics  | Evolutionary and Genomic Microbiology December 2014 | Volume 5 | Article 429 | 20
Valverde, A., Delvasto, P., Peix, A., Velaquez, E., Santa-Regina, I., Ballesta, A., et al. (2006). *Burkholderia ferrariae* sp. nov., isolated from an iron ore in Brazil. *Int. J. Syst. Evol. Microbiol.* 56, 2421–2425. doi: 10.1099/ijs.0.64498-0

Vandamme, P., and Dawyndt, P. (2011). Classification and identification of the *Burkholderia cæpacia* complex: past, present and future. *Syst. Appl. Microbiol.* 34, 87–95. doi: 10.1016/j.syapm.2010.10.002

Vandamme, P., De Brandt, E., Houf, K., Salles, J. F., van Elsas, J. D., Spilker, T., et al. (2013). *Burkholderia humi* sp. nov., *Burkholderia choica* sp. nov., *Burkholderia telluris* sp. nov., *Burkholderia terrestris* sp. nov. and *Burkholderia udeis* sp. nov.: *Burkholderia* glathei-like bacteria from soil and rhizosphere soil. *Int. J. Syst. Evol. Microbiol.* 63(Pt 12), 4707–4718. doi: 10.1099/00207713-48-2-549

Wiersinga, W. J., van der Poll, T., White, N. J., Day, N. P., and Peacock, S. J. (2006). Melioidosis: insights into the pathogenicity of *Burkholderia pseudomallei*. *Nat. Rev. Microbiol.* 4, 272–282. doi: 10.1038/nrmicro1385

Yang, H.-C., Im, W.-T., Kim, K. K., An, D.-S., and Lee, S.-T. (2006). *Burkholderia terrei* sp. nov., isolated from a forest soil. *Int. J. Syst. Evol. Microbiol.* 56, 453–457. doi: 10.1099/ijs.0.63968-0

Yang, H.-C., Im, W.-T., Kim, K. K., An, D.-S., and Lee, S.-T. (2006). *Burkholderia terrei* sp. nov., isolated from a forest soil. *Int. J. Syst. Evol. Microbiol.* 56, 453–457. doi: 10.1099/ijs.0.63968-0

Yarza, P., Richter, M., Pelphes, E., Euzéby, J., Amann, R., Schleifer, K. H., et al. (2008). The All-Species Living Tree project: A 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst. Appl. Microbiol.* 31, 241–250. doi: 10.1016/j.syapm.2008.07.001

Zhang, H., Hanada, S., Shigematsu, T., Shibuya, K., Kanagata, Y., Kanagawa, T., et al. (2000). *Burkholderia kururiensis* sp. nov., a trichloroethylene (TCE)-degrading bacterium isolated from an aquifer polluted with TCE. *Int. J. Syst. Evol. Microbiol.* 50, 743–749. doi: 10.1099/ijs.0.0207713-50-2-743

Zhu, B., Zhou, S., Lou, M., Zhu, J., Li, B., Xie, G., et al. (2011). Characterization and inference of gene gain/loss along *burkholderia* evolutionary history. *Evol. Bioinform. Online* 7, 191. doi: 10.4137/EBO.S7510

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 18 September 2014; paper pending published: 24 October 2014; accepted: 21 November 2014; published online: 19 December 2014.

Citation: Sawana A, Adeolu M and Gupta RS (2014) Molecular signatures and phylogenetic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species. *Front. Genet.* 5:429. doi: 10.3389/fgene.2014.00429

This article was submitted to Evolutionary and Genomic Microbiology, a section of the journal Frontiers in Genetics.

Copyright © 2014 Sawana, Adeolu and Gupta. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.