Phylogenomic Study of *Burkholderia glathei*-like Organisms, Proposal of 13 Novel *Burkholderia* Species and Emended Descriptions of *Burkholderia sordidicola*, *Burkholderia zhejiangensis*, and *Burkholderia grimmiae*

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Partial *gyrB* gene sequence analysis of 17 isolates from human and environmental sources revealed 13 clusters of strains and identified them as *Burkholderia glathei* clade (BGC) bacteria. The taxonomic status of these clusters was examined by whole-genome sequence analysis, determination of the G+C content, whole-cell fatty acid analysis and biochemical characterization. The whole-genome sequence-based phylogeny was assessed using the Genome Blast Distance Phylogeny (GBDP) method and an extended multilocus sequence analysis (MLSA) approach. The results demonstrated that these 17 BGC isolates represented 13 novel *Burkholderia* species that could be distinguished by both genotypic and phenotypic characteristics. BGC strains exhibited a broad metabolic versatility and developed beneficial, symbiotic, and pathogenic interactions with different hosts. Our data also confirmed that there is no phylogenetic subdivision in the genus *Burkholderia* that distinguishes beneficial from pathogenic strains. We therefore propose to formally classify the 13 novel BGC *Burkholderia* species as *Burkholderia arvi* sp. nov. (type strain LMG 29317T = CCUG 68412T), *Burkholderia hypogea* sp. nov. (type strain LMG 29322T = CCUG 68407T), *Burkholderia pterechthonis* sp. nov. (type strain LMG 29326T = CCUG 68403T), *Burkholderia glebae* sp. nov. (type strain LMG 29325T = CCUG 68404T), *Burkholderia pedi* sp. nov. (type strain LMG 29323T = CCUG 68406T), *Burkholderia arationis* sp. nov. (type strain LMG 29324T = CCUG 68405T), *Burkholderia fortuita* sp. nov. (type strain LMG 29320T = CCUG 68409T), *Burkholderia temeraria* sp. nov. (type strain LMG 29319T = CCUG 68410T), *Burkholderia calidae* sp. nov. (type strain LMG 29321T = CCUG 68408T), *Burkholderia concitans* sp. nov. (type strain LMG 29315T = CCUG 68414T), *Burkholderia turbans* sp. nov. (type strain LMG 29316T = CCUG 68413T), *Burkholderia catudaia*...
INTRODUCTION
The genus Burkholderia currently comprises 90 validly named species (Euzéby, 1997) and several uncultured Candidatus species (Van Oevelen et al., 2004; Verstraete et al., 2011; Lemaire et al., 2012) which occupy very diverse niches (Coenye and Vandamme, 2003). Many Burkholderia species have thus far only been isolated as free-living organisms but a growing body of literature reveals that they live in close interaction with numerous plant, animal, fungal or even amoebozoan hosts (Marolda et al., 1999; Van Borm et al., 2002; Kikuchi et al., 2011; Verstraete et al., 2013; Stopnisek et al., 2016; Xu et al., 2016). Burkholderia species may be beneficial to their hosts because some strains can fix nitrogen, produce plant hormones or siderophores, or lower pathogen-related ethylene levels; hence they have been exploited for plant growth promotion and biocontrol of plant diseases (Compant et al., 2008; Vial et al., 2011). Yet, other Burkholderia species are notorious pathogens in plants, animals and humans (Mahenthiralingam et al., 2008). This ecological diversity is likely attributed to their large, multireplicon genomes (typically between 6 and 9 Mb) which also confer a metabolic versatility allowing them to degrade a wide range of recalcitrant xenobiotics (Parke and Gurian-Sherman, 2001; Coenye and Vandamme, 2003).

Phylogenetic analyses based on the 16S rRNA and protein-coding genes showed that Burkholderia glathei clade (BGC) species are phylogenetically divergent from other Burkholderia species and form a separate clade (Sawana et al., 2014; Vandamme et al., 2014). Although this clade thus far includes only 12 formally named species, its functional diversity is impressive. In this clade too, most species have been isolated from bulk and rhizosphere soil (Zolg and Ottow, 1975; Viallard et al., 1998; Vandamme et al., 2013; Draghi et al., 2014; Baek et al., 2015), but also from contaminated soil and sludge from a wastewater treatment system (Lu et al., 2012; Vandamme et al., 2013; Liu et al., 2014). Two BGC species were associated with less studied hosts like fungi (Burkholderia sordidicola) and mosses (Burkholderia grimmiae) (Lim et al., 2003; Tian et al., 2013) but numerous, mostly uncultivated BGC species adopted endosymbiotic lifestyles in insect guts (Kikuchi et al., 2011; Tago et al., 2015; Xu et al., 2016) or plant leaf tissue (Verstraete et al., 2013; Carlier et al., 2015) and many additional unclassified B. glathei-like bacteria have been reported (Nogales et al., 2001; Salles et al., 2006; Pumphrey and Madsen, 2008; Draghi et al., 2014; Verstraete et al., 2014; Peeters et al., 2016).

The present study aimed to perform a phylogenomic study of established and novel species in the B. glathei clade, to formally name the latter and to make reference cultures and whole-genome sequences of each of these versatile bacteria publicly available. The genome sequence-based phylogeny was assessed using the Genome Blast Distance Phylogeny (GBDP) method (Meier-Kolthoff et al., 2013) and an extended multilocus sequence analysis (MLSA) approach. For phenotypic characterization, whole-cell fatty acid profiling and biochemical analyses were performed.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions
Table 1 lists the sources of the 17 studied isolates. Details of type strains of each of the present BGC species were described previously (Zolg and Ottow, 1975; Lim et al., 2003; Lu et al., 2012; Tian et al., 2013; Vandamme et al., 2013; Draghi et al., 2014; Liu et al., 2014; Baek et al., 2015). Strains were grown aerobically on buffered nutrient agar (Oxoid, pH 6.8) and incubated at 28°C. Cultures were preserved in MicroBank™ vials at −80°C.

16S rRNA Gene Sequence Analysis
Nearly complete sequences were obtained as described previously (Peeters et al., 2013).

gyrB Gene Sequence Analysis
Partial gyrB gene sequences were obtained as described previously (Spilker et al., 2009; Peeters et al., 2013). Sequence assembly was performed using BioNumerics v7.5 (Applied Maths). Sequences (589–1182 bp) were aligned based on amino acid sequences using Muscle (Edgar, 2004) in MEGA6 (Tamura et al., 2013). All positions with less than 95% site coverage were eliminated, resulting in a total of 570 positions in the final dataset. Phylogenetic analysis was conducted in MEGA6 (Tamura et al., 2013).

Whole-Genome Sequencing
Genomic DNA of 20 strains (Table 2) was prepared as described by Pitcher et al. (1989). Genomic libraries were prepared using the Nextera kit following the methods introduced by Baym et al. (2015) and the 151 bp paired-end libraries were sequenced on the Illumina HiSeq platform of the University of New Hampshire Hubbard Center for Genomics Studies.
with an average insert size of 386 bp. Quality reports were created by FastQC. Adaptors and low-quality reads were trimmed using Trimmomatic (Bolger et al., 2014) with the following options: ILLUMINA CLIP:NexteraPE-PE.fa:2:30:10 MAXINFO:60:0:4 MINLEN:60. Assembly was performed using SPAdes (Bankevich et al., 2012) with default k-mer sizes (21, 33, 55, 77) and mismatch correction (option—careful). Contigs with length <500 bp and coverage <2 were discarded from the resulting assemblies. Raw reads were mapped against the assemblies using bwa-mem (Li, 2013) and contigs were polished using Pilon (Walker et al., 2014) with default parameters. Quast (Gurevich et al., 2013) was used to create quality reports of the resulting assemblies. Annotation was performed using Prokka as described above.

### Publicly Available Genomes

Twenty three publicly available whole-genome sequences of BGC bacteria were downloaded from the NCBI database (Table 2).

**Phylogenomic Analysis**

The latest version of the Genome Blast Distance Phylogeny (GBDP) approach was applied (Meier-Kolthoff et al., 2013) to calculate the intergenomic distance between each pair of genomes (based on the nucleotide data) and included the calculation of 100 replicate distances to assess pseudo-bootstrap support (Meier-Kolthoff et al., 2014a). Distance calculations were conducted under the recommended settings of the Genome-to-Genome Distance Calculator (GGDC 2.1; http://ggdc.dsmz.de), as described earlier (Meier-Kolthoff et al., 2013). The GBDP trimming algorithm and formula \( d_5 \) were chosen because of...
## TABLE 2 | Genomes included in the present study.

| Strain                         | Project   | Contigs<sup>a</sup> | Size (bp) | %GC  | References                  |
|-------------------------------|-----------|----------------------|-----------|------|-----------------------------|
| B. glathei LMG 14190<sup>T</sup> | PRJEB6934 | 139                  | 8,049,485 | 64.7 | Stopnisek et al., 2016     |
| B. sorodiola LMG 22029<sup>T</sup> | PRJEB12475 | 72                   | 6,874,511 | 60.2 | This study                  |
| B. zhejiangensis OP-1<sup>T</sup> | PRJNA238427 | 116                 | 7,767,215 | 62.7 | Liu et al., 2014            |
| B. grimmiae R27<sup>T</sup>    | PRJNA238424 | 160                  | 6,704,301 | 63.0 | Liu et al., 2014            |
| B. choica LMG 22940<sup>T</sup> | PRJEB12479 | 657                  | 9,776,207 | 62.7 | This study                  |
| B. humi LMG 22934<sup>T</sup>  | PRJEB12476 | 272                  | 7,619,203 | 62.8 | This study                  |
| B. telluris LMG 22936<sup>T</sup> | PRJEB12477 | 163                  | 7,056,109 | 64.0 | This study                  |
| B. terrestris LMG 22937<sup>T</sup> | PRJEB12478 | 645                  | 8,201,357 | 62.6 | This study                  |
| B. udeis LMG 27134<sup>T</sup> | PRJEB12480 | 242                  | 10,051,569| 60.0 | This study                  |
| B. cordobensis LMG 27620<sup>T</sup> | PRJEB12481 | 74                   | 8,208,096 | 63.7 | This study                  |
| B. yangsuensis MP-1<sup>T</sup> | PRJNA238425 | 168             | 8,611,053 | 62.6 | Liu et al., 2014            |
| B. megalochromosomata JC2949<sup>T</sup> | PRJNA241423 | 285              | 9,506,519 | 62.7 | Baek et al., 2015          |
| B. vs. sp. nov. LMG 29317T     | PRJEB12482 | 351                  | 9,665,767 | 62.4 | This study                  |
| B. hypogaia sp. nov. LMG 29322<sup>T</sup> | PRJEB12491 | 94                   | 8,333,271 | 63.2 | This study                  |
| B. pterochothoris sp. nov. LMG 29326<sup>T</sup> | PRJEB12495 | 117                 | 7,714,803 | 64.2 | This study                  |
| B. glebae sp. nov. LMG 29325<sup>T</sup> | PRJEB12494 | 194                 | 7,842,312 | 62.7 | This study                  |
| B. ped<sup>b</sup> sp. nov. LMG 29323<sup>T</sup> | PRJEB12492 | 142                 | 9,141,307 | 61.9 | This study                  |
| B. arationis sp. nov. LMG 29324<sup>T</sup> | PRJEB12493 | 629                 | 9,377,494 | 62.8 | This study                  |
| B. fortuita sp. nov. LMG 29320<sup>T</sup> | PRJEB12489 | 50                   | 7,360,810 | 62.9 | This study                  |
| B. teneraria sp. nov. LMG 29319<sup>T</sup> | PRJEB12488 | 129                 | 8,325,519 | 62.7 | This study                  |
| B. caetae sp. nov. LMG 29321<sup>T</sup> | PRJEB12490 | 379                 | 9,609,693 | 62.5 | This study                  |
| B. concitans sp. nov. LMG 29315<sup>T</sup> | PRJEB12483 | 47                  | 6,166,171 | 63.2 | This study                  |
| B. turbans sp. nov. LMG 29316<sup>T</sup> | PRJEB12484 | 120                 | 7,352,555 | 63.1 | This study                  |
| B. catudaia sp. nov. LMG 29314<sup>T</sup> | PRJEB12486 | 156                 | 7,726,733 | 62.8 | This study                  |
| B. beredens sp. nov. LMG 29314<sup>T</sup> | PRJEB12482 | 78                  | 6,762,081 | 63.1 | This study                  |
| B. cordobensis V123            | PRJNA74517 | 6                   | 8,896,411 | 63.3 | Lim et al., 2012           |
| Burkholderia sp. PML1(12)     | PRJNA53985 | 377                  | 9,368,249 | 60.1 | Uroz and Oger, 2015       |
| Burkholderia sp. S170          | PRJNA248610 | 216              | 10,261,891| 59.6 | Liao et al., 2014         |
| B. zhejiangensis CEIB S4-3     | PRJNA241423 | 285              | 9,506,519 | 62.7 | Hernandez-Mendoza et al., 2014 |
| B. zhejiangensis SJ88          | PRJNA241423 | 14          | 7,878,727 | 62.7 | Kumar et al., 2012       |
| Burkholderia sp. Leaf177       | PRJNA297956 | 27             | 6,308,288 | 59.2 | Bai et al., 2015         |
| B. consicins sp. nov. MR1      | PRJDB1103  | 5                   | 6,019,671 | 63.3 | Pawitwar et al., 2015    |
| Burkholderia sp. RPE64         | PRJDB1103  | 5                   | 6,964,487 | 63.2 | Shibata et al., 2013     |
| Ca. B. kirkii UZHbot1           | PRJNA9825  | 305                | 3,900,738 | 62.9 | Carlier and Ebert, 2012  |
| Ca. B. kirkii UZHbot2           | PRJNA253356 | 48              | 3,914,712 | 64.0 | Pinto-Carbo et al., 2016  |
| Ca. B. pumila UZHbot3           | PRJNA253357 | 519            | 3,681,223 | 59.3 | Pinto-Carbo et al., 2016  |
| Ca. B. verschuerenii UZHbot4    | PRJNA253359 | 446           | 6,188,480 | 61.9 | Pinto-Carbo et al., 2016  |
| Ca. B. humilis UZHbot5          | PRJNA253360 | 354          | 5,148,994 | 60.1 | Pinto-Carbo et al., 2016  |
| Ca. B. calva UZHbot6            | PRJNA253361 | 307          | 4,208,605 | 61.4 | Pinto-Carbo et al., 2016  |
| Ca. B. brachyanthoides UZHbot7  | PRJNA253362 | 684       | 3,545,532 | 61.2 | Pinto-Carbo et al., 2016  |
| Ca. B. schumannianae UZHbot8    | PRJNA253363 | 283          | 2,362,726 | 63.1 | Pinto-Carbo et al., 2016  |
| Ca. B. crenata UZHbot9          | PRJNA253365 | 643          | 2,843,741 | 59.0 | Carlier et al., 2015     |

<sup>a</sup>Status complete: RPE64, RPE67, YI23; status draft assembly: all other genomes. <sup>b</sup>Genome sequence not publicly available, contig sequences were provided by J. Chun (<sup>Baek et al., 2015</sup>).

Moreover, pairwise digital DNA-DNA hybridization (dDDH) values and their confidence intervals were also determined using GGDC 2.1 under recommended settings (<sup>Meier-Kolthoff et al., 2013</sup>). The potential affiliation of the novel strains to existing species was determined by clustering using a 70% dDDH radius around each of the 12 BGC type strains as previously applied (<sup>Liu et al., 2014</sup>).
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Visualization and annotation of the phylogenetic tree was performed using iTOL (Letunic and Bork, 2011). As an alternative for the GBDP method, an extended MLSA analysis was performed in which a whole-genome phylogeny was calculated based on single-copy orthologous genes as described previously (Pinto-Carbo et al., 2016). In short, single-copy orthologs were identified using blastp and OrthoMCL v2.0.9 (with e-value cutoff 1e−6 and 50% match cutoff; Fischer et al., 2011) and aligned based on their amino acid sequences using MUSCLE. The alignments were trimmed using TrimAl (removing positions with gaps in more than 50% of the sequences) and concatenated to construct a Maximum Likelihood tree using RaXML v7.4.2 (Stamatakis, 2014) with the WAG amino acid substitution model and 100 rapid bootstrap analyses.

Phenotypic Characterization

Phenotypic and cellular fatty acid analyses were performed as described previously (Draghi et al., 2014).

RESULTS

16S rRNA Gene Sequence Analysis

The 16S rRNA gene sequences determined in the present study are publicly available through the GenBank/EMBL/DDBJ accession numbers LT158612-LT158624.

gyrB Gene Sequence Analysis

Partial gyrB gene sequences were compared to those of the type strains of the 12 validly named BGC species (Figure 1). The 17 uncultivated isolates represented 13 taxa which showed 83.4–96.2% pairwise identity with the gyrB sequences of the type strains of other BGC species. The gyrB gene sequences determined in the present study are publicly available through the GenBank/EMBL/DDBJ accession numbers LT158625-LT158641.

Whole-Genome Sequencing

To further characterize the taxonomic status of these 13 taxa, we determined the whole-genome sequence of one strain per gyrB cluster and of B. sordidicola LMG 22029T, B. choica LMG 22940T.

FIGURE 1 | Phylogenetic tree based on partial gyrB sequences of the 17 isolates in this study and type strains of phylogenetically related Burkholderia species. The optimal tree (highest log likelihood) was constructed using the Maximum Likelihood method and General Time Reversible model in MEGA6 (Tamura et al., 2013). A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5462)] and allowed for some sites to be evolutionarily invariable (I+G, 37.9331% sites). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches if greater than 50%. For B. megalochromosomata JC2949T the gyrB gene sequence was extracted from the genome sequence. The gyrB sequence of B. kururiensis LMG 19447T was used as outgroup. The scale bar indicates the number of substitutions per site.
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B. humi LMG 22934T, B. telluris LMG 22936T, B. terrestris LMG 22937T, B. udeis LMG 27134T, and B. cordobensis LMG 27620T. The assembly of the Illumina HiSeq 150 bp paired end reads resulted in assemblies with 47–657 contigs and a total of 6,166,171–10,051,569 bp (Table 2). The annotated assemblies of these 20 genomes were submitted to the European Nucleotide Archive and are publicly available through the GenBank/EMBL/DDBJ accession numbers listed in Table 2 and the species descriptions. The genome sequences of the remaining five BGC type strains and of 18 additional strains were publicly available (Table 2).

**DNA Base Composition**
The G+C content of all type strains was calculated from their genome sequences and ranged from 62.4 to 64.2 mol% (Table 2).

**Phylogenomic Analysis**
The pairwise intergenomic distances and dDDH estimates of the 44 genome sequences are listed in Supplementary Table 1. The phylogenetic tree inferred from the intergenomic distances (Figure 2) was well resolved and most branches showed a very high bootstrap support (average support: 94.8%). Species delineation based on the pairwise dDDH values and a 70% dDDH radius around each type strain yielded 39 species which included the present 12 validly named species as well as the 13 novel species delineated by means of partial gyrB gene sequences (Figure 1).

For the extended MLSA approach, we identified 332 single-copy orthologs that were present in all 44 genomes. The Maximum-Likelihood phylogenetic tree based on the concatenated amino acid alignment (Figure 3) was well resolved and showed a high bootstrap support on almost all branches.

The topologies of the two phylogenomic trees (Figures 2, 3) were very similar and both revealed six clusters of species (A-F). The main difference in tree topology related to the phylogenetic position of the Candidatus species in cluster C. This cluster was supported by a 100% bootstrap value in both analyses but its relative position to cluster D species differed in the two trees (Figures 2, 3). Additionally, the internal branching order of cluster C, E and F species differed minimally between both analyses. Both phylogenomic analyses showed that strain MR1 clustered with B. concitans sp. nov. and that strain RPE67 clustered with B. cordobensis. Finally, the large distances between strains PML1(12) and S170, and the type strains of B. glathei and B. sordidicola, respectively, indicated that both strains were misidentified and wrongly annotated in the NCBI database as B. glathei and B. sordidicola, respectively (Figures 2, 3). Both strains

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**FIGURE 2 | Whole-genome sequence based phylogenomic tree of all BGC genomes inferred by GBDP.** The outer column shows the isolation source of the strains. Pseudo-bootstrap support values above 60% are shown. The tree reveals a high average support of 94.8%. Long terminal branches are due to the distinct scaling used by GBDP’s formula $d_5$. B. gladioli PSR3 was used as outgroup. Red capital letters define subtrees that also occur in the tree depicted in Figure 3.
occupy unique positions in the phylogenomic trees and represent additional novel BGC species.

**Cellular Fatty Acid Analysis**

The fatty acid profiles of all strains are shown in Table 3. Branched chain fatty acids have not been reported in members of the genus *Burkholderia* and therefore summed features 2 and 3 very likely represent C14:0 3-OH and C16:1 ω7c, respectively (Yabuuchi et al., 1992). The main fatty acid components are C16:0, C18:1 ω7c and summed feature 3 (most probably representing C16:1 ω7c).

**Biochemical Characterization**

An overview of biochemical characteristics useful for distinguishing the BGC species is shown in Table 4.

**DISCUSSION**

While soil is a well-known source of free-living *Burkholderia* species, these organisms often live in close interaction with plants, animals, fungi, or amoebae (Marolda et al., 1999; Van Borm et al., 2002; Kikuchi et al., 2011; Verstraete et al., 2013; Stopnisek et al., 2016; Xu et al., 2016). The BGC represents a poorly known line of descent within the genus *Burkholderia* and most of the 12 validly named BGC species have been isolated from soil. Yet, publicly available sequence data indicate that the taxonomic diversity in this clade is severely underestimated (Nogales et al., 2001; Salles et al., 2006; Pumphrey and Madsen, 2008; Draghi et al., 2014; Verstraete et al., 2014; Peeters et al., 2016; Xu et al., 2016). In the present study, gyrB gene sequence analysis was used to screen our strain collection and 17 isolates from human and environmental samples were identified as *B. glathei*-like bacteria. The gyrB sequence similarity levels toward other BGC species suggested that the 17 isolates...
### TABLE 3 | Mean fatty acid composition of all examined strains of BGC species.

| Fatty acid     | C16:0 | C16:1ω7c | C16:1ω6c | C16:1ω5c | C16:1ω9c | C16:1ω8c | C17:0 | C17:1ω7c | C18:0 | C18:1ω7c | C18:1ω9c | C18:1ω5c | C18:1ω6c | C18:1ω9c | C18:1ω6c | C18:1ω7c | C18:1ω9c | Summed feature 2 | Summed feature 3 |
|----------------|-------|----------|----------|----------|----------|----------|-------|----------|-------|----------|----------|----------|----------|----------|----------|----------|---------------|---------------|
| C12:0          | TR    | TR       | ND       | ND       | ND       | ND       | ND    | ND       | ND    | ND       | ND       | ND       | ND       | ND       | ND       | ND             | ND             |
| C14:0          | 3.70  | 3.95     | 3.71     | 3.71     | 3.77     | 3.66     | ND    | ND       | ND    | ND       | ND       | ND       | ND       | ND       | ND       | ND             | ND             |
| C15:0          | 0.63  | 1.89     | 1.24     | 1.54     | 1.96     | 1.67     | 1.29  | 1.22     | 1.27  | 1.27     | 1.27     | 1.27     | 1.27     | 1.27     | 1.27     | 1.27             | 1.27             |
| C16:0          | 3.77  | 1.97     | 3.38     | 1.69     | 1.69     | 1.69     | 1.69  | 1.69     | 1.69  | 1.69     | 1.69     | 1.69     | 1.69     | 1.69     | 1.69     | 1.69             | 1.69             |
| C17:1ω7c       | ND    | ND       | ND       | ND       | ND       | ND       | ND    | ND       | ND    | ND       | ND       | ND       | ND       | ND       | ND       | ND             | ND             |
| C18:0          | 3.82  | 1.89     | 3.38     | 1.69     | 1.69     | 1.69     | 1.69  | 1.69     | 1.69  | 1.69     | 1.69     | 1.69     | 1.69     | 1.69     | 1.69     | 1.69             | 1.69             |
| C18:1ω7c       | 4.06  | 1.97     | 3.38     | 1.69     | 1.69     | 1.69     | 1.69  | 1.69     | 1.69  | 1.69     | 1.69     | 1.69     | 1.69     | 1.69     | 1.69     | 1.69             | 1.69             |

Species: 1, B. glathei (strain); 2, B. clypeata (strain); 3, B. chinensis (strain); 4, B. grammica (strain); 5, B. chacei (strain); 6, B. humida (strain); 7, B. flava (strain); 8, B. berenice (strain); 9, B. megabacteriachromatoidis (strain); 10, B. bacteriachromatoidis (strain); 11, B. pagunculosa (strain); 12, B. pseudotuberculosis (strain); 13, B. pseudoaeruginosa (strain); 14, B. pseudomallei (strain); 15, B. pseudobacteriachromatoidis (strain); 16, B. pseudodurans (strain); 17, B. pseudosassoonii (strain); 18, B. pseudogama (strain); 19, B. pseudomaria (strain); 20, B. pseudomorai (strain); 21, B. pseudotuberculatiphilica (strain).

<% are not included, therefore, the percentages may not add up to 100%. TR, trace amount (<0.01). ND, not detected. Summed feature 2 comprises iso-C15:0 2-OH and/or C14:0 3-OH; summed feature 3 comprises C16:1ω7c 3-OH.
In this study represented 13 novel species (Figure 1). To further characterize the taxonomic status of these isolates, we analyzed the genome sequence of 13 isolates representative for the 13 gyrB sequence clusters and of 7 BGC type strains and compared those to 23 whole-genome sequences of BGC strains that were publicly available. Additionally, we also studied their chemotaxonomic and biochemical properties to comply with the polyphasic taxonomic consensus approach to bacterial systematics (Vandamme et al., 1996).

In this genomics era, state-of-the-art sequencing technologies enable direct access to the information contained in whole-genome sequences and it is no longer adequate to deduce genome relatedness through traditional DNA-DNA hybridization experiments (Vandamme and Peeters, 2014; Whitman, 2015). Genomic taxonomy can be studied through various parameters including average nucleotide identity (ANI), GBDP, Maximal Unique Matches index (MUMi), and core gene identity (CGI) (Konstantinidis and Tiedje, 2005; Goris et al., 2007; Deloger et al., 2009; Vanlaere et al., 2009; Meier-Kolthoff et al., 2013). Although, there is a general consensus that genome sequencing could revolutionize prokaryotic systematics (Sutcliffe et al., 2013; Meier-Kolthoff et al., 2014b; Peeters et al., 2015).
TABLE 5 | G+C content (mol%) of validly named BGC species.

| Strain                        | Wet-lab calculation | Calculation from WGS |
|-------------------------------|---------------------|----------------------|
| B. glathei LMG 14190<sup>T</sup> | 64.8 (Zolg and Ottow, 1975) | 64.7 |
| B. sordidicola LMG 22029<sup>T</sup> | 61.3 (Lim et al., 2003) | 60.2 |
| B. zhejiangensis OP-1<sup>T</sup> | 59.4 (Lu et al., 2012) | 62.7 |
| B. grimmiae R27<sup>T</sup> | 64.6 (Tian et al., 2013) | 63.0 |
| B. choica LMG 22940<sup>T</sup> | 63 (Vandamme et al., 2013) | 62.7 |
| B. humi LMG 22034<sup>T</sup> | 63 (Vandamme et al., 2013) | 62.8 |
| B. telluris LMG 22938<sup>T</sup> | 64 (Vandamme et al., 2013) | 64.0 |
| B. terrestris LMG 22937<sup>T</sup> | 62 (Vandamme et al., 2013) | 62.6 |
| B. udeis LMG 27134<sup>T</sup> | 60 (Vandamme et al., 2013) | 60.0 |
| B. cordobensis LMG 27620<sup>T</sup> | 63.6 (Draghi et al., 2014) | 63.7 |
| B. jiangsuensis MP-1<sup>T</sup> | – | 62.6 |
| B. megalochromosomata JC2949<sup>T</sup> | – | 62.7 |

identified strain MR1, which was isolated from Florida golf course soil and which was shown to reduce the herbicide methylarsenate, as *B. concitans* sp. nov. Next to strain YI23, which was previously identified as *B. cordobensis* by Draghi et al. (2014), the present study also identified strain RPE67, which was isolated from the gut of a stink bug, as *B. cordobensis*. Finally, both phylogenomic analyses also showed that strain PML1(12), an ectomycorrhizosphere-inhabiting bacterium with mineral-weathering ability (Uroz and Oger, 2015), strain S170, a potential plant growth promoter isolated from coniferous forest soil (Llado et al., 2014), strain RPE64, a bacterial symbiont of the bean bug *Riptortus pedestris* (Shibata et al., 2013) and strain Leaf177, an *Arabidopsis* leaf isolate (Bai et al., 2015) all represent novel BGC species.

*Burkholderia* genomes vary in size from 3.75 Mb (*B. rhizoxinica* HKI 454) to 11.3 Mb (*B. terrae* BS001), are characterized by a high G+C content (60–68%) and consist of multiple replicons (Winsor et al., 2008; Ussery et al., 2009). The DNA G+C content of the 13 novel species was calculated from their genome sequences and was in the range of that reported for other BGC species (60–65 mol%). For 10 of the 12 established BGC species, the G+C content was previously calculated by traditional wet-lab methods and the reported values differed by 0.1–3.3 mol% from the values calculated from their genome sequences (*Table 5*). As reported by Meier-Kolthoff et al., the G+C content calculations based on genome sequences show a higher precision than calculations based on traditional wet-lab methods because the latter methods do not count nucleotides but estimate the genomic G+C content based on the physical properties of the extracted and/or digested genomic DNA (Meier-Kolthoff et al., 2014b). The difference between literature data (Lim et al., 2003; Lu et al., 2012; Tian et al., 2013) and the genome sequence-based G+C content values of *B. sordidicola* LMG 22029<sup>T</sup>, *B. zhejiangensis* OP-1<sup>T</sup> and *B. grimmiae* R27<sup>T</sup> is larger than 1% and we therefore present emended descriptions of these species. The genome sizes of the type strains of the 13 novel species ranged from 6.2 Mb (*B. concitans* sp. nov. LMG 29315<sup>T</sup>) to 9.7 Mb (*B. arvi* sp. nov. LMG 29317<sup>T</sup>) and corresponded with the genome sizes of other free-living BGC species (*Table 2*). Consistent with reductive genome evolution in obligatory symbionts, the smallest BGC genomes belong to the obligatory leaf endosymbionts (2.4–6.2 Mb; Carlier and Eberl, 2012; Carlier et al., 2015; Pinto-Carbo et al., 2016).

Biochemically, these novel species are similar to their nearest neighbors. However, tests particularly useful for distinguishing BGC species are growth at 37°C and at pH 8, hydrolysis of tween 60 and 80, nitrate reduction, assimilation of arabinose, caprate and citrate, beta-galactosidase activity and C4 lipase (*Table 4*). The most discriminating fatty acids are C<sub>16:0</sub> 3-OH, C<sub>17:0</sub> cyclo, C<sub>19:0</sub> cyclo <i>ω</i>9c and summed features 2 and 3 (*Table 3*). The overall fatty acid profiles of the novel taxa are similar to those of their nearest neighbors and support their placement in the genus *Burkholderia* (Yabuuchi et al., 1992).

The present study again underscores the multifaceted nature of *Burkholderia* bacteria (Coenye and Vandamme, 2003; Mahenthiralingam et al., 2005) and highlights that also BGC species have evolved a broad range of interactions with different
hosts. *B. cordobensis* is a striking example of phenotypic and geographic breadth: it was recovered from agricultural soil in Argentina (strain LMG 27620\textsuperscript{T}) (Draghi et al., 2014), from golf course soil in South Korea (strain YI23) (Lim et al., 2012) and from the gut of the bean bug *Riptortus pedestris* in Japan (strain RPE67) (Takeshita et al., 2014). The two latter strains (YI23 and RPE67) have fenitrothion degrading properties. The former two strains (LMG 27620\textsuperscript{T} and YI23) were free-living but the latter (RPE67) is an endosymbiont of stink bugs that is not vertically transmitted but acquired from soil by the nymphal insect (Kikuchi et al., 2007). The insecticide resistance to fenitrothion in the pest insects was shown to be established by the endosymbiotic *Burkholderia* strain in the insect gut (Kikuchi et al., 2012) and was shown to emerge as a consequence of repeated insecticide use (Tago et al., 2015). The *Riptortus pedestris-B. cordobensis* association thus appears to be a rather young endosymbiosis and contrasts with the symbiosis observed between plant species of the *Rubiaceae* and *Primulaceae* families and several *Candidatus Burkholderia* species. The *Candidatus* designation is a provisional taxonomic status for organisms that have been characterized but that cannot be cultivated at present (Schleifer, 2009). These obligate leaf endosymbionts are vertically transmitted and represent an obligatory symbiosis which was estimated to originate millions of years ago (Lemaire et al., 2011).

BGC species harbor both beneficial and pathogenic strains. Strains PML1(12) and S170 show biotechnological potential for mineral-weathering and plant growth promotion, respectively, and are exemplary for the metabolic versatility of *Burkholderia* organisms (Lladó et al., 2014; Uroz and Oger, 2015). Mineral-weathering bacteria dissolve key nutrients from minerals and thereby increase the bioavailability of chemical nutrients in the environment (Uroz et al., 2009). On the other hand, three strains analyzed in the present study were isolated from human clinical samples, i.e., blood, pleural fluid and lung tissue (Table 1) and were classified as two novel species (*Burkholderia concitans* sp. nov. and *Burkholderia turbans* sp. nov.). They represent, to our knowledge, the first examples of human clinical isolates in the *B. glathei* clade. Strikingly, strain MR1, which was isolated from Florida golf course soil and shown to reduce the herbicide methyarsenate, was also identified as *Burkholderia concitans* sp. nov., and this species thus represents yet another human clinical *Burkholderia* species with interesting biotechnological properties (Coenye et al., 2001; Coenye and Vandamme, 2003; Goris et al., 2004; Mahenthiralingam et al., 2005). This study therefore further underscores that there is no phylogenetic subdivision in the genus *Burkholderia* that distinguishes beneficial from pathogenic strains (Angus et al., 2014; Sawana et al., 2014; Estrada-de los Santos et al., 2016; Dobriska and Samadpour, 2016).

In summary, the present study provides genotypic, chemotaxonomic and phenotypic data which enable the differentiation of 13 novel species in the genus *Burkholderia* and we propose the names *Burkholderia arvi* sp. nov., *Burkholderia hypogeia* sp. nov., *Burkholderia ptereochthonis* sp. nov., *Burkholderia glebae* sp. nov., *Burkholderia pedti* sp. nov., *Burkholderia arationis* sp. nov., *Burkholderia fortuita* sp. nov., *Burkholderia temeraria* sp. nov., *Burkholderia calidiae* sp. nov., *Burkholderia concitans* sp. nov., *Burkholderia turbans* sp. nov., *Burkholderia catudaia* sp. nov. and *Burkholderia peredens* sp. nov., with strains LMG 29317\textsuperscript{T}, LMG 29322\textsuperscript{T}, LMG 29326\textsuperscript{T}, LMG 29325\textsuperscript{T}, LMG 29323\textsuperscript{T}, LMG 29324\textsuperscript{T}, LMG 29320\textsuperscript{T}, LMG 29319\textsuperscript{T}, LMG 29321\textsuperscript{T}, LMG 29315\textsuperscript{T}, LMG 29316\textsuperscript{T}, LMG 29318\textsuperscript{T}, and LMG 29314\textsuperscript{T} as type strains, respectively. By making reference cultures and whole-genome sequences of each of these versatile bacteria publicly available, we aim to contribute to future knowledge about the metabolic versatility and pathogenicity of *Burkholderia* organisms.

**Description of Burkholderia arvi** sp. nov. *Burkholderia arvi* (ar’vi. L. gen. n. *arvi* of a field).

Cells are Gram-negative, non-motile rods (less than 1 \(\mu\)m wide and about 1 \(\mu\)m long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on tryptcose soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 6–7 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for nitrate reduction, beta-galactosidase (PNPG) (weak) and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, gluconate, malate, citrate (weak), and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction and assimilation of maltose, caprate, and adipate. When tested using API ZYM strips, positive for alkaline phosphatase, leucyl arylamidase, acid phosphatase, and phosphoamidase (weak); negative for C4 lipase, C8 lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucononidase, alpha-galactosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase. The following fatty acids are present: \(C\textsubscript{16:0}, C\textsubscript{16:1} 3\text{-OH}, C\textsubscript{18:1} \omega\text{7c}\text{, summed feature 2 (most likely } C\textsubscript{14:0} 3\text{-OH), and summed feature 3 (most likely } C\textsubscript{16:1} \omega\text{7c}\text{ in moderate amounts (>5%), and } C\textsubscript{14:0}, C\textsubscript{16:0} 2\text{-OH, } C\textsubscript{17:0} \text{ cyclo, and } C\textsubscript{19:0} \text{ cyclo } \omega\text{8c in minor amounts (1–5%).}

The type strain is LMG 29317\textsuperscript{T} (=CCUG 68412\textsuperscript{T}) and was isolated from agricultural soil in Argentina in 2010 (Draghi et al., 2014). Its G+C content is 62.4 mol% (calculated based on its genome sequence). The 16S rRNA, *gyrB* and whole-genome sequence of LMG 29317\textsuperscript{T} are publicly available through the accession numbers LT158615, LT158628, and FCOM02000000, respectively.

**Description of Burkholderia hypogeia** sp. nov. *Burkholderia hypogeia* (hy.po.ge’ia. Gr. adj. hypogeos subterraneous; N. L. fem. adj. hypogeia, subterraneous, earth-born).

Cells are Gram-negative, non-motile rods (about 1 \(\mu\)m wide and 1–2 \(\mu\)m long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on tryptcose soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-creamy color. The type strain is LMG 29317\textsuperscript{T} (=CCUG 68412\textsuperscript{T}) and was isolated from agricultural soil in Argentina in 2010 (Draghi et al., 2014). Its G+C content is 62.4 mol% (calculated based on its genome sequence). The 16S rRNA, *gyrB* and whole-genome sequence of LMG 29317\textsuperscript{T} are publicly available through the accession numbers LT158615, LT158628, and FCOM02000000, respectively.
margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 6 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for nitrate reduction and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, gluconate, malate, and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose, caprate, adipate and citrate. When tested using API ZYM strips, positive for alkaline phosphatase (weak), C4 lipase, C8 lipase (weak), leucyl arylamidase, acid phosphatase and phosphomonoesterase (weak); negative for C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, alpha-fucosidase. The following fatty acids are present: C_{16:0}, C_{18:0} cyclo, C_{18:1}ω7c, summed feature 2 (most likely C_{14:0} 3-OH) and summed feature 3 (most likely C_{16:1} ω7c) in moderate amounts (>5%), and C_{14:0}, C_{16:0} 2-OH, C_{16:1} 2-OH, and C_{19:0} cyclo ω8c in minor amounts (1–5%).

The type strain is LMG 29322T (=CCUG 68407T) and was isolated from botanical garden soil in Belgium in 2014 (Peeters et al., 2016). Its G+C content is 64.2 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29322T are publicly available through the accession numbers LT158624, LT158637, and FC0B02000000, respectively.

**Description of Burkholderia glebae sp. nov.**

*Burkholderia glebae* (gle'bae. L. gen. n. glebae from a lump or clod of earth, soil).

Cells are Gram-negative, non-motile rods (less than 1 μm wide and about 1 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round, tiny (typically less than 0.5 mm in diameter), non-translucent, with a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–28°C and at pH 7–8 in NB at 28°C (for the type strain only at pH 7). Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for nitrate reduction and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, gluconate, malate, citrate, and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose, caprate, and adipate. When tested using API ZYM strips, positive for leucyl arylamidase, acid phosphatase and phosphomonoesterase; negative for C8 lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase; strain-dependent reactions for alkaline phosphatase (type strain negative) and C4 lipase (type strain weak). The following fatty acids are present in all isolates: C_{16:0}, C_{18:0} 3-OH, C_{17:0} cyclo, C_{18:1}ω7c, summed feature 2 (most likely C_{14:0} 3-OH) and summed feature 3 (most likely C_{16:1} ω7c) in moderate amounts (>5%), and C_{14:0}, C_{16:0} 2-OH, C_{16:1} 2-OH, and C_{19:0} cyclo ω8c in minor amounts (1–5%) (mean value of all isolates).

The type strain is LMG 29325T (=CCUG 68404T) and was isolated from greenhouse soil in Belgium in 2014 (Peeters et al., 2016). Its G+C content is 62.7 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29325T are publicly available through the accession numbers LT158620, LT158633, and FC0A0200000, respectively.

**Description of Burkholderia pterechonthis sp. nov.**

*Burkholderia pterechonthis* (pte.re.o.chto'nis Gr. n. *pteris* fern; Gr. n. *chthon* soil; N. L. gen. n. *pterechonthis*, from fern soil).

Cells are Gram-negative, non-motile rods (less than 1 μm wide and about 1 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round, tiny (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 7 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for the assimilation of glucose, mannose, mannitol, N-acetyl-glucosamine, gluconate, malate, and phenylacetate; negative for nitrate reduction, production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of arabinose, maltose, caprate, adipate, and citrate. When tested using API ZYM strips, positive for alkaline phosphatase, C4 lipase, leucyl arylamidase, acid phosphatase and phosphomonoesterase (weak); negative for C8 lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase. The following fatty acids are present: C_{16:0}, C_{18:0} 3-OH, C_{17:0} cyclo, C_{18:1}ω7c, summed feature 2 (most likely C_{14:0} 3-OH) and summed feature 3 (most likely C_{16:1} ω7c) in moderate amounts (>5%), and C_{14:0}, C_{16:0} 2-OH, C_{16:1} 2-OH, and C_{19:0} cyclo ω8c in minor amounts (1–5%).

The type strain is LMG 29325T (=CCUG 68404T) and was isolated from botanical garden soil in Belgium in 2014 (Peeters et al., 2016). Its G+C content is 64.2 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29325T are publicly available through the accession numbers LT158620, LT158633, and FC0B0200000, respectively. An additional strain has been isolated from soil in the Netherlands (Table 1).
Cells are Gram-negative, non-motile rods (less than 1 µm wide and 1–2 µm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a beige color. Grows on MacConkey agar. Growth occurs at 15–28°C and at pH 6–8 in NB at 28°C (type strain only in pH 6–7). Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein; when tested using API 20NE strips, positive for hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose (type strain negative) and the assimilation of caprate (type strain negative) and the assimilation of maltose. When tested using API ZYM strips, positive for alkaline phosphatase, leucyl arylamidase, acid phosphatase, and phosphoamidase; negative for C14 lipase, cystine arylamidase, trypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase; strain-dependent reactions for C8 lipase (type strain negative), valine arylamidase (type strain negative), and chymotrypsin (type strain negative). The following fatty acids are present in all isolates: C14:0, C16:0 3-OH, C17:0 cyclo, C18:1ω7c, summed feature 2 (most likely C14:0 3-OH), and summed feature 3 (most likely C16:1ω7c) in moderate amounts (>5%), and C14:0, C16:0 2-OH, C16:1 2-OH, and C19:0 cyclo ω8c in minor amounts (1–5%) (mean value of all isolates).

The type strain is LMG 29324T (=CCUG 68406T) and was isolated from botanical garden soil in Belgium in 2014 (Peeters et al., 2016). Its G+C content is 62.8 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB, and whole-genome sequence of LMG 29324T are publicly available through the accession numbers LT158622, LT158635, and FCO02000000, respectively. An additional strain has been isolated from soil in the Netherlands (Table 1).

Description of Burkholderia arationis sp. nov.

Burkholderia arationis (a.ra.ti.o’nis. L. gen. n. arationis from a field).

Cells are Gram-negative, non-motile rods (less than 1 µm wide and about 1 µm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, translucent, with entire margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–28°C and at pH 6 in NB at 28°C (the type strain did not grow in liquid NB medium). Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, gluconate, caprate (weak), adipate (weak), malate, citrate, and phenylacetate; negative for nitrate reduction, production of indol, fermentation of glucose, arginine dihydrodrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG), and assimilation of maltose. When tested using API ZYM strips, positive for alkaline phosphatase, C4 lipase, leucyl arylamidase, acid phosphatase and phosphoamidase; negative for C14 lipase, cystine arylamidase, trypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase; strain-dependent reactions for C8 lipase (type strain negative), valine arylamidase (type strain negative), and chymotrypsin (type strain negative). The following fatty acids are present in all isolates: C16:0, C16:0 3-OH, C18:1ω7c, summed feature 2 (most likely C14:0 3-OH), and summed feature 3 (most likely C16:1ω7c) in moderate amounts (>5%), and C14:0 in minor amounts (1–5%) (mean value of all isolates).

The type strain is LMG 29324T (=CCUG 68406T) and was isolated from botanical garden soil in Belgium in 2014 (Peeters et al., 2016). Its G+C content is 62.8 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB, and whole-genome sequence of LMG 29324T are publicly available through the accession numbers LT158622, LT158635, and FCO02000000, respectively. An additional strain has been isolated from soil in the Netherlands (Table 1).
The type strain is LMG 29320T (=CCUG 68409T) and was isolated from Fadogia homblei rhizosphere soil in South Africa in 2013 (Verstraete et al., 2014). Its G+C content is 62.9 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29320T are publicly available through the accession numbers LT158618, LT158631, and FCNX02000000, respectively.

**Description of Burkholderia temeraria sp. nov.**

*Burkholderia temeraria* (te.me.ra’ri.a. L. fem. adj. *temeraria* accidental, inconsiderate; referring to its accidental isolation when searching for *Burkholderia caledonica* endophytes).

Cells are Gram-negative, non-motile rods (less than 1 μm wide and about 1 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-cream color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 6–7 in NB at 28°C. Catalase and oxidase activities are present. Does not hydrolyze tween 60, tween 80, starch and casein. When tested using API 20NE strips, positive for nitrate reduction and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, gluconate, caprate, malate, citrate (weak), and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose and adipate. When tested using API ZYM strips, positive for alkaline phosphatase (weak), C8 lipase (weak), leucyl arylamidase (weak), acid phosphatase and phosphoamidase (weak); negative for C4 lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-gluconidase, alpha-gluconidase, beta-gluconidase, N-acetyl-beta-gluconamidase, alpha-mannosidase, and alpha-fucosidase. The following fatty acids are present: C16:0, C18:1ω6c, summed feature 2 (most likely C14:0 3-OH) and summed feature 3 (most likely C16:1ω7c) in moderate amounts (>5%), and C14:0, C16:0 2-OH, C16:0 3-OH, and C17:0 cyclo in minor amounts (1–5%).

The type strain is LMG 29321T (=CCUG 68408T) and was isolated from greenhouse pond water in Belgium in 2013 (Peeters et al., 2016). Its G+C content is 62.5 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29321T are publicly available through the accession numbers LT158619, LT158632, and FCOX02000000, respectively.

**Description of Burkholderia concitans sp. nov.**

*Burkholderia concitans* (con.ci’tans. L. fem. part. pres. *concitans* disturbing, upsetting; because the isolation of this bacterium from human sources, including blood, further disturbs the image of this lineage of *Burkholderia* species as benign bacteria).

Cells are Gram-negative, non-motile rods (less than 1 μm wide and about 1 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-cream color. Grows on MacConkey agar. Growth occurs at 15–28°C and at pH 6–7 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API ZYM strips, positive for alkaline phosphatase, C4 lipase, C8 lipase (weak), leucyl arylamidase, valine arylamidase, acid phosphatase, and phosphoamidase; negative for C14 lipase,
trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase; strain-dependent reactions for cystine arylamidase (type strain negative). The following fatty acids are present in all isolates: C\textsubscript{16:0}, C\textsubscript{16:0} 3-OH, C\textsubscript{17:0} cyclo, C\textsubscript{18:1}ω7c, C\textsubscript{19:0} cyclo ω8c, summed feature 2 (most likely C\textsubscript{14:0} 3-OH) and summed feature 3 (most likely C\textsubscript{16:1} ω7c) in moderate amounts (>5%), and C\textsubscript{14:0}, C\textsubscript{16:0} 2-OH, and C\textsubscript{16:1} 2-OH in minor amounts (1–5%) (mean value of all isolates).

The type strain is LMG 29315\textsuperscript{T} (=CCUG 68414\textsuperscript{T}) and was isolated from human lung tissue in the USA in 2006. Its G+C content is 63.2 mol%. The 16S rRNA, gyrB, and whole-genome sequence of LMG 29315\textsuperscript{T} are publicly available through the accession numbers LT158613, LT158626 and FCNV02000000, respectively. An additional strain has been isolated from human blood in the USA in 2010 (Table 1).

### Description of Burkholderia turbans sp. nov.

**Burkholderia turbans** (tur’bans. L. fem. part. pres. turbans disturbing, agitating, because the isolation of this bacterium from human pleural fluid further disturbs the image of this lineage of *Burkholderia* species as benign bacteria).

Cells are Gram-negative, non-motile rods (about 1 μm wide and 1–1.5 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 6–7 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for nitrate reduction and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, glucuronate, malate, and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose, caprate, adipate, and citrate. When tested using API ZYM strips, positive for alkaline phosphatase (weak), leucyl arylamidase, acid phosphatase, and phosphoamidase (weak); negative for C4 lipase, C8 lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase. The following fatty acids are present: C\textsubscript{16:0}, C\textsubscript{16:0} 3-OH, C\textsubscript{18:1}ω7c, summed feature 2 (most likely C\textsubscript{14:0} 3-OH) and summed feature 3 (most likely C\textsubscript{16:1} ω7c) in moderate amounts (>5%), and C\textsubscript{14:0}, C\textsubscript{16:0} 2-OH, C\textsubscript{17:0} cyclo, and C\textsubscript{19:0} cyclo ω8c in minor amounts (1–5%).

The type strain is LMG 29318\textsuperscript{T} (=CCUG 68411\textsuperscript{T}) and was isolated from *Fadogia homblei* rhizosphere soil in South Africa in 2013 (Verstraete et al., 2014). Its G+C content is 62.8 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29318\textsuperscript{T} are publicly available through the accession numbers LT158616, LT158629, and FCOF02000000, respectively.

### Description of Burkholderia catudaia sp. nov.

**Burkholderia catudaia** (ca.tu.da’ia. Gr. adj. catudaios subterraneous; N. L. fem. adj. *catudaia*, earth-born).

Cells are Gram-negative, non-motile rods (about 1 μm wide and 1–2 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 6–7 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for nitrate reduction and assimilation of glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, glucuronate, malate, and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease, esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose, caprate, adipate, and citrate. When tested using API ZYM strips, positive for alkaline phosphatase (weak), leucyl arylamidase, acid phosphatase, and phosphoamidase (weak); negative for C4 lipase, C8 lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase, alpha-mannosidase, and alpha-fucosidase. The following fatty acids are present: C\textsubscript{16:0}, C\textsubscript{16:0} 3-OH, C\textsubscript{18:1}ω7c, summed feature 2 (most likely C\textsubscript{14:0} 3-OH) and summed feature 3 (most likely C\textsubscript{16:1} ω7c) in moderate amounts (>5%), and C\textsubscript{14:0}, C\textsubscript{16:0} 2-OH, C\textsubscript{17:0} cyclo, and C\textsubscript{19:0} cyclo ω8c in minor amounts (1–5%).

The type strain is LMG 29318\textsuperscript{T} (=CCUG 68411\textsuperscript{T}) and was isolated from *Fadogia homblei* rhizosphere soil in South Africa in 2013 (Verstraete et al., 2014). Its G+C content is 62.8 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29318\textsuperscript{T} are publicly available through the accession numbers LT158616, LT158629, and FCOF02000000, respectively.

### Description of Burkholderia peredens sp. nov.

**Burkholderia peredens** (per.e’dens. L. fem. part. pres. *peredens* consuming, devouring; referring to the capacity of this bacterium to degrade fenitrothion).

Cells are Gram-negative, non-motile rods (about 1 μm wide and 1–2 μm long) with rounded ends that occur as single units or in pairs. After 48 h of incubation on trypticase soy agar at 28°C, colonies are round (typically less than 1 mm in diameter), smooth, shiny, non-translucent, with entire margins and a white-creamy color. Grows on MacConkey agar. Growth occurs at 15–37°C and at pH 7 in NB at 28°C. Catalase and oxidase activities are present. Hydrolyses tween 60, but not tween 80, starch and casein. When tested using API 20NE strips, positive for the assimilation of glucose, arabinose (weak), mannose, mannitol, N-acetyl-glucosamine, glucuronate, malate, and phenylacetate; negative for production of indol, fermentation of glucose, arginine dihydrolase, urease. 

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esculin hydrolysis, gelatin liquefaction, beta-galactosidase (PNPG) and assimilation of maltose, caprate, adipate, and citrate. When tested using API ZYM strips, positive for alkaline phosphatase, C4 lipase (weak), C8 lipase (weak), leucyl arylamidase, acid phosphatase, and phosphoamidase (weak); negative for C14 lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alpha-galactosidase, beta-galactosidase, beta-gluconidase, alpha-glucosidase, beta-gluconidase, N-acetyl-beta-gluconamidase, alpha-mannosidase, and alpha-fucosidase. The following fatty acids are present: C\textsubscript{16:0}, C\textsubscript{16:1} 3-OH, C\textsubscript{18:1} 07c, summed feature 2 (most likely C\textsubscript{14:0} 3-OH) and summed feature 3 (most likely C\textsubscript{16:1} 07c) in moderate amounts (>5%), and C\textsubscript{16:0}, C\textsubscript{16:0} 2-OH, C\textsubscript{16:1} 2-OH and C\textsubscript{17:0} cyclo in minor amounts (1–5%).

The type strain is LMG 29314\textsuperscript{T} (=CCUG 68415\textsuperscript{T}) and was isolated from soil in Japan (Hayatsu et al., 2000). Its G+C content is 63.1 mol% (calculated based on its genome sequence). The 16S rRNA, gyrB and whole-genome sequence of LMG 29314\textsuperscript{T} are publicly available through the accession numbers LT158612, LT158625, and FCOH02000000, respectively.

**Emended Description of the Species *Burkholderia sordidicola* (Lim et al., 2003)**

The description of the species *Burkholderia sordidicola* is the one given by Lim et al. (2003) with the following modification. The G+C content of the type strain is 60.2%.

**Emended Description of the Species *Burkholderia zhejiangensis* (Lu et al., 2012)**

The description of the species *Burkholderia zhejiangensis* is the one given by Lu et al. (2012) with the following modification. The G+C content of the type strain is 62.7%.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2016.00877

**Supplementary Table 1 | Pairwise GGD and dDDH values.** For each pair of genomes the intergenicomic distance (GGD), dDDH and 95% confidence interval (CI) is given, sorted from high to low dDDH. dDDH values above the 70% cut-off for species delineation are shown in bold type.
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**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.

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