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Phylogenomic analysis supports the reclassification of *Burkholderia novacaledonica* as *Caballeronia novacaledonica* comb. nov.

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Abstract

Burkholderia novacaledonica is a Betaproteobacterial species isolated from ultramafic soils in New Caledonia. The characterization and classification of this species into the *Burkholderia* genus was done simultaneously with the proposal of the new genus *Caballeronia*, initially composed of closely related *Burkholderia glathei*-like species. Thereafter, some reports based on the use of phylogenetic marker genes suggested that *B. novacaledonica* forms part of *Caballeronia* genus. Lacking a formal validation, and with the availability of its genome sequence, a genome-based phylogeny of *B. novacaledonica* was obtained to unravel its taxonomic position in *Burkholderia sensu lato*. A partial *gyrB* gene phylogeny, extended multilocus sequence typing on homologous protein sequences, and genomic distance-based phylogeny, all support the placement of this species in the *Caballeronia* genus. Therefore, the reclassification of *B. novacaledonica* to *Caballeronia novacaledonica* comb. nov. is proposed.

Since the *Pseudomonas* Group II was transferred to the *Burkholderia* genus in 1992 [1] several new species belonging to this bacterial genus have been described (https://lpsn.dsmz.de/genus/burkholderia). The rising number of new species determined, and the availability of several bacterial genome sequences, led to a continuously revised taxonomy of this genus. Thus, Estrada de los Santos *et al.* [2], using a multilocus sequencing analysis (MLSA), and Sawana *et al.* [3], using conserved sequence indels (CSIs), proposed the split of the *Burkholderia* genus in two genera: the *Burkholderia* genus, composed mainly of human, plant and animal pathogens (including species of the *Burkholderia cepacia* complex as well as *Burkholderia pseudomallei* complex), and the *Paraburkholderia* genus, mainly composed of environmental and beneficial species. Later on, Dobritsa and colleagues [4] proposed transferring the species phylogenetically related to *Burkholderia glathei* to a new genus, *Caballeronia*. More recently, a phylogenetic approach using conserved protein sequences reaffirmed the previous findings and proposed two new genera into the *Burkholderia* clade: *Mycetohabitans* gen. nov., and *Trinickia* gen. nov. [5], along with the reclassification and description of *Robbsia* gen. nov and *Pararobbsia* gen. nov [6, 7]. Thus, the last decade has been a dynamic scenario for *Burkholderia sensu lato* related species in order to unequivocally define their taxonomic placement.

During the course of defining these different genera several species have been described as belonging to *Burkholderia*, however they were later transferred and emended to accommodate their rightful placement in these newly proposed bacterial genera [4, 8, 9]. In this period *Burkholderia ultramafica* and *Burkholderia novacaledonica* were described, which are plant-associated species isolated from rhizospheric samples of *Costularia* (*Cyperaceae*) adapted to extreme edaphic conditions in New Caledonia [5]. These species were classified as belonging to *Burkholderia* by a polyphasic approach, but the phylogenetic information obtained by analysing single marker genes, as well as the presence of specific CSI, suggested their transfer to *Paraburkholderia* and *Caballeronia*, respectively [9, 10]. In fact, and based on its phylogenomic analysis, *B. ultramafica* was formally proposed to be transferred to the *Paraburkholderia* genus [11]. The taxonomic rank of *B. novacaledonica* remains, however, unresolved. Thus, based on previous observations, and with the availability of the genome sequence for the type strain of *B. novacaledonica*, a phylogenomic analysis was performed to unravel its taxonomic position.

The phylogenetic position of *B. novacaledonica* through the 16S rDNA gene sequence has been previously determined [9, 10]. This analysis showed that the type strain (*B.*

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Abbreviations: GBDP, Genome Blast Distance Phylogeny; *gyrB*, DNA gyrase subunit B; MLSA, multilocus sequence analysis.

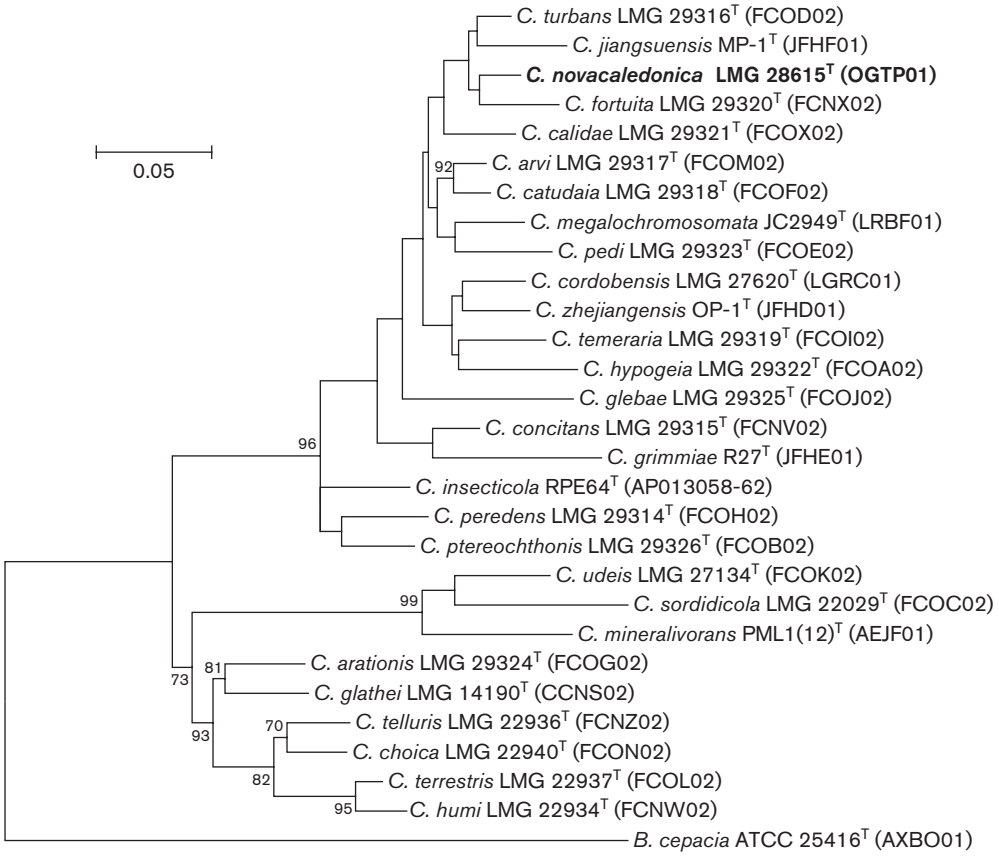

Fig. 1. Molecular phylogenetic analysis of partial *gyrB* gene sequences. The evolutionary history was inferred by using the maximum-likelihood method based on the general time reversible model. The tree with the highest log likelihood (−4565.7758) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches, determined on 1000 bootstrap replicates. Bootstrap values higher than 70% are shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter=0.3202)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 31.1162% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. A total of 654 positions in the final dataset were analysed. Evolutionary analyses were conducted using MEGA 7 [20].

novacaledonica STM10272^T) is phylogenetically related to the *Caballeronia*. In fact, pairwise sequence similarity values determined through EzBioCloud database [12] showed values higher than 99% of 16S rDNA sequence identity with its closest related species: *C. turbans* LMG 29316^T (99.59%), *C. peredens* LMG 29314^T (99.38%), *C. insecticola* RPE64^T (99.38%), and *C. ptereochthonis* LMG 29326^T (99.17%). To improve upon discriminant ability of the 16S rDNA, it has been recommended to use partial *gyrB* as a molecular marker of *Burkholderia glathei*-related species [13]. Thus, the phylogenetic analysis of the partial *gyrB* sequence was performed (Fig. 1), showing that *B. novacaledonica* STM10272^T belongs to the *Caballeronia* genus, with its closest relatives *C. turbans* LMG 29316^T (96.97%) and *C. calidae* LMG 29321^T (96.1%) according to the *gyrB* identity values. Thus, single gene phylogenetic trees support the transfer of *B. novacaledonica* to the *Caballeronia* genus.

Furthermore, a phylogenomic analysis was performed to improve upon the resolution of the single gene-based phylogeny, using the available genome sequence (GenBank assembly accession no. GCA_900258035.1). Firstly, an extended MLSA using single-copy orthologous genes was performed. To obtain these genes homologous gene families were determined through the GET_HOMOLOGUES software, using bidirectional best hit (BDBH) and clusters of orthologous groups (COG) algorithms with default parameters and minimal coverage of 90% between aligned sequences [14]. The resulting core genome was analysed through the GET_PHYLOMARKERS pipeline v1.3.1 [15] to identify high-quality marker genes for robust phylogenomic analyses, thereby excluding recombinant gene alignments and alignments producing anomalous or poorly resolved trees. Thus, 321 marker genes were selected and aligned based on their amino acid sequences to compute a phylogenetic tree. As shown in Fig. 2, the position of *B. novacaledonica* STM 10272^T in the *Caballeronia* genus is confirmed, and it appears to be most closely related to *C. megalochromosomata* JC2949^T

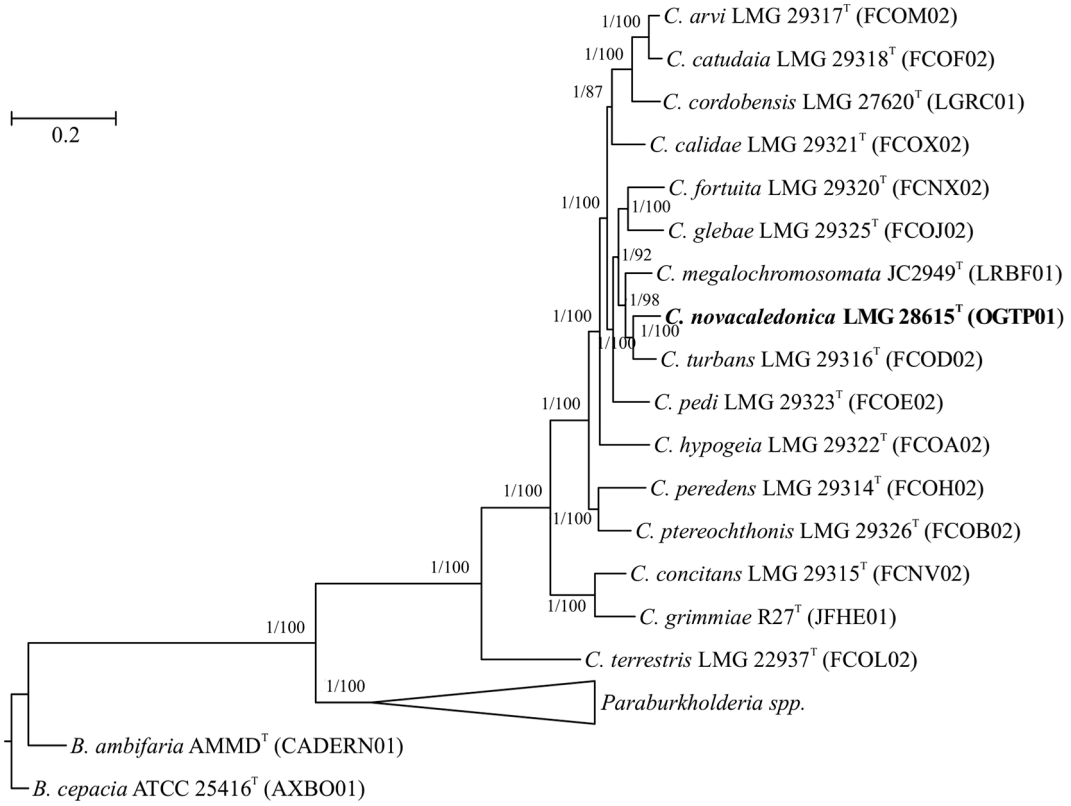

Fig. 2. Core-genome phylogeny for the genus *Caballeronia* and related species. The figure shows the best maximum-likelihood species tree inferred from the supermatrix of 321 top-scoring markers (39537 amino-acid residues, *Lnl* –856076.399). Core genome genes were selected using bidirectional best hit (BDBH) and clusters of orthologous groups (COG) algorithms implemented in the GET_HOMOLOGUES software [14] with default parameters and a minimal coverage of 90% between aligned sequences. Out of 1024 core genes 321 were selected after evaluation of recombination signal and outliers gene trees, using Phi-test and kdetrees R package, as implemented in GET_PHYLOMARKERS [15]. Concatenated top-scoring phylogenetic markers were used to infer maximum-likelihood phylogenies using IQ-Tree v.1.6.3 [21]. Node numbers indicate the approximate Bayesian posterior probabilities (aBypp)/UFBoot2 support values, determined on 1000 bootstrap replicates. The scale bar represents the number of expected substitutions per site under the best-fitting LG+F+R3 model, selected using ModelFinder (as implemented in IQ-Tree v 1.6.3).

(branch support value 1/92) and *C. turbans* LMG 29316^T (branch support value 1/100).

Additionally, by employing the Type Strain Genome Server (TYGS) database a Genome BLAST Distance Phylogeny (GBDP) approach was followed to calculate intergenomic distances between each pair of genomes, based on the nucleotide data. The resulting intergenomic distances were used to infer a balanced minimum evolution tree (inferred by the distance formula d5 [16]), with branch support (FASTME 2.1.6.1 [17]) inferred from 100 pseudo-bootstrap replicates each and applied to the *Caballeronia* genus. As shown in Fig. 3, the phylogenomic inference showed that *B. novacaledonica* belongs to the *Caballeronia* genus, being closely related to *C. jianguensis* MP-1^T, *C. turbans* LMG 29316^T, and *C. megalochromosomata* JC2949^T. This pattern association was also confirmed through Average Nucleotide Identity (ANI) values [18], showing the highest values with these *Caballeronia* species (92.68, 92.12 and 91.97% regarding *C. jianguensis* MP-1^T, *C. turbans* LMG 29316^T, and *C. megalochromosomata* JC2949^T, respectively). Finally, percent G+C was calculated from the genome sequence, which showed a slightly lower value (63.3 mol%) than the wet-lab determination (63.6 mol%) [19].

The phylogenomic analysis performed, as well as single marker gene phylogenies [9, 10] support the transfer of *B. novacaledonica* STM20272^T to the *Caballeronia* genus, so the reclassification of *B. novacaledonica* as *Caballeronia novacaledonia* comb. nov. is proposed.

EMENDED DESCRIPTION OF *CABALLERONIA NOVACALEDONICA* COMB. NOV.

Caballeronia novacaledonica (no.va.ca.le.do'ni.ca. L. masc. adj. *novus* new; L. fem. n. *Caledonia* Latin name for the Scottish Highlands; N.L. fem. adj. *novacaledonica* of New Caledonia from where the strains were isolated).

Basonym: *Burkholderia novacaledonica* Guentas *et al.* 2020.

The description of the species *Caballeronia novacaledonica* is the one given by Guentas *et al.* [19] with the following

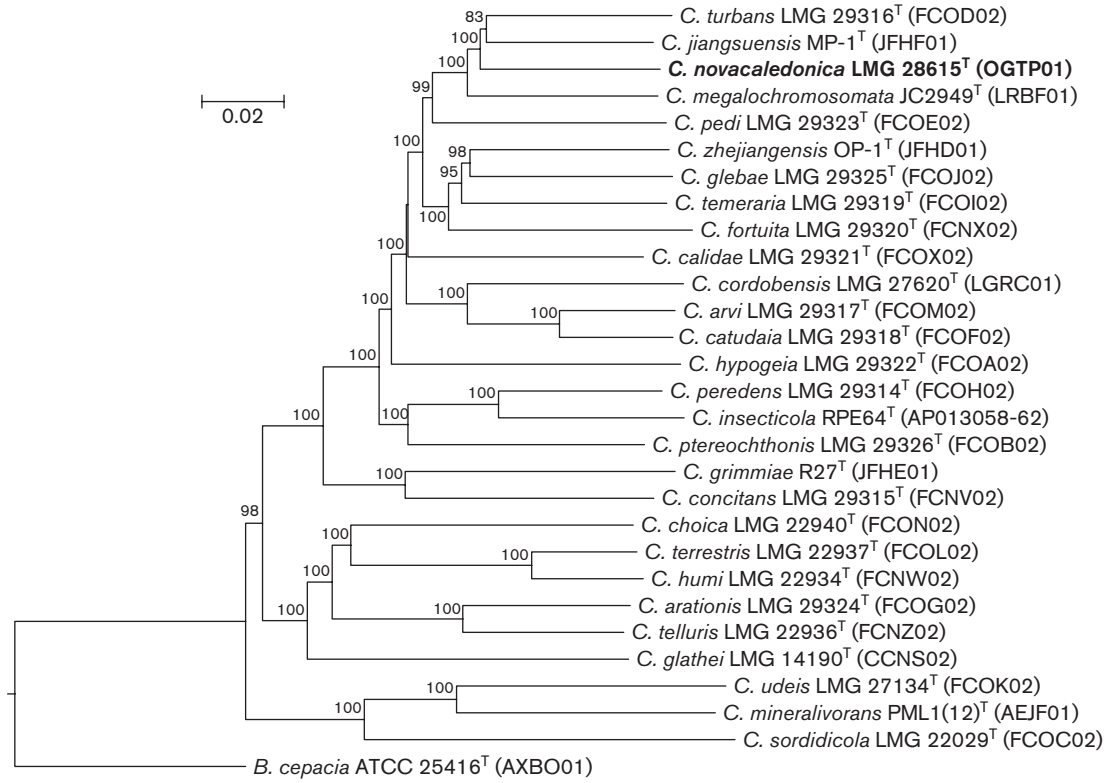

Fig. 3. Whole-genome sequence based phylogenomic tree of *Caballeronia* type strains inferred by GBDP. Tree inferred with FastME 2.1.6.1 [17] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values >60% from 100 replications, with an average branch support of 96.9%.

modification. The G+C content of the type strain is 63.3 mol%. The type strain is STM10272^T (=CIP110887^T=LMG28615^T).

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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