Systematic and Applied Microbiology 37 (2014) 194–199

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Intraspecific variation in *Burkholderia caledonica*: Europe vs. Africa and soil vs. endophytic isolates

Brecht Verstraete[a,*], Charlotte Peeters[b], Braam van Wyk[c], Erik Smets[a,d], Steven Dessein[a,e], Peter Vandamme[b]

[a] *Plant Conservation and Population Biology, KU Leuven, 3001 Leuven, Belgium*
[b] *Laboratory of Microbiology, Ghent University, 9000 Ghent, Belgium*
[c] *H.G.W.J. Schweickerdt Herbarium, University of Pretoria, 0083 Hatfield, South Africa*
[d] *Naturalis Biodiversity Center, Leiden University, 2300 RA Leiden, The Netherlands*
[e] *National Botanic Garden of Belgium, 1860 Meise, Belgium*

ARTICLE INFO

Article history:
Received 12 July 2013
Received in revised form
29 November 2013
Accepted 16 December 2013

Keywords:
Biogeography
Burkholderia caledonica
Endophyte
Rhizosphere
Rubiaceae
Soil

ABSTRACT

The best-known interaction between bacteria and plants is the *Rhizobium*-legume symbiosis, but other bacteria–plant interactions exist, such as between *Burkholderia* and Rubiaceae (coffee family). A number of bacterial endophytes in Rubiaceae are closely related to the soil bacterium *Burkholderia caledonica*. This intriguing observation is explored by investigating isolates from different geographic regions (Western Europe vs. sub-Saharan Africa) and from different niches (free-living bacteria in soil vs. endophytic bacteria in host plants). The multilocus sequence analysis shows five clades, of which clade 1 with two basal isolates deviates from the rest and is therefore not considered further. All other isolates belong to the species *B. caledonica*, but two genetically different groups are identified. Group A holds only European isolates and group B holds isolates from Africa, with the exception of one European isolate. Although the European and African isolates are considered one species, some degree of genetic differentiation is evident. Endophytic isolates of *B. caledonica* are found in certain members of African Rubiaceae, but only in group B. Within this group, the endophytes cannot be distinguished from the soil isolates, which indicates a possible exchange of bacteria between soil and host plant.

Introduction

Non-pathological interactions between bacteria and plants are ubiquitous and it is assumed that not a single plant species is devoid of a bacterial partner [18]. The specialized mutualistic interaction between α-rhizobia and legumes through the formation of root nodules is well known and has been exhaustively studied [17]. Other types of interactions between many different bacteria and various plant groups are also known, but most are poorly studied. One example is the occurrence of bacterial endophytes in specialized leaf nodules of certain tropical plants [15,16]. Such intimate association between bacteria and plants occurs in at least 500 species of the huge flowering plant family Rubiaceae (coffee family), more specifically in members of the genera *Pavetta*, *Psychotria* and *Sericanthe* [15]. Besides the occurrence of clearly differentiated nodules harbouring the bacterial endophyte, other Rubiaceae genera exist that house endophytic bacteria freely in the intercellular spaces between the mesophyll cells of their leaves [23–25]. In both instances, the endophytes belong to the genus *Burkholderia* [15,23–25]. Some of them were attributed new scientific names (e.g. "*Candidatus* B. schumannianae" in Lemaire et al. [16]), while others are provisionally referred to by merely an informal descriptive phrase (e.g. "endophyte of *Vangueria infausta*" in Verstraete et al. [23]). At least 15 species of Rubiaceae share a common, non-nodulated leaf endophyte. Based on 16S rRNA gene sequence similarity, this endophyte was considered to belong to *Burkholderia caledonica*, a species that was originally isolated from soil [25].

16S rRNA gene sequencing does not always provide enough resolution to differentiate among bacterial species, nor among sequence-discrete populations within a bacterial species. Such intraspecific variation may result from genomic adaptations to unique geographical and ecological conditions [2]. Multilocus sequence analysis (MLSA) is a more useful method for discriminating among closely related bacterial species and isolates, because using multiple DNA markers reduces the chance of stochastic clustering and consequently provides a more reliable hypothesis on the evolutionary relatedness of different isolates [10]. The use

* Corresponding author at: Plant Conservation and Population Biology, KU Leuven, Kasteelpark Arenberg 31, PO Box 2435, 3001 Leuven, Belgium. Tel.: +32 16321536.
E-mail address: brecht.verstraete@bio.kuleuven.be (B. Verstraete).

0723-2020/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.syapm.2013.12.001

of a set of seven housekeeping genes was shown to be successful in distinguishing among bacterial isolates in environmental samples [4].

In the present study, we investigate *B. caledonica* isolates from different geographic regions, namely Western Europe and sub-Saharan Africa, and from ecologically distinct niches, namely free-living in soil and endophytic in host plants. Our first objective is to explore whether there is a genetic differentiation among bacteria found in geographical distinct regions (regardless of niche). The second objective is to establish the evolutionary relatedness between free-living and endophytic representatives of *B. caledonica* (regardless of geography).

Materials and methods

Origin of host plant material and bacterial isolates

Host plant material of the Rubiaceae species was collected during several botanical expeditions in Africa and vouchers have been deposited at the herbarium of the National Botanic Garden of Belgium (BR). All required permits for the collection of these plants were obtained and are deposited at the National Botanic Garden of Belgium. Host plant leaves were collected from live plants in the wild and immediately put on silica gel to allow rapid dehydration and DNA preservation. The leaves, together with the silica gel, were kept in airtight plastic bags.

The presence of endophytic *Burkholderia* bacteria in different representatives of the tribe Vanguerieae (Rubiaceae) was noticed earlier [24,25]. For the present study, we only focus on the interaction between plants and *B. caledonica*, and we therefore compiled 35 plant samples representing 13 plant species of which the endophyte has been identified as *B. caledonica*. The plants were collected in different regions covering a wide geographic range.

Free-living isolates from previous studies that bear the name *B. caledonica* were used. These strains were originally isolated from soil samples collected in the Netherlands and Scotland [3,19]. For the present study, an additional 11 isolates were isolated from rhizosphere soil samples of *Fadogia homblei* collected near Pretoria, South Africa.

Two closely related *Burkholderia* species, namely *Burkholderia phymatum* and *Burkholderia phytofirmans*, were used as outgroup for the phylogenetic analysis and their corresponding DNA sequences were obtained from GenBank.

Detailed information on all isolates is provided in Table 1.

Analysis of endophytic B. caledonica

A cultivation-independent screening method was set up to investigate the presence and identity of endophytic bacteria in the Rubiaceae host plants. Silica-dried leaves were handled with sterile tweezers on a sanitized workbench and rinsed with 70% ethanol to remove debris and epiphytes from the leaf surfaces. This technique has been applied successfully and proven to be adequate in previous studies on *Burkholderia* endophytes in Rubiaceae [14–16,23–25]. To confirm the endophytic lifestyle of the bacteria investigated, scanning electron microscopy has been previously used to show the presence of the bacteria between the mesophyll cells of the leaves ([23], their Fig. 1).

Isolation of B. caledonica from soil

Soil samples were collected from the rhizosphere of *F. homblei* near Pretoria, South Africa. To extract the soil bacteria, 25 g of each soil sample together with 225 ml physiological saline was transferred to sterile plastic bags and the samples were homogenized in a Stomacher laboratory blender for 30 s at high speed (Bag-Mixer, Interscience). Aliquots (50 μl) of the serial dilution series (1:10) in physiological saline were plated on the selective medium PCAT [1] with 200 ppm cycloheximide and incubated at 28 °C for 5 days. Colonies were randomly picked up and subcultivated on buffered Nutrient Agar (Oxoid, pH 6.8). Dereplication was done using MALDI-TOF MS as previously described [8] and isolates were identified using *recA* gene sequence analysis as described below.

DNA extraction, amplification and sequencing

Extraction of bacterial DNA from the silica-dried leaves of the host plants was performed using the E.Z.N.A.™ HP Plant DNA Mini Kit (Omega Bio-Tek) according to the manufacturer's instructions. Primers and annealing temperatures for the amplification of the seven MLSA markers, namely *atpD*, *gltB*, *gyrB*, *lepA*, *phaC*, *recA* and *trpB*, are based on the multilocus sequence typing scheme proposed by Spilker et al. [20]. PCR products were send to Macrogen for sequencing. Total DNA from the cultured *B. caledonica* isolates was prepared by heating one to two colonies at 95 °C for 15 min in 20 μl buffer containing 0.25% (v/v) sodium dodecyl sulphate (SDS) and 0.05 M NaOH. Following cell lysis, 180 μl distilled water was added to the lysis buffer and the DNA solutions were stored at −20 °C. The MLSA genes were amplified using the primers mentioned above and sequenced on an Applied Biosystems 3130xl DNA Sequencer and protocols of the manufacturer using the BigDye Terminator Cycle Sequencing Ready kit. All new DNA sequences are deposited in GenBank and the accession numbers can be found in Table S1.

Phylogenetic and network analyses

All obtained sequences were assembled and edited using the DNA analysis software platform Geneious v5.4.7. A preliminary sequence alignment was performed with MUSCLE under default parameters [6] as implemented in Geneious, followed by manual fine-tuning resulting in an unequivocal alignment. Phylogenetic trees were constructed using Maximum Likelihood analysis in PhyML [9]. The DNA substitution GTR + I + G was selected under the Akaike Information Criterion using jModelTest 2.1.3 [5]. Non-parametric bootstrap analysis with 1000 iterations was carried out to calculate the relative support for individual clades found in the likelihood analysis. To detect cluster patterns within the bacterial species *B. caledonica*, a phylogenetic network analysis was performed using the NeighborNet method implemented in SplitsTree4 [11]. The splits graph was produced from distances computed as the proportion of positions at which two sequences differ (UncorrectedP option). Bootstrap analysis with 100 iterations was performed to compute the relative support of the edges.

Results

We investigated 59 different isolates originating from two geographic regions: 13 from Western Europe and 46 from sub-Saharan Africa. Moreover, the African isolates were obtained from two ecologically distinct niches, namely 11 free-living bacteria isolated from soil, four endophytic isolates from *F. homblei* host plants, and 31 uncultured endophytic bacteria found in various rubiaceous host species (Table 1). A multilocus sequence analysis with seven concatenated DNA markers (*atpD*, *gltB*, *gyrB*, *lepA*, *phaC*, *recA* and *trpB* with a total length of 4422 bp) was performed on all isolates. The analysis of the evolutionary relatedness between these 59 isolates resulted in a robust phylogenetic tree showing two basal isolates and two large groups. The isolates R-23313 and R-23319, both from The Netherlands, fall at the base of the phylogenetic tree (Fig. 1 clade 1) and their concatenated sequence similarity with the type

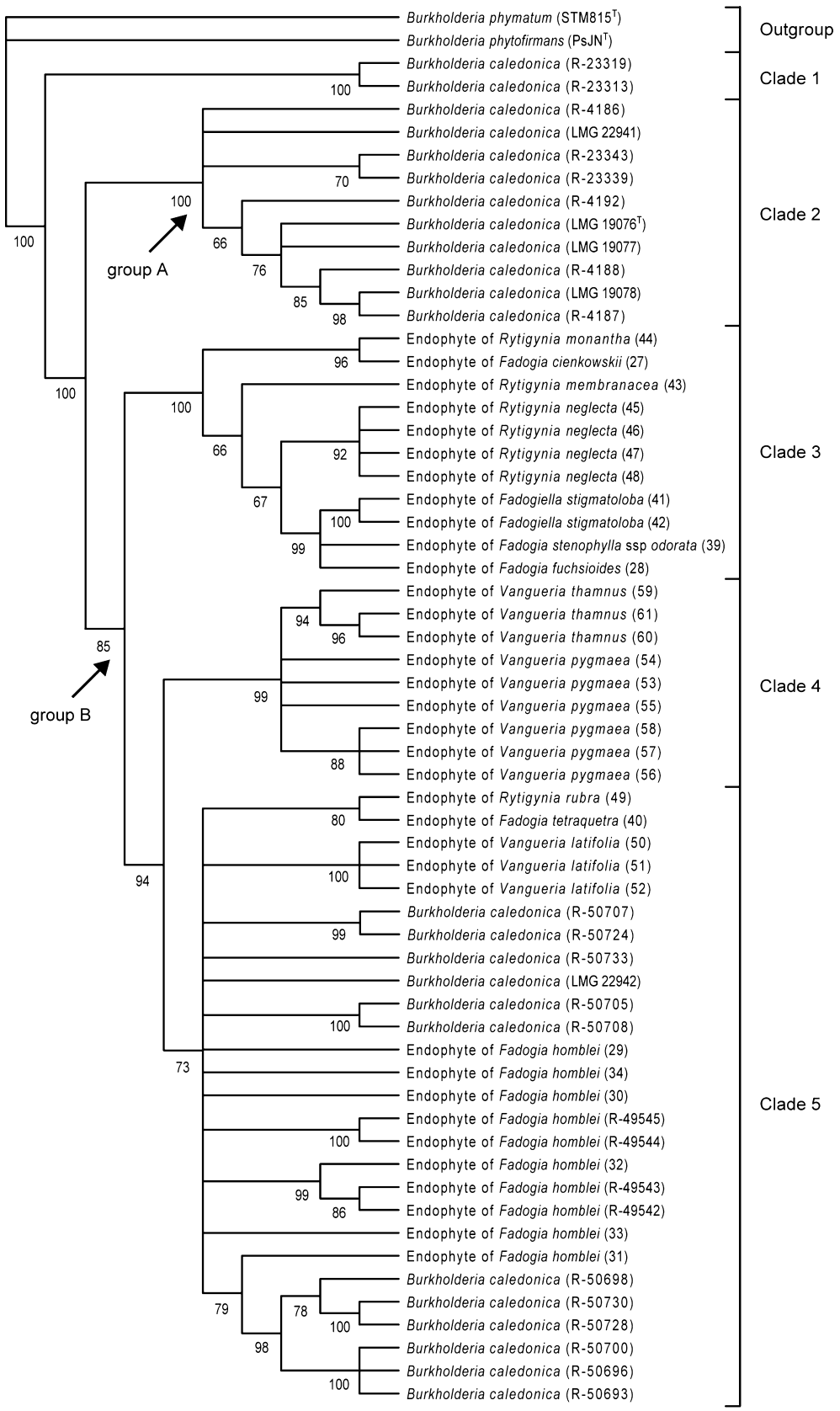

Fig. 1. Phylogenetic relationships within the bacterial species *Burkholderia caledonica* based on seven housekeeping genes. Bootstrap values are indicated below the branches and numbers between brackets refer to Table 1. Group A with exclusively European isolates is distinct from group B that contains African soil isolates and endophytes and a single European isolate. Within group B, three clades are evident and soil bacteria are only found in clade 5.

Table 1
Detailed information on the *B. caledonica* isolates used in this study, with data on isolate/voucher, country, environment, and origin of DNA.

N	Taxon	Isolate/voucher	Country	Environment	DNA origin
1	*Burkholderia phymatum*	STM815 type	French Guiana	Root endophyte	Isolated strain
2	*Burkholderia phytofirmans*	PsJN type	(Canada?)	Root endophyte	Isolated strain
3	*Burkholderia caledonica*	LMG 19077	Scotland	Rhizosphere soil	Isolated strain
4	*Burkholderia caledonica*	R-4186	Scotland	Rhizosphere soil	Isolated strain
5	*Burkholderia caledonica*	R-4187	Scotland	Rhizosphere soil	Isolated strain
6	*Burkholderia caledonica*	R-4188	Scotland	Rhizosphere soil	Isolated strain
7	*Burkholderia caledonica*	R-4192	Scotland	Rhizosphere soil	Isolated strain
8	*Burkholderia caledonica*	LMG 19078	Scotland	Rhizosphere soil	Isolated strain
9	*Burkholderia caledonica*	LMG 19076 type	Scotland	Rhizosphere soil	Isolated strain
10	*Burkholderia caledonica*	R-23313	Netherlands	Bulk soil	Isolated strain
11	*Burkholderia caledonica*	R-23319	Netherlands	Bulk soil	Isolated strain
12	*Burkholderia caledonica*	LMG 22941	Netherlands	Rhizosphere soil	Isolated strain
13	*Burkholderia caledonica*	R-23339	Netherlands	Rhizosphere soil	Isolated strain
14	*Burkholderia caledonica*	LMG 22942	Netherlands	Rhizosphere soil	Isolated strain
15	*Burkholderia caledonica*	R-23343	Netherlands	Rhizosphere soil	Isolated strain
16	*Burkholderia caledonica*	R-50693	South Africa	Rhizosphere soil	Isolated strain
17	*Burkholderia caledonica*	R-50696	South Africa	Rhizosphere soil	Isolated strain
18	*Burkholderia caledonica*	R-50698	South Africa	Rhizosphere soil	Isolated strain
19	*Burkholderia caledonica*	R-50700	South Africa	Rhizosphere soil	Isolated strain
20	*Burkholderia caledonica*	R-50705	South Africa	Rhizosphere soil	Isolated strain
21	*Burkholderia caledonica*	R-50707	South Africa	Rhizosphere soil	Isolated strain
22	*Burkholderia caledonica*	R-50708	South Africa	Rhizosphere soil	Isolated strain
23	*Burkholderia caledonica*	R-50724	South Africa	Rhizosphere soil	Isolated strain
24	*Burkholderia caledonica*	R-50728	South Africa	Rhizosphere soil	Isolated strain
25	*Burkholderia caledonica*	R-50730	South Africa	Rhizosphere soil	Isolated strain
26	*Burkholderia caledonica*	R-50733	South Africa	Rhizosphere soil	Isolated strain
27	Endophyte of *Fadogia cienkowskii*	Dessein et al. 258 (BR)	Zambia	Leaf endophyte	Metagenomic DNA
28	Endophyte of *Fadogia fuchsioides*	Dessein et al. 1083 (BR)	Zambia	Leaf endophyte	Metagenomic DNA
29	Endophyte of *Fadogia homblei*	Lemaire & Verstraete 9 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
30	Endophyte of *Fadogia homblei*	Lemaire & Verstraete 22 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
31	Endophyte of *Fadogia homblei*	Lemaire & Verstraete 30 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
32	Endophyte of *Fadogia homblei*	Lemaire & Verstraete 50 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
33	Endophyte of *Fadogia homblei*	Lemaire & Verstraete 57 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
34	Endophyte of *Fadogia homblei*	Lemaire & Verstraete 292 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
35	Endophyte of *Fadogia homblei*	R-49542	South Africa	Leaf endophyte	Isolated strain
36	Endophyte of *Fadogia homblei*	R-49543	South Africa	Leaf endophyte	Isolated strain
37	Endophyte of *Fadogia homblei*	R-49544	South Africa	Leaf endophyte	Isolated strain
38	Endophyte of *Fadogia homblei*	R-49545	South Africa	Leaf endophyte	Isolated strain
39	Endophyte of *Fadogia stenophylla* ssp. *odorata*	Lovett 2267 (BR)	Tanzania	Leaf endophyte	Metagenomic DNA
40	Endophyte of *Fadogia tetraquetra*	Lemaire & Verstraete 223 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
41	Endophyte of *Fadogiella stigmatoloba*	Dessein et al. 337 (BR)	Zambia	Leaf endophyte	Metagenomic DNA
42	Endophyte of *Fadogiella stigmatoloba*	Gillett 17403 (BR)	Tanzania	Leaf endophyte	Metagenomic DNA
43	Endophyte of *Rytigynia membranacea*	Lachenaud et al. 739 (BR)	Cameroon	Leaf endophyte	Metagenomic DNA
44	Endophyte of *Rytigynia monantha*	Niyongabo 53 (BR)	Burundi	Leaf endophyte	Metagenomic DNA
45	Endophyte of *Rytigynia neglecta*	Dessein et al. 2958 (BR)	Cameroon	Leaf endophyte	Metagenomic DNA
46	Endophyte of *Rytigynia neglecta*	Dessein et al. 2961 (BR)	Cameroon	Leaf endophyte	Metagenomic DNA
47	Endophyte of *Rytigynia neglecta*	Dessein et al. 3053 (BR)	Cameroon	Leaf endophyte	Metagenomic DNA
48	Endophyte of *Rytigynia neglecta*	Dessein et al. 3056 (BR)	Cameroon	Leaf endophyte	Metagenomic DNA
49	Endophyte of *Rytigynia rubra*	Dessein et al. 2541 (BR)	Cameroon	Leaf endophyte	Metagenomic DNA
50	Endophyte of *Vangueria latifolia*	Lemaire & Verstraete 69 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
51	Endophyte of *Vangueria latifolia*	Lemaire & Verstraete 74 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
52	Endophyte of *Vangueria latifolia*	Lemaire & Verstraete 141 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
53	Endophyte of *Vangueria pygmaea*	Dessein et al. 666 (BR)	Zambia	Leaf endophyte	Metagenomic DNA
54	Endophyte of *Vangueria pygmaea*	Dessein et al. 726 (BR)	Zambia	Leaf endophyte	Metagenomic DNA
55	Endophyte of *Vangueria pygmaea*	Lemaire & Verstraete 26A (BR)	South Africa	Leaf endophyte	Metagenomic DNA
56	Endophyte of *Vangueria pygmaea*	Lemaire & Verstraete 26B (BR)	South Africa	Leaf endophyte	Metagenomic DNA
57	Endophyte of *Vangueria pygmaea*	Lemaire & Verstraete 28 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
58	Endophyte of *Vangueria pygmaea*	Lemaire & Verstraete 42 (BR)	South Africa	Leaf endophyte	Metagenomic DNA
59	Endophyte of *Vangueria thamnus*	Bester 10538 (PRE)	South Africa	Leaf endophyte	Metagenomic DNA
60	Endophyte of *Vangueria thamnus*	Lemaire & Verstraete 25B (BR)	South Africa	Leaf endophyte	Metagenomic DNA
61	Endophyte of *Vangueria thamnus*	Steyn 1835 (PRE)	South Africa	Leaf endophyte	Metagenomic DNA

strain of *B. caledonica* is 96.4%. This is lower than the 3% threshold value applied for species delineation in the *Burkholderia cepacia* complex [22]. The two well-defined groups are: group A holding 10 European soil isolates including the *B. caledonica* type strain LMG 19076^T (Fig. 1 clade 2) and group B comprising 31 uncultured endophytic isolates, 4 endophytic *F. homblei* isolates (R-49542, R-49543, R-49544, and R-49545), 11 African soil isolates, and a single aberrant European soil isolate LMG 22942 (Fig. 1 clades 3, 4, and 5). Bootstrap values are indicated below every branch and these two main groups are highly supported. Group A holding the European soil isolates, including the type strain, forms a strongly supported clade separate from group B (Fig. 1 clade 2). The similarity of the concatenated sequences within this group A ranges from 99.7% to 99.9%. The genetic variation in this group is thus very low. The similarity of the concatenated sequences within group B ranges from 97.5% to 100%. When comparing group A versus group B, the total concatenated sequence similarity is always higher than 97% (between 97.6% and 98.4%).

The result of the phylogenetic network analysis is concordant with the Maximum Likelihood analysis. The splits graph shows the same clusters: two aberrant soil isolates separated from the rest (Fig. 2 clade 1), a coherent group A exclusively consisting of

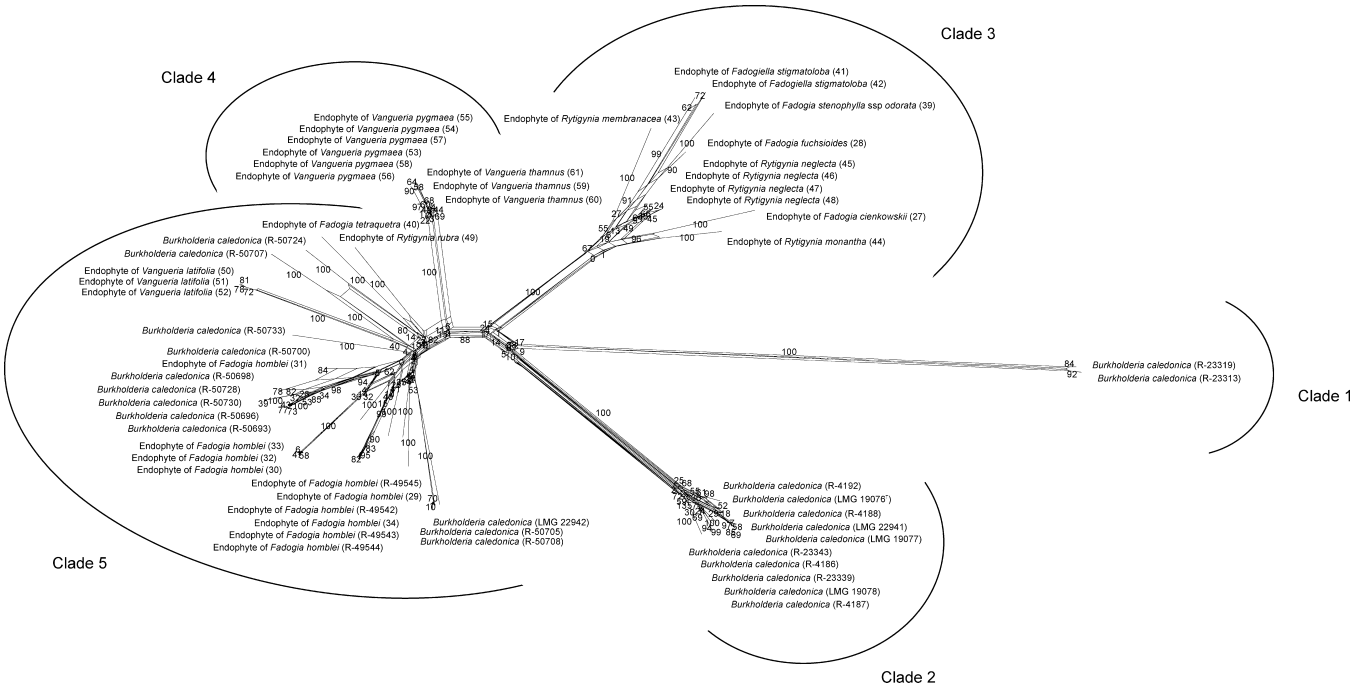

Fig. 2. Splits graph of the *Burkholderia caledonica* isolates based on the concatenated sequences of seven DNA markers. Numbers between brackets refer to Table 1. Clade 1 holds two aberrant European bulk soil isolates. The other European rhizosphere soil isolates, including the type strain, are found in clade 2. Clades 3, 4 and 5 all hold African soil bacteria or endophytes, with the exception of the European soil isolate LMG 22942 in clade 5.

European soil isolates (Fig. 2 clade 2), and a large group B with African soil isolates, endophytic *B. caledonica* and one European soil isolate (Fig. 2 clades 3, 4, and 5). The first clade holds the two soil isolates from the Netherlands that deviate considerably from the original *B. caledonica* type strain and that are set apart from all other investigated isolates as are evident from the length of the edges. Clade 2 is well supported as a separate group due to the length of the edges, and these soil isolates from the Netherlands and Scotland are closely related to the *B. caledonica* type strain. Group B comprises three clades that contain the endophytes of particular host plants, the African soil isolates and a European soil isolate. Different endophytic isolates originating from the same host plant species are always found in the same clade (e.g. the endophyte of *Vangueria pygmaea* is only found in clade 4). The free-living soil bacteria that were isolated from the rhizosphere of *F. homblei* are grouping with the endophytes of *F. homblei* and fall in the same clade (Fig. 2 clade 5).

Discussion

In the first comprehensive survey of non-nodulated bacteria in host plants of Rubiaceae, the identity of the endophytes was revealed as members of *Burkholderia* [25]. Some of these bacteria were shown to be genetically distinct from known species, but others had a high 16S rRNA gene sequence similarity with free-living *B. caledonica* strains [23]. The close relatedness, yet small genetic difference, of these endophytic bacteria to a free-living strain drew our attention. In an attempt to unravel the intraspecific variation within *B. caledonica* and to possibly discriminate between isolates based on geography and/or niche, we applied multilocus sequence analysis (MLSA). This technique has been increasingly used for classification, as well as in population biology studies of *Burkholderia* strains [21].

The phylogenetic tree of contains several clades: two free-living soil isolates from Europe at the base (Fig. 1 clade 1), a clade of exclusively European rhizosphere soil bacteria (Fig. 1 clade 2), two clades of African endophytes (Fig. 1 clades 3 and 4), and a clade holding several endophytes together with African rhizosphere soil bacteria and one European soil isolate (Fig. 1 clade 5). Apart from clade 1, two large groups are evident: group A holds exclusively European rhizosphere soil bacteria and group B contains mainly African rhizosphere soil bacteria and endophytes and one soil isolate from Europe. The existence of the small genetic difference between the two groups may be the result of geographic isolation and independent evolution since group A represents bacteria from Western Europe, while group B mainly holds isolates from sub-Saharan Africa. However, when comparing the concatenated sequences of these two groups and applying the 3% threshold value used for species delineation in the *B. cepacia* complex [22], all these isolates represent a single species. Although there is a clear trend, we cannot conclude that an absolute geographic pattern is present in *B. caledonica*.

Clade 1 holds the isolates R-23313 and R-23319 and is considered separate from group A and group B. These two strains were originally isolated from bulk soil collected from arable fields in the Netherlands as part of a larger study on the diversity of *Burkholderia* in soil [19]. They were included in the current analysis because they were assigned to *B. caledonica* because of their similarity in whole cell protein profile towards the *B. caledonica* type strain [19]. Moreover, their 16S rRNA gene sequence is 99.1% similar to that of the *Burkholderia bryophila* type strain, a species described after the Salles et al. [19] study, compared to 99% similarity to that of the *B. caledonica* type strain. An analysis of *B. bryophila* and other closely related *Burkholderia* species through MLSA is warranted in order to reassess the taxonomic status of these two strains which are therefore not considered further in the discussion of *B. caledonica* below.

The Scottish isolates (in clade 2) were assigned to *B. caledonica* in a polyphasic taxonomic study that involved DNA-DNA hybridization experiments of two representative isolates only, namely LMG 19076^{T} and LMG 19077; the remaining isolates were assigned to the same species on the basis of whole-cell protein pattern similarity and other phenotypic characteristics [3]. The isolates from the Netherlands (in clade 2) were assigned to *B. caledonica*, again

primarily on the basis of whole-cell protein pattern similarity [19]. Our MLSA data supports the conclusion that the investigated isolates represent a single species.

Clades 3 and 4 represent solely endophytic bacteria found in different African Rubiaceae plants, while clade 5 is represented by both soil-dwelling as endophytic bacteria (Figs. 1 and 2). Clade 3 comprises the endophytes of *Rytigynia* and *Fadogiella*, which are plants found in forests or woodland, while clade 4 contains the endophytes of *V. pygmaea* and *Vangueria thamnus*, which are plants found in grassland or savannah.

Clade 5 includes the endophytes of four different host plants together with several free-living bacteria. It has been suggested that combining MLSA data with ecological data allows for more meaningful taxonomic assignments [7]. If we apply this to clade 5, we find that two distinct niches are present, namely endophytic versus soil-dwelling. Although the endophytic isolates in clade 5 were originally isolated from within their host plants [23], they are capable of surviving and growing outside this particular habitat (e.g. the cultivated endophytes R-49542 to R-49545). This suggests that the endophytes are most probably not dependent on particular components from the host plant for survival, and that the interaction is – at least for the bacteria – probably not obligatory. The soil bacteria in clade 5 were isolated from the rhizosphere of *F. homblei*. The soil is red and sandy, with an acidic pH of around 4. Based on the analysis of seven housekeeping genes, these soil isolates do not differ significantly from the endophytes. The high similarity between soil bacteria and endophytes suggests that a possible exchange exists between the soil habitat and the host plant. Such horizontal infection events would explain the promiscuity of the endophytes and the presence of the same bacterial endophyte in different host species (clades 3, 4 and 5). A similar horizontal transmission of *Burkholderia* bacteria has been observed in stinkbugs, where the endosymbionts are acquired from the soil [12]. The phylogenetic analysis of these gut symbionts shows that they do not form a coherent clade, indicating host-symbiont promiscuity [13].

The isolate LMG 22942 is a soil bacterium isolated from rhizosphere soil in the Netherlands [19], but the phylogenetic analysis assigns it to group B (Fig. 1). It is the only exception in an otherwise exclusively African group. In spite of this aberrant strain, there is a clear trend of a biogeographic pattern within *B. caledonica*. The presence of the aberrant European soil isolate in the 'African' group B may suggest the existence of strains with a global distribution as observed in some *B. cepacia* complex species as well (see http://pubmlst.org/bcc/) and may therefore complement, rather than invalidate the strong trend in biogeographic separation revealed by the MLSA data (Figs. 1 and 2).

Acknowledgements

This research was supported financially by the Research Foundation – Flanders (FWO, G.0343.09N) and by the King Leopold-III Fund for Nature Exploration and Conservation. BV holds a PhD research grant from the Agency for Innovation by Science and Technology (IWT, N° 91158).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2013.12.001.

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