

## Supplemental Information

### **Expanding the *Burkholderia pseudomallei* complex with the addition of two novel species: *Burkholderia mayonis* sp. nov. and *Burkholderia savannae* sp. nov.**

Running Title: *Burkholderia mayonis* and *B. savannae* sp. nov.

Carina M. Hall,<sup>a</sup> Anthony L. Baker,<sup>b</sup> Jason W. Sahl,<sup>a</sup> Mark Mayo,<sup>c</sup> Holger C. Scholz,<sup>d</sup> Mirjam Kaestli,<sup>c</sup> James Schupp,<sup>c</sup> Madison Martz,<sup>a</sup> Erik W. Settles,<sup>a</sup> Joseph D. Busch,<sup>a</sup> Lindsay Sidak-Loftis,<sup>a</sup> Astrid Thomas,<sup>d</sup> Lisa Kreutzer,<sup>d</sup> Enrico Georgi,<sup>d</sup> Herbert P. Schweizer,<sup>a</sup> Jeffrey M. Warner,<sup>b</sup> Paul Keim,<sup>a</sup> Bart J. Currie,<sup>b</sup> David M. Wagner<sup>a#</sup>

<sup>a</sup>Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, Arizona, USA

<sup>b</sup>Discipline of Biomedicine and Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia

<sup>c</sup>Menzies School of Health Research, Darwin, Northern Territory, Australia

<sup>d</sup>Bundeswehr Institute of Microbiology, Munich, Germany

<sup>e</sup>Translational Genetics and Genomics, Flagstaff, Arizona, USA

## MALDI-TOF

Preparations of bacterial isolates (BDU6<sup>T</sup>, BDU8, MSMB266<sup>T</sup>, MSMB852, BDU18, BDU19) for matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were done according to the ethanol/formic acid extraction protocol recommended by the manufacturer (Bruker Daltonics) and as previously described (1). Briefly, strains were grown on Columbia agar supplemented with 5% sheep blood then a loopful of bacterial material was evenly dissolved in 300 µl analytical grade water, and 900 µl pure ethanol was added. The cell suspension was centrifuged at 13,000 × g for 2 minutes and the supernatant was discarded. The centrifugation was repeated, and the residual ethanol was discarded. The pellet was air dried and thoroughly resuspended in 5-50 µl 70% formic acid depending on the size, and, finally, an equal volume of acetonitrile was added. After centrifugation at 13,000 × g for 2 minutes, 1 µl of the supernatant was transferred to the MALDI target plate. After air-drying at room temperature 1 µl of matrix solution (saturated solution of α-cyano-hydroxy-cinnamic acid in 50% aqueous acetonitrile containing 2.5% trifluoroacetic acid) per spot was applied. MALDI-TOF MS was conducted using a Microflex LT mass spectrometer (Bruker Daltonics) equipped with a nitrogen laser. All spectra were recorded in linear, positive ion mode across a mass/charge ratio (m/z) of 2,000 to 20,000. The acceleration voltage was 20 kV. Spectra were collected as a sum of 240 shots across a spot. Main spectra were calculated from 8 spectra per strain and used for construction of a score oriented dendrogram by using the BioTyper software (version 2.3, Bruker Daltonics). In these analyses *B. mayonis* sp. nov. and *B. savannae* sp. nov. strains grouped together with other members of the *B. pseudomallei* complex and with *B. ubonensis* from the *B. cepacia* complex and (Fig. S4).

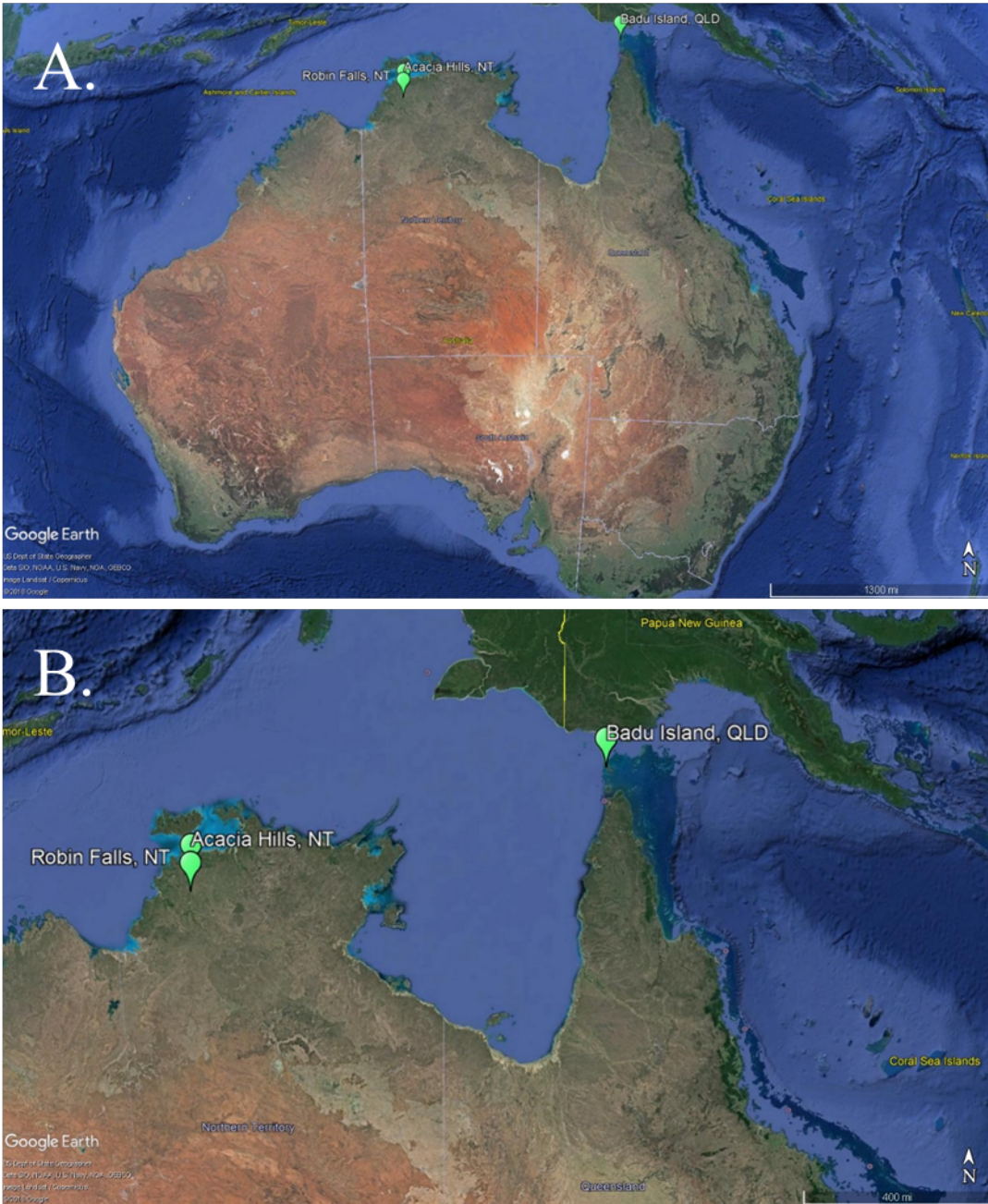


Fig. S1. Geographic origin of *B. mayonis* sp. nov. (Badu Island) and *B. savannae* sp. nov. (Acacia Hills, Badu Island, and Robin Falls) isolates from the environment (Google Earth, US Dept of State Geographer, Data SIO, NOAA, US Navy, NGA, GEBCO, Image Landsat/Copernicus, ©2018 Google). Panel B is a zoomed in version of panel A.

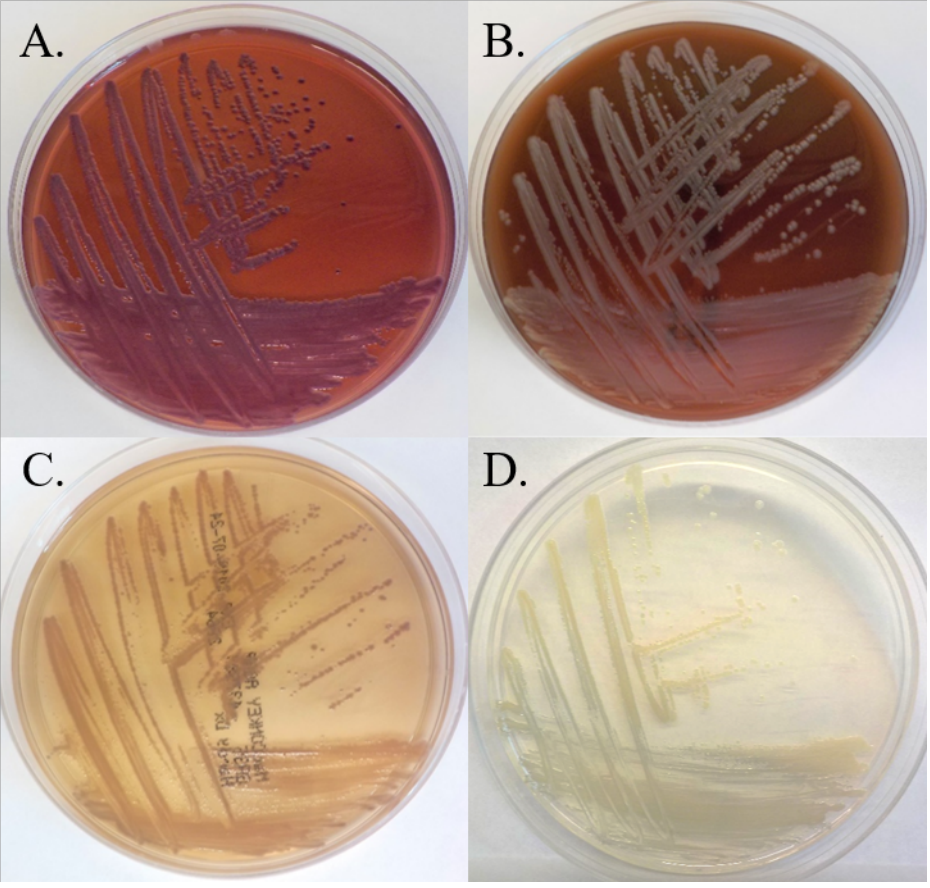


Fig. S2. *B. mayonis* sp. nov. (BDU6<sup>T</sup>) on four media types after 96 hours of incubation at 25°C. Panels A. Ashdown's Agar; B. Columbia Blood Agar; C. MacConkey Agar; D. Luria-Bertani Agar.

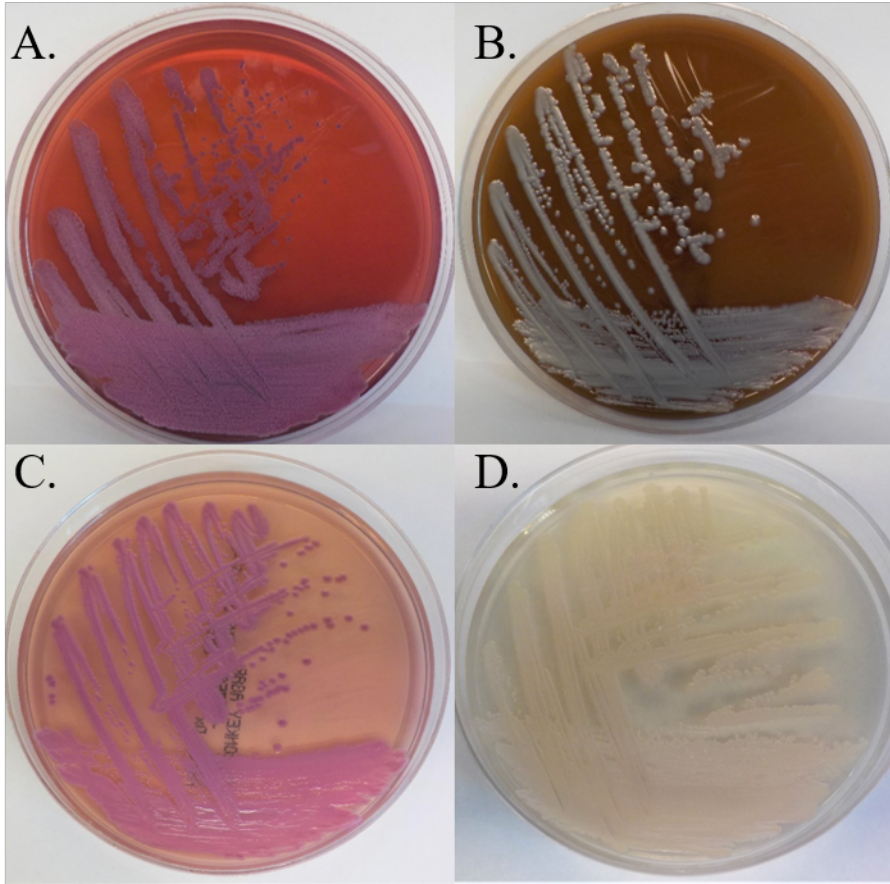


Fig. S3. *B. savannae* sp. nov. (MSMB266<sup>T</sup>) on four media types after 96 hours of incubation at 25°C. Panels A. Ashdown's Agar; B. Columbia Blood Agar; C. MacConkey Agar; D. Luria-Bertani Agar.



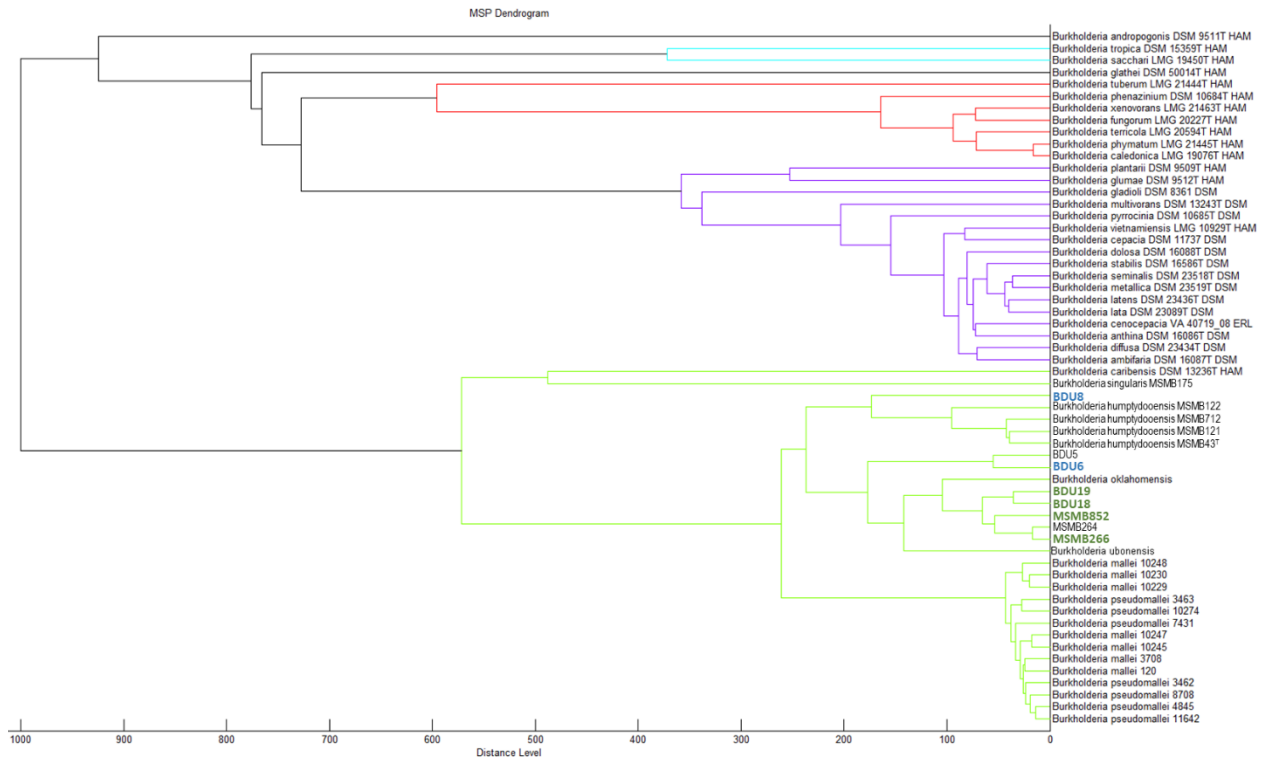


Fig. S4. Dendrogram demonstrating strain relatedness revealed by MALDI – TOF analysis. *B. mayonis* sp. nov. strains are in blue text while *B. savannae* sp. nov. strains are in green text. Although BDU5 was not described in this study the MALDI-TOF analysis suggests that this may be an additional *B. mayonis* sp. nov. strain and this is further supported by BDU5, BDU6, and BDU8 all reported as putative species 2 in a previous study (2). MSMB264 was also not described in this study but the MALDI-TOF analysis suggests that it may be an additional *B. savannae* sp. nov. strain.

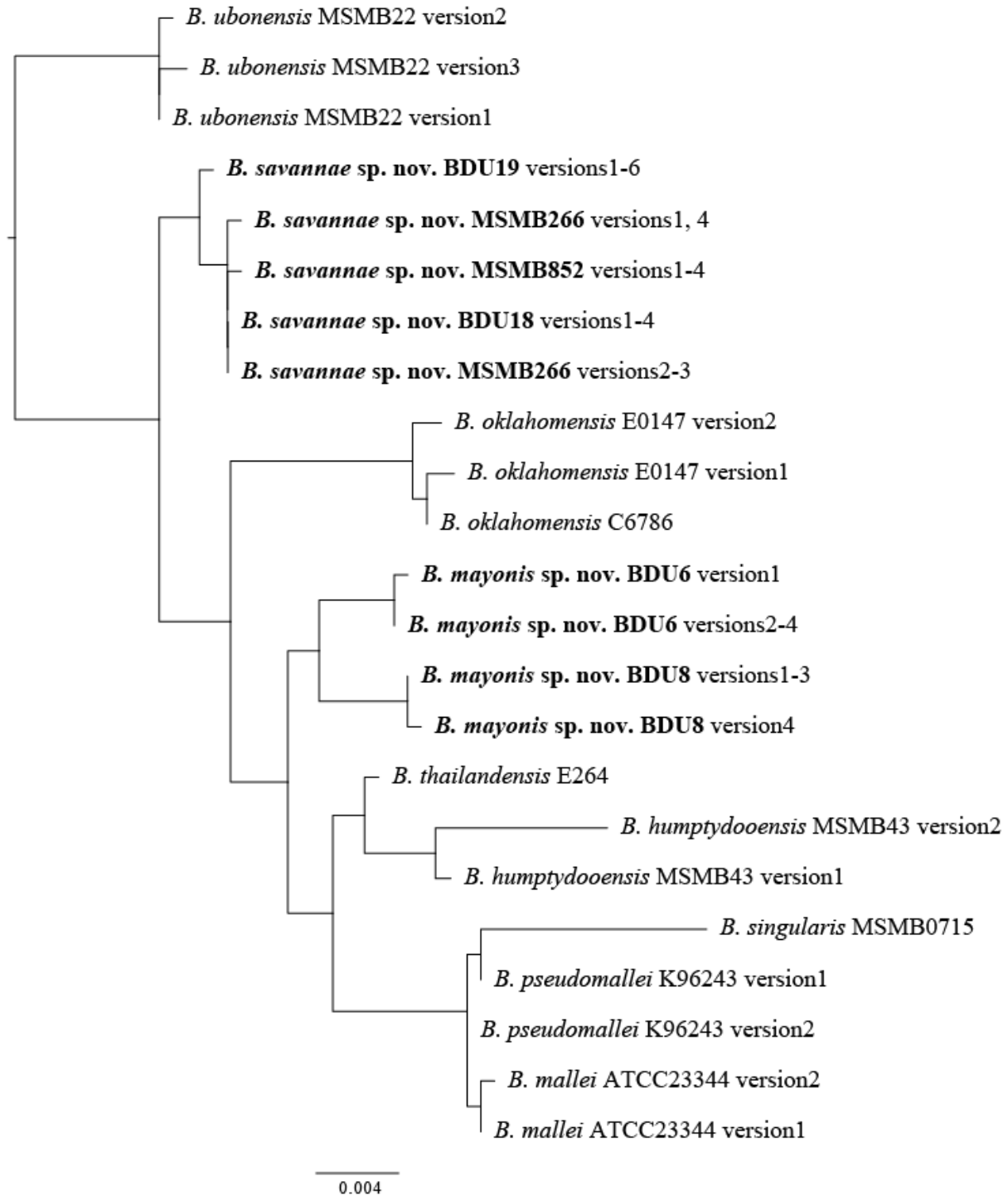


Fig. S5. 16S maximum likelihood phylogeny using unique copies of 16S from *B. mayonis* sp. nov. and *B. savannae* sp. nov. strains, bolded (1,529 bp and 23 sequences). Rooted with *B. ubonensis*.

Table S1. The blast score ratios of three virulence genes, *bimA*, the type III secretion system (T3SS), and the type VI secretion system 5 (T6SS-5) across the *B. mayonis* sp. nov. and *B. savannae* sp. nov. strains. The blast score ratios are show where “0” (white) represents the locus fully absent and “1” (red) represents the locus fully present for that strain.

Locus	Region	<i>B. mayonis</i>		<i>B. savannae</i>				<i>B. pseudomallei</i>	
		BDU8	BDU6 <sup>T</sup>	MSMB266 <sup>T</sup>	BDU19	BDU18	MSMB852	K96243	1026b
BPSS1492	bimA	0.30	0.19	0.31	0.19	0.19	0.19	1.00	1.00
BPSS1390	T3SS	0.52	0.19	0.53	0.53	0.52	0.53	1.00	1.00
BPSS1391	T3SS	0.34	0.05	0.33	0.33	0.33	0.33	1.00	1.00
BPSS1392	T3SS	0.47	0.19	0.45	0.45	0.45	0.45	1.00	1.00
BPSS1393	T3SS	0.27	0.12	0.29	0.28	0.29	0.28	1.00	1.00
BPSS1394	T3SS	0.74	0.49	0.74	0.74	0.74	0.74	1.00	1.00
BPSS1395	T3SS	0.35	0.07	0.36	0.36	0.36	0.36	1.00	1.00
BPSS1396	T3SS	0.17	0.07	0.18	0.18	0.18	0.18	1.00	1.00
BPSS1397	T3SS	0.54	0.26	0.53	0.53	0.53	0.53	1.00	1.00
BPSS1398	T3SS	0.26	0.00	0.28	0.28	0.28	0.28	1.00	0.99
BPSS1399	T3SS	0.30	0.00	0.28	0.29	0.29	0.29	1.00	1.00
BPSS1400	T3SS	0.50	0.24	0.49	0.49	0.50	0.49	1.00	1.00
BPSS1401	T3SS	0.67	0.36	0.68	0.68	0.67	0.68	1.00	1.00
BPSS1402	T3SS	0.21	0.08	0.21	0.21	0.21	0.21	1.00	0.99
BPSS1403	T3SS	0.23	0.00	0.21	0.21	0.21	0.21	1.00	1.00
BPSS1404	T3SS	0.79	0.46	0.79	0.79	0.79	0.79	1.00	1.00
BPSS1405	T3SS	0.60	0.34	0.60	0.60	0.60	0.60	1.00	1.00
BPSS1406	T3SS	0.09	0.07	0.11	0.11	0.12	0.12	1.00	0.96
BPSS1407	T3SS	0.28	0.07	0.30	0.30	0.30	0.31	1.00	0.99
BPSS1408	T3SS	0.32	0.20	0.32	0.32	0.33	0.32	1.00	1.00
BPSS1409	T3SS	0.00	0.18	0.16	0.18	0.18	0.17	1.00	0.96
BPSS1410	T3SS	0.73	0.00	0.72	0.72	0.72	0.72	1.00	1.00
BPSS0091	T6SS-5	0.21	0.29	0.81	0.81	0.81	0.81	1.00	0.99



BPSS0092	T6SS-5	0.28	0.41	0.91	0.90	0.91	0.90	1.00	1.00
BPSS0093	T6SS-5	0.28	0.32	0.89	0.89	0.89	0.89	1.00	1.00
BPSS0094	T6SS-5	0.00	0.26	0.85	0.84	0.84	0.84	1.00	1.00
BPSS0095	T6SS-5	0.08	0.00	0.77	0.77	0.77	0.77	1.00	0.99
BPSS0096	T6SS-5	0.06	0.07	0.83	0.84	0.84	0.84	1.00	0.99
BPSS0097	T6SS-5	0.76	0.76	0.98	0.98	0.98	0.98	1.00	1.00
BPSS0098	T6SS-5	0.86	0.86	0.99	0.99	0.99	0.99	1.00	1.00
BPSS0099	T6SS-5	0.69	0.70	0.96	0.96	0.96	0.96	1.00	0.99
BPSS0100	T6SS-5	0.44	0.45	0.89	0.89	0.89	0.89	1.00	0.99
BPSS0101	T6SS-5	0.64	0.64	0.99	0.99	0.99	0.99	1.00	1.00
BPSS0102	T6SS-5	0.39	0.39	0.93	0.93	0.93	0.93	1.00	1.00
BPSS0103	T6SS-5	0.44	0.44	0.91	0.91	0.91	0.91	1.00	1.00
BPSS0104	T6SS-5	0.00	0.00	0.86	0.86	0.86	0.86	1.00	0.99
BPSS0105	T6SS-5	0.28	0.28	0.85	0.86	0.86	0.85	1.00	0.97
BPSS0106	T6SS-5	0.24	0.20	0.82	0.82	0.82	0.82	1.00	1.00
BPSS0108	T6SS-5	0.00	0.00	0.64	0.64	0.64	0.64	1.00	0.99
BPSS0109	T6SS-5	0.00	0.00	0.48	0.47	0.47	0.47	1.00	1.00
BPSS0110	T6SS-5	0.39	0.39	0.92	0.92	0.92	0.92	1.00	0.99
BPSS0111	T6SS-5	0.53	0.53	0.96	0.96	0.96	0.95	1.00	1.00
BPSS0112	T6SS-5	0.65	0.66	0.99	0.99	0.99	0.99	1.00	1.00
BPSS0113	T6SS-5	0.43	0.43	0.93	0.93	0.93	0.93	1.00	1.00
BPSS0114	T6SS-5	0.37	0.36	0.90	0.90	0.90	0.90	1.00	1.00
BPSS0115	T6SS-5	0.05	0.05	0.82	0.82	0.82	0.82	1.00	0.99
BPSS0116	T6SS-5	0.79	0.79	0.92	0.92	0.92	0.92	1.00	1.00
BPSS0117	T6SS-5	0.85	0.14	0.87	0.87	0.87	0.87	1.00	1.00

Table S2. Pairwise matrix of 16S rRNA sequence percent identity and SNPs. Top half of matrix lists the number of SNPs and the bottom half of the matrix lists the percent identity of the 16S rRNA gene sequences. Species: Bma, *Burkholderia mallei*; Bp, *B. pseudomallei*; Bt, *B. thailandensis*; Bo, *B. oklahomensis*; Bh, *B. humptydoensis*; Bm, *B. mayonis* sp. nov.; Bs, *B. savannae* sp. nov.; Bsi, *B. singularis*; Bu, *B. ubonensis*.

	Bo E0	Bo E0	Bo C6	Bu MS	Bu MS	Bu MS	Bs B	Bs MS	Bs MS	Bs B	Bs MS	Bsi MS	B m	B m	B m	B m	Bma AT	Bma AT	Bp K9	Bp K9	Bh MS	Bt E2	Bh MS
	14	14	78	M	M	M	D	MB	MB	D	MB	MB	B	B	B	B	CC2	CC2	62	62	M	64	M
	7	7	6	B2	B2	B2	U1	266	852	U1	266	715	D	D	D	D	334	334	43	43	B4		B4
	co	co		2	2	2	9	copi	copi	8	copi		U6	U6	U8	U8	4	4	co	co	3		3
	py	py		cop	cop	cop		es1,	es	co	es2-		co	co	co	co	copy	copy	py	py	cop		cop
	1	2		y3	y1	y2		4	1-4	pie	s		py	pie	pie	py	2	1	1	2	y2		y1
										1-4					3								
Bo E0147 copy1	■	5	2	33	31	32	22	22	22	23	23	30	24	23	21	22	25	24	24	23	26	23	27
Bo E0147 copy2	99.7	■	3	31	29	30	20	20	20	21	21	30	24	23	19	20	24	23	23	22	25	21	25
Bo C6786	99.9	99.8	■	31	29	30	20	20	20	21	21	28	24	23	19	20	23	22	22	21	24	21	25
Bu MSMB2 2 copy3	97.8	98.0	98.0	■	2	3	22	24	24	23	23	29	31	32	34	35	27	26	24	25	21	26	26
Bu MSMB2 2 copy1	98.0	98.1	98.1	99.9	■	1	20	22	22	21	21	27	29	30	32	33	25	24	22	23	19	24	24
Bu MSMB2 2 copy2	97.9	98.0	98.0	99.8	99.9	■	21	23	23	22	22	28	30	31	33	34	26	25	23	24	20	25	25
Bs BDU19	98.6	98.7	98.7	98.6	98.7	98.6	■	4	4	3	3	23	16	17	20	21	23	22	20	21	22	18	22
Bs MSMB2 66	98.6	98.7	98.7	98.4	98.6	98.5	99.7	■	2	1	1	21	20	21	16	17	25	24	22	23	24	20	24

copies1, 4																								
Bs MSMB8 52 copies 1-4	98.6	98.7	98.7	98.4	98.6	98.5	99.7	99.9		1	1	21	20	21	16	17	25	24	22	23	24	20	24	
Bs BDU18 copies 1- 4	98.5	98.6	98.6	98.5	98.6	98.6	99.8	99.9	99.9			0	20	19	20	17	18	24	23	21	22	23	19	23
Bs MSMB2 66 copies2-3	98.5	98.6	98.6	98.5	98.6	98.6	99.8	99.9	99.9	10.0			20	19	20	17	18	24	23	21	22	23	19	23
Bsi MSMB7 15	98.0	98.0	98.2	98.1	98.2	98.2	98.5	98.6	98.6	98.7	98.7			27	28	26	27	17	16	14	15	31	22	27
Bm BDU6 copy1	98.4	98.4	98.4	98.0	98.1	98.0	99.0	98.7	98.7	98.8	98.8	98.2			1	12	13	20	19	17	18	29	14	19
Bm BDU6 copies2-4	98.5	98.5	98.5	97.9	98.0	98.0	98.9	98.6	98.6	98.7	98.7	98.2	99.9			11	12	19	18	18	17	28	13	18
Bm BDU8 copies1-3	98.6	98.8	98.8	97.8	97.9	97.8	98.7	99.0	99.0	98.9	98.9	98.3	99.2	99.3			1	22	21	21	20	27	12	19
Bm BDU8 copy4	98.6	98.7	98.7	97.7	97.8	97.8	98.6	98.9	98.9	98.8	98.8	98.2	99.1	99.2	99.9			23	22	22	21	28	13	20
Bma ATCC23 344 copy2	98.4	98.4	98.5	98.2	98.4	98.3	98.5	98.4	98.4	98.4	98.4	98.9	98.7	98.8	98.6	98.5			1	3	2	26	14	17
Bma ATCC23 344 copy1	98.4	98.5	98.6	98.3	98.4	98.4	98.6	98.4	98.4	98.5	98.5	99.0	98.8	98.8	98.6	98.6	99.9			2	1	25	13	16
Bp K96243 copy1	98.4	98.5	98.6	98.4	98.6	98.5	98.7	98.6	98.6	98.6	98.6	99.1	98.9	98.8	98.6	98.6	99.8	99.9			1	25	13	16

Bp K96243 copy2	98 .5	98. 6	98. 6	98. 4	98. 5	98. 4	98. 6	98. 5	98. 5	98. 6	98. 6	99. 0	98. 8	98. 9	98. 7	98. 6	99.9	99.9	99. 9		24	12	15
Bh MSMB4 3 copy2	98 .3	98. 4	98. 4	98. 6	98. 8	98. 7	98. 6	98. 4	98. 4	98. 5	98. 5	98. 0	98. 1	98. 2	98. 2	98. 2	98.3	98.4	98. 4	98. 4		15	12
Bt E264	98 .5	98. 6	98. 6	98. 3	98. 4	98. 4	98. 8	98. 7	98. 7	98. 8	98. 8	98. 6	99. 1	99. 1	99. 2	99. 1	99.1	99.1	99. 1	99. 2	99. 0		7
Bh MSMB4 3 copy1	98 .2	98. 4	98. 4	98. 3	98. 4	98. 4	98. 6	98. 4	98. 4	98. 5	98. 5	98. 2	98. 8	98. 8	98. 8	98. 7	98.9	99.0	99. 0	99. 0	99. 2	99. 5	

Table S3. List of the 66 assemblies used to construct the WGS phylogeny for Figure 1.

<b>NCBI assembly accession number</b>	<b><i>Burkholderia species</i></b>	<b>Strain ID</b>	<b>Assembly type</b>
GCA_001522635.2	<i>B. mayonis</i> sp. nov.	BDU8	Complete
GCA_001523745.2	<i>B. mayonis</i> sp. nov.	BDU6	Complete
GCA_001462435.1	<i>B. humptydooensis</i>	MSMB122	Chromosome
GCA_002888035.1	<i>B. humptydooensis</i>	MSMB121	Contig
GCA_002888095.1	<i>B. humptydooensis</i>	MSMB122	Contig
GCA_000170355.1	<i>B. oklahomensis</i>	EO147	Contig
GCA_000755985.1	<i>B. oklahomensis</i>	EO147	Complete
GCA_001522105.2	<i>B. oklahomensis</i>	EO147	Complete
GCA_000170375.1	<i>B. oklahomensis</i>	C6786	Contig
GCA_001522135.2	<i>B. oklahomensis</i>	C6786	Complete
GCA_000959365.1	<i>B. oklahomensis</i>	C6786	Complete
GCA_900608545.1	<i>B. oklahomensis</i>	LMG_23618	Contig
GCA_900446275.1	<i>B. oklahomensis</i>	NCTC13388	Contig
GCA_000011545.1	<i>B. pseudomallei</i>	K96243	Complete
GCA_001524445.2	<i>B. savannae</i> sp. nov.	MSMB0266	Complete
GCA_001546955.1	<i>B. savannae</i> sp. nov.	BDU19	Contig
GCA_001546915.1	<i>B. savannae</i> sp. nov.	BDU18	Contig
GCA_001524625.2	<i>B. savannae</i> sp. nov.	MSMB0852	Complete
GCA_001523725.1	<i>B. singularis</i>	TSV85	Contig
GCA_900176645.1	<i>B. singularis</i>	LMG_28154	Contig
GCA_000012365.1	<i>B. thailandensis</i>	E264	Complete
GCA_000567925.1	<i>B. thailandensis</i>	2002721723	Complete
GCA_002588555.1	<i>B. thailandensis</i>	FDAARGOS_425	Contig
GCA_002888235.1	<i>B. thailandensis</i>	2002721723	Contig
GCA_000959425.1	<i>B. thailandensis</i>	2002721643	Complete
GCA_002891195.1	<i>B. thailandensis</i>	FDAARGOS_240	Contig
GCA_002891115.1	<i>B. thailandensis</i>	FDAARGOS_236	Contig
GCA_003568605.1	<i>B. thailandensis</i>	E264	Complete
GCA_000152285.1	<i>B. thailandensis</i>	E264	Chromosome
GCA_002888315.1	<i>B. thailandensis</i>	2002721643	Contig
GCA_000567945.1	<i>B. thailandensis</i>	E444	Complete
GCA_002888205.1	<i>B. thailandensis</i>	E0444	Contig
GCA_003614835.1	<i>B. thailandensis</i>	FDAARGOS	Complete
GCA_000706745.1	<i>B. thailandensis</i>	USAMRU_Malaysia20	Complete
GCA_001555555.1	<i>B. thailandensis</i>	Malaysia20	Contig
GCA_003614815.1	<i>B. thailandensis</i>	FDAARGOS_241	Complete
GCA_000877335.1	<i>B. thailandensis</i>	Phuket4W1	Scaffold

GCA_002888355.1	<i>B. thailandensis</i>	Phuket4W1	Contig
GCA_003020025.1	<i>B. thailandensis</i>	FDAARGOS_237	Complete
GCA_000765375.1	<i>B. thailandensis</i>	E254	Complete
GCA_002891095.1	<i>B. thailandensis</i>	FDAARGOS_244	Contig
GCA_002888335.1	<i>B. thailandensis</i>	E254	Contig
GCA_000170395.1	<i>B. thailandensis</i>	Bt4	Contig
GCA_000764595.1	<i>B. thailandensis</i>	MSMB59	Complete
GCA_001718595.1	<i>B. thailandensis</i>	MSMB59	Complete
GCA_001524345.1	<i>B. thailandensis</i>	MSMB60	Contig
GCA_001524325.1	<i>B. thailandensis</i>	E1	Contig
GCA_000170315.1	<i>B. thailandensis</i>	TXDOH	Contig
GCA_000808035.2	<i>B. thailandensis</i>	2003015869	Complete
GCA_001718295.1	<i>B. thailandensis</i>	2003015869	Complete
GCA_002888425.1	<i>B. thailandensis</i>	TXDOH	Contig
GCA_000179515.1	<i>B. thailandensis</i>	E555	Contig
GCA_002803565.1	<i>B. thailandensis</i>	2_1	Contig
GCA_000567905.1	<i>B. thailandensis</i>	H0587	Complete
GCA_002888395.1	<i>B. thailandensis</i>	H0587	Contig
GCA_003020185.1	<i>B. thailandensis</i>	FDAARGOS_238	Complete
GCA_001718615.1	<i>B. thailandensis</i>	2002721121	Complete
GCA_000959605.1	<i>B. thailandensis</i>	34	Complete
GCA_002891155.1	<i>B. thailandensis</i>	FDAARGOS_243	Contig
GCA_001718635.1	<i>B. thailandensis</i>	2002721643	Complete
GCA_001555485.1	<i>B. thailandensis</i>	2002721621	Contig
GCA_000170495.1	<i>B. thailandensis</i>	MSMB43	Contig
GCA_000266985.1	<i>B. thailandensis</i>	MSMB43	Scaffold
GCA_000385525.1	<i>B. thailandensis</i>	MSMB121	Complete
GCA_002386205.1	<i>B. thailandensis</i>	FDAARGOS_426	Complete
GCA_000959245.1	<i>B. ubonensis</i>	MSMB22	Complete

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