

# **All ANIs are not created equal: Implications for prokaryotic species boundaries and integration of ANIs into polyphasic taxonomy**

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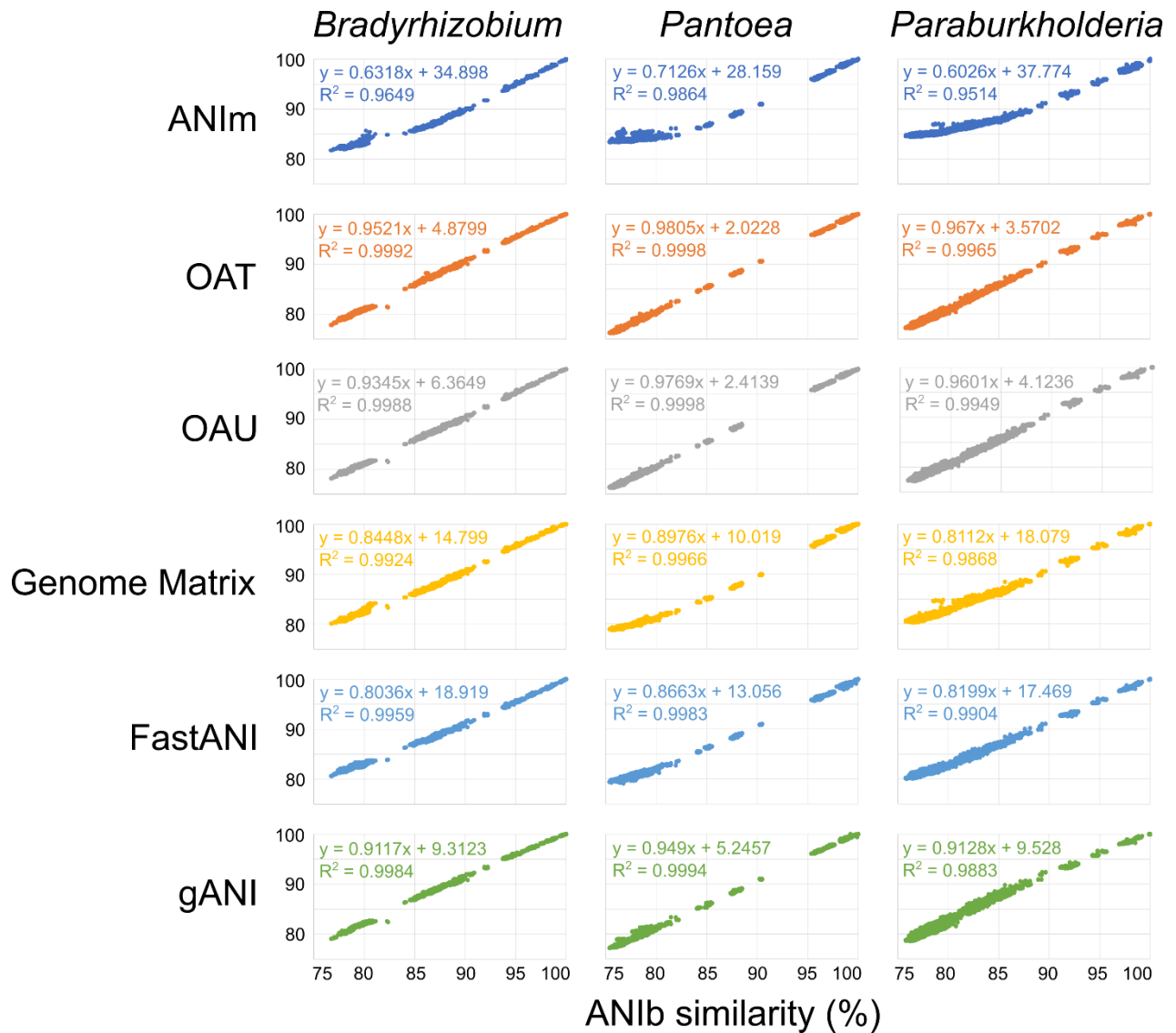
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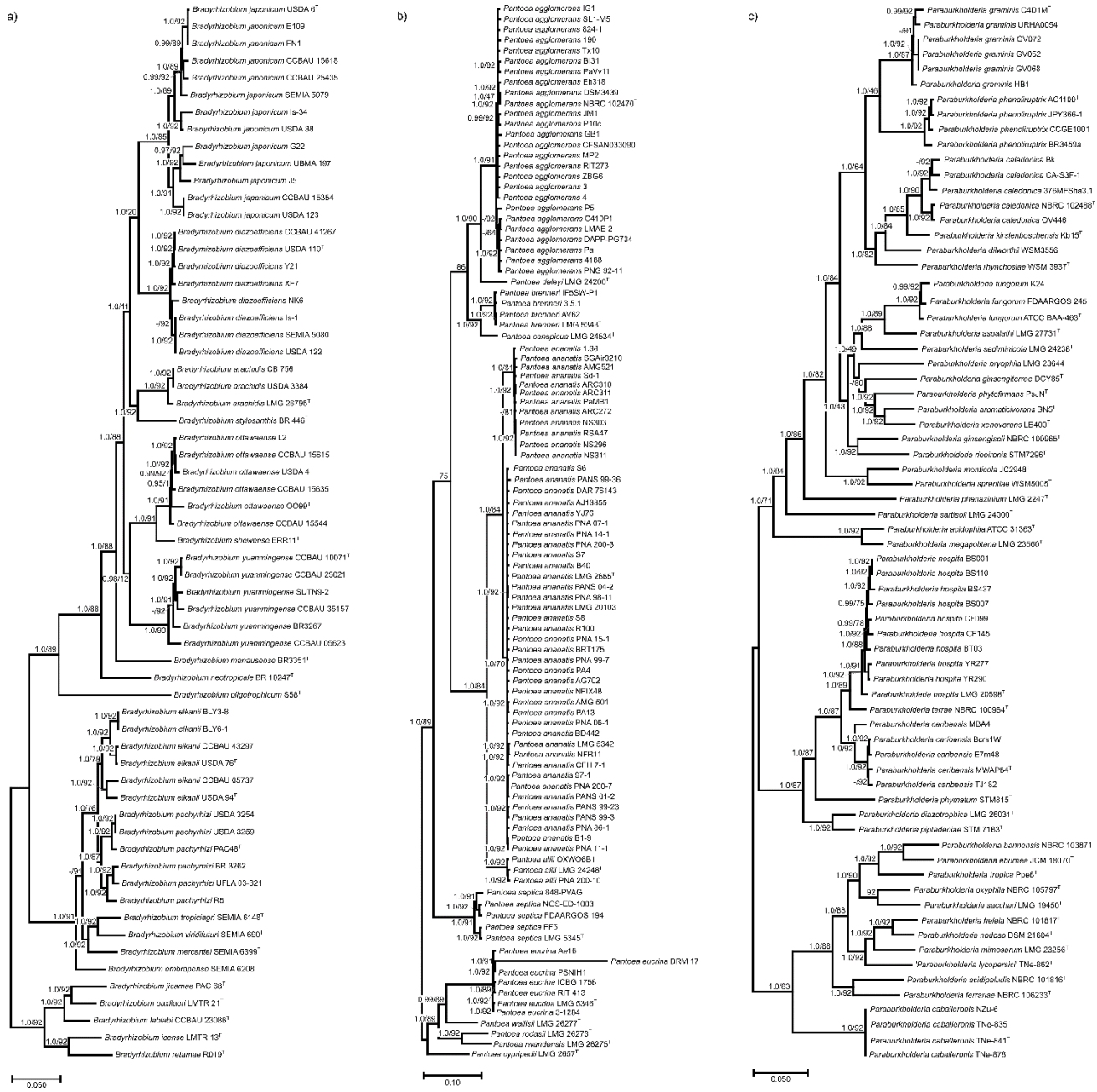
Supplementary Table S1. Genome sequence information of isolates used in the study

Class in GTDB	Order in GTDB	Genus	Species	Strain <sup>a</sup>	NCBI classification	GTDB classification	CheckM Completeness	Genome size (bp)	Accession number			
Alphaproteobacteria	Rhizobiales	Bradyrhizobium	<i>Bradyrhizobium arachidis</i>	LMG 26795 <sup>1</sup>	<i>Bradyrhizobium arachidis</i>	<i>Bradyrhizobium arachidis</i>	100.00%	9,793,799	GCA_000116675.1			
			<i>Bradyrhizobium arachidis</i>	USDA 3384	<i>Bradyrhizobium arachidis</i>	<i>Bradyrhizobium arachidis</i>	100.00%	9,800,269	GCA_000472685.1			
			<i>Bradyrhizobium arachidis</i>	CB756	<i>Bradyrhizobium</i> sensp. SA-4 str. CB756	<i>Bradyrhizobium arachidis</i>	100.00%	9,826,880	GCA_000473125.1			
			<i>Bradyrhizobium diazoefficiens</i>	USDA 110 <sup>1</sup>	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.99%	9,105,823	GCA_000211365.1			
			<i>Bradyrhizobium diazoefficiens</i>	CCBAU 41267	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.60%	9,121,826	GCA_000261765.1			
			<i>Bradyrhizobium diazoefficiens</i>	USDA 122	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.99%	9,976,980	GCA_000473025.1			
			<i>Bradyrhizobium diazoefficiens</i>	SEMIA 5080	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.99%	9,085,533	GCA_000648595.2			
			<i>Bradyrhizobium diazoefficiens</i>	Is-1	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.99%	9,983,878	GCA_001280585.1			
			<i>Bradyrhizobium diazoefficiens</i>	NK6	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.29%	10,475,157	GCA_001549695.1			
			<i>Bradyrhizobium diazoefficiens</i>	Y21	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.99%	9,941,773	GCA_002531995.1			
			<i>Bradyrhizobium diazoefficiens</i>	Y57	<i>Bradyrhizobium diazoefficiens</i>	<i>Bradyrhizobium diazoefficiens</i>	99.44%	9,260,701	GCA_001493845.1			
			<i>Bradyrhizobium elkanii</i>	USDA 76 <sup>1</sup>	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium elkanii</i>	100.00%	9,484,767	GCA_000379145.1			
			<i>Bradyrhizobium elkanii</i>	CCBAU 05737	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium elkanii</i>	99.83%	9,773,178	GCA_000261505.1			
			<i>Bradyrhizobium elkanii</i>	CCBAU 43297	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium elkanii</i>	99.32%	9,347,700	GCA_000261525.1			
			<i>Bradyrhizobium elkanii</i>	USDA 94	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium elkanii</i>	99.96%	9,558,895	GCA_000519275.1			
			<i>Bradyrhizobium elkanii</i>	BLV6-1	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium elkanii</i>	100.00%	9,202,572	GCA_001718185.1			
			<i>Bradyrhizobium elkanii</i>	BLV3-8	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium elkanii</i>	100.00%	9,198,916	GCA_001718205.1			
			<i>Bradyrhizobium tense</i>	SEMIA 6208	<i>Bradyrhizobium tense</i>	<i>Bradyrhizobium tense</i>	99.96%	9,265,322	GCA_000213945.1			
			<i>Bradyrhizobium tense</i>	LMTR 13 <sup>1</sup>	<i>Bradyrhizobium tense</i>	<i>Bradyrhizobium tense</i>	99.90%	9,322,773	GCA_001493385.1			
			<i>Bradyrhizobium japonicum</i>	USDA 6 <sup>1</sup>	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	100.00%	9,207,384	GCA_000483475.1			
			<i>Bradyrhizobium japonicum</i>	CCBAU 15354	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	99.31%	10,126,594	GCA_000261545.1			
			<i>Bradyrhizobium japonicum</i>	CCBAU 15618	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	99.65%	9,824,012	GCA_000261585.1			
			<i>Bradyrhizobium japonicum</i>	CCBAU 25435	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	99.59%	9,460,795	GCA_000261605.1			
			<i>Bradyrhizobium japonicum</i>	USDA 38	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	99.70%	9,608,975	GCA_000472745.1			
			<i>Bradyrhizobium japonicum</i>	USDA 123	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	99.85%	10,407,465	GCA_000278125.1			
			<i>Bradyrhizobium japonicum</i>	SEMIA 5079	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	99.83%	9,583,021	GCA_000661915.1			
			<i>Bradyrhizobium japonicum</i>	Is-34	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	100.00%	10,326,597	GCA_000773865.1			
			<i>Bradyrhizobium japonicum</i>	E109	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	100.00%	9,224,208	GCA_000807315.1			
			<i>Bradyrhizobium japonicum</i>	FN1	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	100.00%	9,138,496	GCA_001038185.1			
			<i>Bradyrhizobium japonicum</i>	JS	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	100.00%	10,138,651	GCA_001887695.1			
			<i>Bradyrhizobium japonicum</i>	URMA197	<i>Bradyrhizobium japonicum</i>	<i>Bradyrhizobium japonicum</i>	100.00%	10,442,239	GCA_002108935.1			
			<i>Bradyrhizobium japonicum</i>	IS22	<i>Bradyrhizobium</i> sp. IS22	<i>Bradyrhizobium japonicum</i>	99.58%	9,091,355	GCA_000213925.1			
			<i>Bradyrhizobium pizoniae</i>	PAC 68 <sup>1</sup>	<i>Bradyrhizobium pizoniae</i>	<i>Bradyrhizobium pizoniae</i>	98.52%	8,738,021	GCA_000420995.1			
			<i>Bradyrhizobium lablabi</i>	CCBAU 23086 <sup>1</sup>	<i>Bradyrhizobium lablabi</i>	<i>Bradyrhizobium lablabi</i>	99.54%	8,817,291	GCA_001440475.1			
			<i>Bradyrhizobium manausense</i>	BR351 <sup>1</sup>	<i>Bradyrhizobium manausense</i>	<i>Bradyrhizobium manausense</i>	98.53%	9,145,311	GCA_001440035.1			
			<i>Bradyrhizobium mercantii</i>	SEMIA 6399 <sup>1</sup>	<i>Bradyrhizobium mercantii</i>	<i>Bradyrhizobium mercantii</i>	99.67%	9,842,857	GCA_001982635.1			
			<i>Bradyrhizobium neotropiale</i>	BR 10247 <sup>1</sup>	<i>Bradyrhizobium neotropiale</i>	<i>Bradyrhizobium neotropiale</i>	99.59%	8,679,329	GCA_001641695.1			
			<i>Bradyrhizobium oligotrophicum</i>	SS8 <sup>1</sup>	<i>Bradyrhizobium oligotrophicum</i>	<i>Bradyrhizobium oligotrophicum</i>	99.35%	8,264,165	GCA_000344805.1			
			<i>Bradyrhizobium ottawaense</i>	OC99 <sup>1</sup>	<i>Bradyrhizobium ottawaense</i>	<i>Bradyrhizobium ottawaense</i>	99.96%	9,606,328	GCA_000278135.1			
			<i>Bradyrhizobium ottawaense</i>	CCBAU 15544	<i>Bradyrhizobium</i> sp. CCBAU 15544	<i>Bradyrhizobium ottawaense</i>	99.79%	9,481,383	GCA_000661935.1			
			<i>Bradyrhizobium ottawaense</i>	CCBAU 15615	<i>Bradyrhizobium</i> sp. CCBAU 15615	<i>Bradyrhizobium ottawaense</i>	99.74%	8,852,539	GCA_000261725.1			
			<i>Bradyrhizobium ottawaense</i>	CCBAU 15635	<i>Bradyrhizobium</i> sp. CCBAU 15635	<i>Bradyrhizobium ottawaense</i>	99.80%	8,288,715	GCA_000261745.1			
			<i>Bradyrhizobium ottawaense</i>	USDA 4	<i>Bradyrhizobium ottawaense</i>	<i>Bradyrhizobium ottawaense</i>	99.96%	8,737,621	GCA_000472725.1			
			<i>Bradyrhizobium ottawaense</i>	L2	<i>Bradyrhizobium ottawaense</i>	<i>Bradyrhizobium ottawaense</i>	99.96%	8,797,357	GCA_002532025.1			
			<i>Bradyrhizobium pachyrrhizii</i>	PAC 48 <sup>1</sup>	<i>Bradyrhizobium pachyrrhizii</i>	<i>Bradyrhizobium pachyrrhizii</i>	99.26%	8,711,973	GCA_001189245.1			
			<i>Bradyrhizobium pachyrrhizii</i>	USDA 3254	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium pachyrrhizii</i>	100.00%	9,979,722	GCA_000278765.1			
			<i>Bradyrhizobium pachyrrhizii</i>	USDA 3259	<i>Bradyrhizobium elkanii</i>	<i>Bradyrhizobium pachyrrhizii</i>	100.00%	9,721,483	GCA_000213905.1			
			<i>Bradyrhizobium pachyrrhizii</i>	BR3262	<i>Bradyrhizobium pachyrrhizii</i>	<i>Bradyrhizobium pachyrrhizii</i>	98.93%	8,965,178	GCA_001440015.1			
			<i>Bradyrhizobium pachyrrhizii</i>	UFLA 03-321	<i>Bradyrhizobium</i> sp. UFLA 03-321	<i>Bradyrhizobium pachyrrhizii</i>	99.70%	8,595,048	GCA_001956825.1			
			<i>Bradyrhizobium pachyrrhizii</i>	R5	<i>Bradyrhizobium</i> sp. R5	<i>Bradyrhizobium pachyrrhizii</i>	99.96%	9,444,915	GCA_000104045.1			
			<i>Bradyrhizobium pavlovi</i>	LMTR 21 <sup>1</sup>	<i>Bradyrhizobium pavlovi</i> LMTR 21	<i>Bradyrhizobium pavlovi</i>	100.00%	8,377,719	GCA_001693515.2			
			<i>Bradyrhizobium retamae</i>	Ro19 <sup>1</sup>	<i>Bradyrhizobium retamae</i> Ro19	<i>Bradyrhizobium retamae</i>	99.85%	8,466,225	GCA_001440415.1			
			<i>Bradyrhizobium shewense</i>	ER11 <sup>1</sup>	<i>Bradyrhizobium shewense</i> ER11	<i>Bradyrhizobium shewense</i>	100.00%	9,163,226	GCA_000594005.1			
			<i>Bradyrhizobium sylvianthii</i>	GI2	<i>Bradyrhizobium sylvianthii</i> BR 446	<i>Bradyrhizobium sylvianthii</i>	99.54%	8,795,050	GCA_000329095.1			
			<i>Bradyrhizobium tropiciagi</i>	SEMIA 6148 <sup>1</sup>	<i>Bradyrhizobium tropiciagi</i> SEMIA 6148	<i>Bradyrhizobium tropiciagi</i>	98.40%	9,767,314	GCA_001189845.1			
			<i>Bradyrhizobium viridifurii</i>	SEMIA 690 <sup>1</sup>	<i>Bradyrhizobium viridifurii</i> SEMIA 690	<i>Bradyrhizobium viridifurii</i>	97.90%	8,811,922	GCA_001238275.1			
			<i>Bradyrhizobium yunnanense</i>	CCBAU 10071 <sup>1</sup>	<i>Bradyrhizobium yunnanense</i> CCBAU 10071	<i>Bradyrhizobium yunnanense</i>	99.90%	8,201,522	GCA_000094975.1			
			<i>Bradyrhizobium yunnanense</i>	SUTN9-2	<i>Bradyrhizobium</i> sp. SUTN9-2	<i>Bradyrhizobium yunnanense</i>	98.51%	8,092,255	GCA_003112645.1			
			<i>Bradyrhizobium yunnanense</i>	BR3267	<i>Bradyrhizobium yunnanense</i> BR3267	<i>Bradyrhizobium yunnanense</i>	99.80%	7,904,309	GCA_001439885.1			
			<i>Bradyrhizobium yunnanense</i>	CCBAU 35157	<i>Bradyrhizobium yunnanense</i> CCBAU 35157	<i>Bradyrhizobium yunnanense</i>	99.47%	8,854,129	GCA_000261825.1			
			<i>Bradyrhizobium yunnanense</i>	CCBAU 21021	<i>Bradyrhizobium yunnanense</i> CCBAU 21021	<i>Bradyrhizobium yunnanense</i>	99.44%	9,981,650	GCA_000329095.1			
			<i>Bradyrhizobium yunnanense</i>	CCBAU 05623	<i>Bradyrhizobium yunnanense</i> CCBAU 05623	<i>Bradyrhizobium yunnanense</i>	99.72%	8,245,751	GCA_000261785.1			
			Gammaproteobacteria	Enterobacteriales	Pantoea	<i>Pantoea agglomerans</i>	NBRC 102470 <sup>1</sup>	<i>Pantoea agglomerans</i> NBRC 102470	<i>Pantoea agglomerans</i>	100.00%	4,652,040	GCA_001598475.1
						<i>Pantoea agglomerans</i>	190	<i>Pantoea agglomerans</i> 190	<i>Pantoea agglomerans</i>	99.81%	5,002,566	GCA_000731125.1
						<i>Pantoea agglomerans</i>	3	<i>Pantoea agglomerans</i> 3	<i>Pantoea agglomerans</i>	100.00%	4,813,581	GCA_001597625.1
						<i>Pantoea agglomerans</i>	4188	<i>Pantoea agglomerans</i> pv. betae 4188	<i>Pantoea agglomerans</i>	99.95%	5,001,997	GCA_001662025.1
						<i>Pantoea agglomerans</i>	4	<i>Pantoea agglomerans</i> 4	<i>Pantoea agglomerans</i>	100.00%	4,827,990	GCA_000473785.2
						<i>Pantoea agglomerans</i>	B24-1	<i>Pantoea agglomerans</i> pv. <i>typhophilae</i> B24-1	<i>Pantoea agglomerans</i>	100.00%	4,981,068	GCA_000329155.1
						<i>Pantoea agglomerans</i>	B31	<i>Pantoea agglomerans</i> B31	<i>Pantoea agglomerans</i>	100.00%	4,047,763	GCA_001583435.1
						<i>Pantoea agglomerans</i>	C410P1	<i>Pantoea agglomerans</i> C410P1	<i>Pantoea agglomerans</i>	100.00%	5,115,241	GCA_001709315.1
						<i>Pantoea agglomerans</i>	CFSAN033090	<i>Pantoea</i> sp. CFSAN033090	<i>Pantoea agglomerans</i>	100.00%	4,942,973	GCA_001264395.1
						<i>Pantoea agglomerans</i>	DAPP-PG734	<i>Pantoea agglomerans</i> DAPP-PG734	<i>Pantoea agglomerans</i>	99.73%	5,365,929	GCA_000710215.1
						<i>Pantoea agglomerans</i>	DSM 3439	<i>Pantoea agglomerans</i> DSM3439 <sup>1</sup>	<i>Pantoea agglomerans</i>	100.00%	4,659,376	GCA_000185875.1
						<i>Pantoea agglomerans</i>	EH318	<i>Pantoea agglomerans</i> EH318	<i>Pantoea agglomerans</i>	100.00%	5,035,839	GCA_000872425.1
						<i>Pantoea agglomerans</i>	GB1	<i>Pantoea agglomerans</i> GB1	<i>Pantoea agglomerans</i>	99.54%	4,795,050	GCA_000329155.1
						<i>Pantoea agglomerans</i>	IG1	<i>Pantoea agglomerans</i> IG1	<i>Pantoea agglomerans</i>	100.00%	4,829,577	GCA_000214285.2
						<i>Pantoea agglomerans</i>	JM1	<i>Pantoea agglomerans</i> JM1	<i>Pantoea agglomerans</i>	100.00%	4,794,872	GCA_002222515.1
						<i>Pantoea agglomerans</i>	LMAE-2	<i>Pantoea agglomerans</i> LMAE-2	<i>Pantoea agglomerans</i>	100.00%	4,981,165	GCA_000814075.1
						<i>Pantoea agglomerans</i>	MP2	<i>Pantoea agglomerans</i> MP2	<i>Pantoea agglomerans</i>	100.00%	4,733,829	GCA_000757415.1
						<i>Pantoea agglomerans</i>	P10c	<i>Pantoea agglomerans</i> P10c	<i>Pantoea agglomerans</i>	100.00%	4,775,916	GCA_001288285.1
						<i>Pantoea agglomerans</i>	P5	<i>Pantoea agglomerans</i> P5	<i>Pantoea agglomerans</i>	100.00%	5,074,260	GCA_002157425.2
						<i>Pantoea agglomerans</i>	P9	<i>Pantoea agglomerans</i> P9	<i>Pantoea agglomerans</i>	99.67%	4,795,157	GCA_000329155.1
						<i>Pantoea agglomerans</i>	PwV11	<i>Pantoea agglomerans</i> PwV11	<i>Pantoea agglomerans</i>	100.00%	4,850,774	GCA_000086865.1
						<i>Pantoea agglomerans</i>	PNG 92-11	<i>Pantoea agglomerans</i> PNG 92-11	<i>Pantoea agglomerans</i>	100.00%	5,058,055	GCA_003149005.1
						<i>Pantoea agglomerans</i>	RIT 273	<i>Pantoea agglomerans</i> RIT273	<i>Pantoea agglomerans</i>	100.00%	5,365,338	GCA_000627115.1
						<i>Pantoea agglomerans</i>	S11-M5	<i>Pantoea agglomerans</i> S11-M5	<i>Pantoea agglomerans</i>	100.00%	4,919,733	GCA_000220605.2
						<i>Pantoea agglomerans</i>	Tx10	<i>Pantoea agglomerans</i> Tx10	<i>Pantoea agglomerans</i>	100.00%	4,856,993	GCA_000475055.1
						<i>Pantoea agglomerans</i>	ZB65	<i>Pantoea agglomerans</i> ZB65	<i>Pantoea agglomerans</i>	100.00%	4,729,004	GCA_002138615.1
						<i>Pantoea allii</i>	LMG 24248 <sup>1</sup>	<i>Pantoea allii</i> LMG 24248	<i>Pantoea allii</i>	100.00%	5,241,139	GCA_000213925.1
						<i>Pantoea allii</i>	QXW0681	<i>Pantoea</i> sp. QXW0681	<i>Pantoea allii</i>	100.00%	5,236,140	GCA_001441335.1
						<i>Pantoea allii</i>	PNA 200-10	<i>Pantoea allii</i> PNA 200-10	<i>Pantoea allii</i>	100.00%	5,000,872	GCA_003148935.1
						<i>Pantoea ananatis</i>	LMG 2665 <sup>1</sup>	<i>Pantoea ananatis</i> LMG 2665	<i>Pantoea ananatis</i>	100.00%	4,938,703	GCA_000710035.1
						<i>Pantoea ananatis</i>	1-38	<i>Pantoea ananatis</i> 1-38	<i>Pantoea ananatis</i>	100.00%	4,869,281	GCA_002224035.1
						<i>Pantoea ananatis</i>	97-1	<i>Pantoea ananatis</i> 97-1	<i>Pantoea ananatis</i>	100.00%	4,994,140	GCA_002952035.2
						<i>Pantoea ananatis</i>	AG702	<i>Pantoea</i> sp. AG702	<i>Pantoea ananatis</i>	100.00%	4,752,726	GCA_003182295.1
						<i>Pantoea ananatis</i>	AJ13555	<i>Pantoea ananatis</i> AJ13555	<i>Pantoea ananatis</i>	100.00%	4,877,279	GCA_000270125.2
						<i>Pantoea ananatis</i>	AMG 501	<i>Pantoea</i> sp. AMG 501	<i>Pantoea ananatis</i>	100.00%	5,102,640	GCA_002215245.1
						<i>Pantoea ananatis</i>	AMG 521	<i>Pantoea ananatis</i> AMG521	<i>Pantoea ananatis</i>	100.00%	4,875,770	GCA_001465955.1
						<i>Pantoea ananatis</i>	ARC272	<i>Pantoea ananatis</i> ARC272	<i>Pantoe</i>			

			<i>Pantoea ananatis</i> PNA 15-1	<i>Pantoea ananatis</i> PNA 15-1	<i>Pantoea ananatis</i>	100.00%	4,995,747	GCA_002959585.1
			<i>Pantoea ananatis</i> PNA 200-3	<i>Pantoea ananatis</i> PNA 200-3	<i>Pantoea ananatis</i>	100.00%	5,008,808	GCA_002959565.1
			<i>Pantoea ananatis</i> PNA 200-7	<i>Pantoea ananatis</i> PNA 200-7	<i>Pantoea ananatis</i>	100.00%	4,951,661	GCA_003148595.1
			<i>Pantoea ananatis</i> PNA 86-1	<i>Pantoea ananatis</i> PNA 86-1	<i>Pantoea ananatis</i>	100.00%	4,821,391	GCA_002168795.1
			<i>Pantoea ananatis</i> PNA 98-11	<i>Pantoea ananatis</i> PNA 98-11	<i>Pantoea ananatis</i>	100.00%	5,037,181	GCA_003182215.1
			<i>Pantoea ananatis</i> PNA 99-7	<i>Pantoea ananatis</i> PNA 99-7	<i>Pantoea ananatis</i>	100.00%	4,971,764	GCA_002959535.1
			<i>Pantoea ananatis</i> R100	<i>Pantoea ananatis</i> R100	<i>Pantoea ananatis</i>	100.00%	5,037,181	GCA_001543055.1
			<i>Pantoea ananatis</i> RSA47	<i>Pantoea ananatis</i> RSA47	<i>Pantoea ananatis</i>	100.00%	4,737,914	GCA_001475885.1
			<i>Pantoea ananatis</i> S6	<i>Pantoea ananatis</i> S6	<i>Pantoea ananatis</i>	100.00%	4,344,781	GCA_001169355.1
			<i>Pantoea ananatis</i> S7	<i>Pantoea ananatis</i> S7	<i>Pantoea ananatis</i>	100.00%	4,490,316	GCA_002168795.1
			<i>Pantoea ananatis</i> S8	<i>Pantoea ananatis</i> S8	<i>Pantoea ananatis</i>	99.93%	4,483,222	GCA_001169395.1
			<i>Pantoea ananatis</i> Sd-1	<i>Pantoea ananatis</i> Sd-1	<i>Pantoea ananatis</i>	100.00%	4,926,744	GCA_00058275.1
			<i>Pantoea ananatis</i> SGAi0210	<i>Pantoea ananatis</i> SGAi0210	<i>Pantoea ananatis</i>	100.00%	4,808,686	GCA_003184965.2
			<i>Pantoea ananatis</i> Y176	<i>Pantoea ananatis</i> Y176	<i>Pantoea ananatis</i>	100.00%	5,146,480	GCA_002224585.2
			<i>Pantoea breneri</i> LMG 5343 <sup>†</sup>	<i>Pantoea breneri</i> LMG 5343	<i>Pantoea breneri</i>	99.30%	4,933,175	GCA_002095305.1
			<i>Pantoea breneri</i> 3.5.1	<i>Pantoea</i> sp. 3.5.1	<i>Pantoea breneri</i>	100.00%	4,964,649	GCA_000731025.2
			<i>Pantoea breneri</i> AW62	<i>Pantoea</i> sp. AW62	<i>Pantoea breneri</i>	99.80%	4,973,366	GCA_002233725.1
			<i>Pantoea breneri</i> JFSSW-P1	<i>Pantoea breneri</i> JFSSW-P	<i>Pantoea breneri</i>	100.00%	5,122,786	GCA_001743465.1
			<i>Pantoea conspicua</i> LMG 24534 <sup>†</sup>	<i>Pantoea conspicua</i> LMG 24534	<i>Pantoea conspicua</i>	99.32%	4,308,858	GCA_002095315.1
			<i>Pantoea cypripedii</i> LMG 2657 <sup>†</sup>	<i>Pantoea cypripedii</i> LMG 2657	<i>Pantoea cypripedii</i>	99.95%	6,553,225	GCA_00209535.1
			<i>Pantoea deleyi</i> LMG 24200 <sup>†</sup>	<i>Pantoea deleyi</i> LMG 24200	<i>Pantoea deleyi</i>	98.89%	4,613,488	GCA_002095375.1
			<i>Pantoea eucrina</i> LMG 5346 <sup>†</sup>	<i>Pantoea eucrina</i> LMG 5346	<i>Pantoea eucrina</i>	99.03%	3,892,376	GCA_002095385.1
			<i>Pantoea eucrina</i> 3_1284	<i>Pantoea</i> sp. 3_1284	<i>Pantoea eucrina</i>	99.05%	4,072,087	GCA_00313015.1
			<i>Pantoea eucrina</i> Aa16	<i>Pantoea</i> sp. Aa16	<i>Pantoea eucrina</i>	99.02%	4,396,619	GCA_002195725.1
			<i>Pantoea eucrina</i> BRM17	<i>Pantoea</i> sp. BRM17	<i>Pantoea eucrina</i>	69.79%	4,271,249	GCA_00219595.1
			<i>Pantoea eucrina</i> ICBG 1758	<i>Pantoea</i> sp. ICBG 1758	<i>Pantoea eucrina</i>	99.28%	4,175,519	GCA_002928235.1
			<i>Pantoea eucrina</i> PSNIH1	<i>Pantoea</i> sp. PSNIH1	<i>Pantoea eucrina</i>	99.25%	4,410,683	GCA_000784875.3
			<i>Pantoea eucrina</i> RIT 413	<i>Pantoea</i> sp. RIT 413	<i>Pantoea eucrina</i>	99.28%	3,836,880	GCA_003057675.2
			<i>Pantoea rodasil</i> LMG26273 <sup>†</sup>	<i>Pantoea rodasil</i> LMG 26273	<i>Pantoea rodasil</i>	99.09%	5,751,456	GCA_002095465.1
			<i>Pantoea rwandensis</i> LMG 26275 <sup>†</sup>	<i>Pantoea rwandensis</i> LMG 26275	<i>Pantoea rwandensis</i>	99.61%	5,775,402	GCA_002095475.1
			<i>Pantoea septica</i> LMG 5345 <sup>†</sup>	<i>Pantoea septica</i> LMG 5345	<i>Pantoea septica</i>	99.96%	4,308,724	GCA_002095575.1
			<i>Pantoea septica</i> B48	<i>Pantoea septica</i> B48	<i>Pantoea septica</i>	99.74%	4,990,319	GCA_00219595.1
			<i>Pantoea septica</i> FDAARGOS 194	<i>Pantoea</i> sp. FDAARGOS 194	<i>Pantoea septica</i>	99.99%	4,877,138	GCA_002131385.2
			<i>Pantoea septica</i> FFS	<i>Pantoea septica</i> FFS	<i>Pantoea septica</i>	99.99%	4,548,084	GCA_000612605.1
			<i>Pantoea septica</i> NGS-ED-1003	<i>Pantoea</i> sp. NGS-ED-1003	<i>Pantoea septica</i>	99.99%	4,809,062	GCA_000738765.1
			<i>Pantoea wallisii</i> LMG 26277 <sup>†</sup>	<i>Pantoea wallisii</i> LMG 26277	<i>Pantoea wallisii</i>	99.12%	4,653,282	GCA_002095485.1
			<i>Paraburkholderia caribensis</i> MWAP64 <sup>†</sup>	<i>Paraburkholderia caribensis</i> DSM 13236	<i>Paraburkholderia</i> (A) <i>caribensis</i>	98.99%	9,032,490	GCA_002092945.1
			<i>Paraburkholderia caribensis</i> BcS1W	<i>Paraburkholderia caribensis</i> BcS1W	<i>Paraburkholderia</i> (A) <i>caribensis</i>	99.96%	5,319,993	GCA_001611015.1
			<i>Paraburkholderia caribensis</i> ET1848	<i>Paraburkholderia</i> sp. ET1848	<i>Paraburkholderia</i> (A) <i>caribensis</i>	99.93%	4,498,799	GCA_00219595.1
			<i>Paraburkholderia caribensis</i> MBA4	<i>Paraburkholderia caribensis</i> MBA4	<i>Paraburkholderia</i> (A) <i>caribensis</i>	99.40%	4,482,704	GCA_000522545.2
			<i>Paraburkholderia caribensis</i> T1182	<i>Paraburkholderia caribensis</i> T1182	<i>Paraburkholderia</i> (A) <i>caribensis</i>	99.40%	9,205,673	GCA_003028645.1
			<i>Paraburkholderia diazotrophica</i> LMG 26031 <sup>†</sup>	<i>Paraburkholderia diazotrophica</i> LMG 26031	<i>Paraburkholderia</i> (A) <i>diazotrophica</i>	99.50%	6,688,577	GCA_000180845.1
			<i>Paraburkholderia hospita</i> LMG 20598	<i>Paraburkholderia hospita</i> DSM 17164	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.19%	11,527,206	GCA_002092965.1
			<i>Paraburkholderia hospita</i> B5001	<i>Paraburkholderia hospita</i> B5001	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.40%	11,294,072	GCA_000265115.1
			<i>Paraburkholderia hospita</i> B5007	<i>Paraburkholderia hospita</i> B5007	<i>Paraburkholderia</i> (A) <i>hospita</i>	98.99%	11,019,888	GCA_002157295.1
			<i>Paraburkholderia hospita</i> B5110	<i>Paraburkholderia hospita</i> B5110	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.40%	11,212,608	GCA_002157295.1
			<i>Paraburkholderia hospita</i> B5437	<i>Paraburkholderia hospita</i> B5437	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.11%	11,297,686	GCA_002157305.1
			<i>Paraburkholderia hospita</i> BT03	<i>Burkholderia</i> sp. BT03	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.09%	10,918,404	GCA_000281995.2
			<i>Paraburkholderia hospita</i> CF099	<i>Burkholderia</i> sp. CF099	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.29%	10,359,601	GCA_000168075.1
			<i>Paraburkholderia hospita</i> CF145	<i>Burkholderia</i> sp. CF145	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.31%	10,860,052	GCA_002245905.1
			<i>Paraburkholderia hospita</i> YR277	<i>Burkholderia</i> sp. YR277	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.09%	10,793,882	GCA_000168175.1
			<i>Paraburkholderia hospita</i> YR290	<i>Burkholderia</i> sp. YR290	<i>Paraburkholderia</i> (A) <i>hospita</i>	99.29%	11,302,107	GCA_000230235.1
			<i>Paraburkholderia phytatum</i> STM815 <sup>†</sup>	<i>Paraburkholderia phytatum</i> STM815	<i>Paraburkholderia</i> (A) <i>phytatum</i>	99.09%	6,676,562	GCA_000020045.1
			<i>Paraburkholderia piptadeniae</i> STM7183 <sup>†</sup>	<i>Paraburkholderia piptadeniae</i> STM 7183	<i>Paraburkholderia</i> (A) <i>piptadeniae</i>	99.50%	9,710,406	GCA_000007165.2
			<i>Paraburkholderia terrae</i> DSM 17804 <sup>†</sup>	<i>Paraburkholderia terrae</i> DSM 17804	<i>Paraburkholderia</i> (A) <i>terrae</i>	99.80%	10,062,489	GCA_002092925.1
			" <i>Paraburkholderia acidophila</i> " ATCC 31363 <sup>†</sup>	<i>Pseudomonas acidophila</i> ATCC 31363	<i>Paraburkholderia acidophila</i>	97.88%	7,170,935	GCA_002362315.1
			<i>Paraburkholderia aromaticivorans</i> BN5 <sup>†</sup>	<i>Paraburkholderia aromaticivorans</i> BN5	<i>Paraburkholderia aromaticivorans</i>	100.00%	4,907,716	GCA_002278075.1
			<i>Paraburkholderia aspalathi</i> LMG 27731 <sup>†</sup>	<i>Paraburkholderia aspalathi</i> LMG 27731	<i>Paraburkholderia aspalathi</i>	99.90%	9,892,286	GCA_000116445.1
			<i>Paraburkholderia bryophila</i> LMG 23644 <sup>†</sup>	<i>Paraburkholderia bryophila</i> LMG 23644	<i>Paraburkholderia bryophila</i>	99.40%	8,014,697	GCA_003269035.1
			<i>Paraburkholderia caledonica</i> NBRC 102488 <sup>†</sup>	<i>Paraburkholderia caledonica</i> NBRC 102488	<i>Paraburkholderia caledonica</i>	99.60%	7,282,355	GCA_000685095.1
			<i>Paraburkholderia caledonica</i> 376MFSna3.1	<i>Paraburkholderia caledonica</i> 376MFSna3.1	<i>Paraburkholderia caledonica</i>	100.00%	8,337,283	GCA_00219595.1
			<i>Paraburkholderia caledonica</i> BK	<i>Burkholderia</i> sp. BK	<i>Paraburkholderia caledonica</i>	100.00%	7,282,355	GCA_000169005.1
			<i>Paraburkholderia caledonica</i> CA-53F-1	<i>Paraburkholderia caledonica</i> CA-53F-1	<i>Paraburkholderia caledonica</i>	99.97%	7,581,410	GCA_002179925.1
			<i>Paraburkholderia caledonica</i> OV446	<i>Paraburkholderia</i> sp. OV446	<i>Paraburkholderia caledonica</i>	100.00%	7,257,342	GCA_003201235.1
			<i>Paraburkholderia dilworthii</i> WSM 3556 <sup>†</sup>	<i>Paraburkholderia dilworthii</i> WSM3556	<i>Paraburkholderia dilworthii</i>	100.00%	7,679,067	GCA_000472525.1
			<i>Paraburkholderia fungorum</i> ATCC BAA-463 <sup>†</sup>	<i>Paraburkholderia fungorum</i>	<i>Paraburkholderia fungorum</i>	99.60%	9,058,983	GCA_000966155.1
			<i>Paraburkholderia fungorum</i> FDAARGOS 245	<i>Paraburkholderia fungorum</i> FDAARGOS_245	<i>Paraburkholderia fungorum</i>	99.60%	9,009,624	GCA_002919075.1
			<i>Paraburkholderia fungorum</i> J4	<i>Burkholderia</i> sp. J4	<i>Paraburkholderia fungorum</i>	99.19%	8,341,181	GCA_000148185.1
			<i>Paraburkholderia ginsengisoli</i> NBRC 100965 <sup>†</sup>	<i>Paraburkholderia ginsengisoli</i> NBRC 100965	<i>Paraburkholderia ginsengisoli</i>	99.90%	6,541,887	GCA_000739735.1
			<i>Paraburkholderia ginsengiterrae</i> DCY85 <sup>†</sup>	<i>Paraburkholderia ginsengiterrae</i> DCY85	<i>Paraburkholderia ginsengiterrae</i>	100.00%	6,533,041	GCA_001645125.1
			<i>Paraburkholderia graminis</i> C4D1M <sup>†</sup>	<i>Paraburkholderia graminis</i> C4D1M	<i>Paraburkholderia graminis</i>	98.67%	7,477,263	GCA_000172415.1
			<i>Paraburkholderia graminis</i> GV052	<i>Paraburkholderia</i> sp. GV052	<i>Paraburkholderia graminis</i>	99.60%	7,284,992	GCA_002308595.1
			<i>Paraburkholderia graminis</i> GV068	<i>Paraburkholderia</i> sp. GV068	<i>Paraburkholderia graminis</i>	99.60%	7,286,732	GCA_003058125.1
			<i>Paraburkholderia graminis</i> GV072	<i>Paraburkholderia</i> sp. GV072	<i>Paraburkholderia graminis</i>	99.60%	7,284,546	GCA_003050715.1
			<i>Paraburkholderia graminis</i> H81	<i>Burkholderia</i> sp. H81	<i>Paraburkholderia graminis</i>	99.60%	7,206,167	GCA_002195945.1
			<i>Paraburkholderia graminis</i> URHAD0054	<i>Burkholderia</i> sp. URHAD0054	<i>Paraburkholderia graminis</i>	99.60%	7,244,804	GCA_000421865.1
			<i>Paraburkholderia kirstenboschensis</i> Kb15 <sup>†</sup>	<i>Paraburkholderia kirstenboschensis</i> Kb15	<i>Paraburkholderia kirstenboschensis</i>	97.15%	8,342,886	GCA_001639975.1
			<i>Paraburkholderia megapolitana</i> LMG 23560 <sup>†</sup>	<i>Paraburkholderia megapolitana</i> LMG 23650	<i>Paraburkholderia megapolitana</i>	98.07%	7,607,319	GCA_000113825.1
			<i>Paraburkholderia monticola</i> JC2948 <sup>†</sup>	<i>Paraburkholderia monticola</i> JC2948	<i>Paraburkholderia monticola</i>	100.00%	7,845,380	GCA_001580545.1
			<i>Paraburkholderia phenazinum</i> LMG2247 <sup>†</sup>	<i>Paraburkholderia phenazinum</i> LMG 2247	<i>Paraburkholderia phenazinum</i>	99.09%	8,597,887	GCA_000100735.1
			<i>Paraburkholderia phenoliruptrix</i> AC1100 <sup>†</sup>	<i>Paraburkholderia phenoliruptrix</i> AC1100	<i>Paraburkholderia phenoliruptrix</i>	100.00%	7,811,030	GCA_000416445.1
			<i>Paraburkholderia phenoliruptrix</i> BR3459a	<i>Paraburkholderia phenoliruptrix</i> BR3459a	<i>Paraburkholderia phenoliruptrix</i>	100.00%	7,651,131	GCA_000300095.1
			<i>Paraburkholderia phenoliruptrix</i> CCGE1001	<i>Burkholderia</i> sp. CCGE1001	<i>Paraburkholderia phenoliruptrix</i>	100.00%	7,251,494	GCA_000176935.3
			<i>Paraburkholderia phenoliruptrix</i> TPY 3166 <sup>†</sup>	<i>Burkholderia</i> sp. TPY3166	<i>Paraburkholderia phenoliruptrix</i>	100.00%	6,780,608	GCA_000515195.1
			<i>Paraburkholderia phytofirmans</i> PsJn <sup>†</sup>	<i>Paraburkholderia phytofirmans</i> PsJn	<i>Paraburkholderia phytofirmans</i>	100.00%	8,214,658	GCA_000020125.1
			<i>Paraburkholderia rhynchosiae</i> WSM 3937 <sup>†</sup>	<i>Paraburkholderia rhynchosiae</i> WSM 3937	<i>Paraburkholderia rhynchosiae</i>	100.00%	8,029,899	GCA_002879865.1
			<i>Paraburkholderia ribeironis</i> STM7296 <sup>†</sup>	<i>Paraburkholderia ribeironis</i> STM7296	<i>Paraburkholderia ribeironis</i>	100.00%	7,279,154	GCA_000019265.2
			<i>Paraburkholderia sartisoli</i> LMG 24000 <sup>†</sup>	<i>Paraburkholderia sartisoli</i> LMG 24000	<i>Paraburkholderia sartisoli</i>	97.38%	5,930,259	GCA_000107885.1
			<i>Paraburkholderia sediminicola</i> LMG 24238 <sup>†</sup>	<i>Paraburkholderia sediminicola</i> LMG 24238	<i>Paraburkholderia sediminicola</i>	99.50%	7,311,602	GCA_000104005.1
			<i>Paraburkholderia sprentioe</i> WSM 5005 <sup>†</sup>	<i>Paraburkholderia sprent</i>				

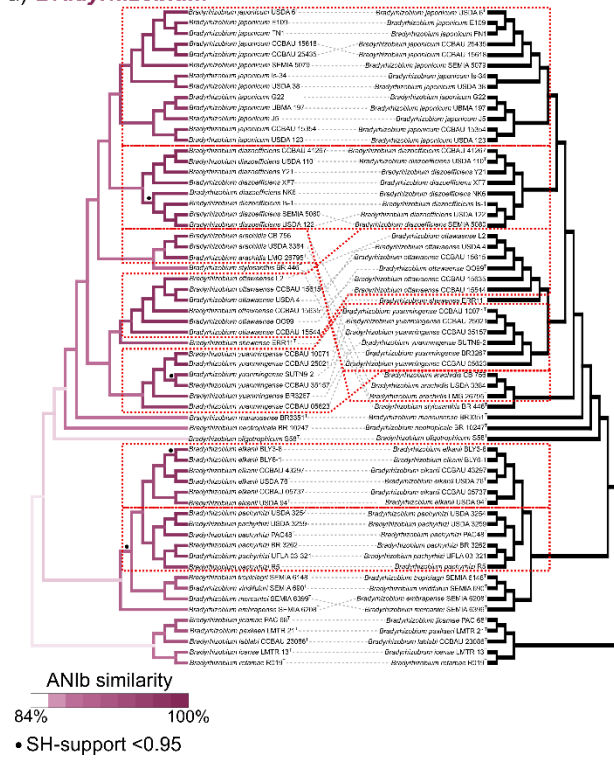


**Figure S1.** Comparison of the different methods used for calculating ANI indicated on individual graphs and separated by taxon set. The x-axis depicts ANIb similarity, while the y-axis indicates the percentage similarity for each of the approaches. The equation and  $R^2$ -value for the linear regression line for each graph is indicated in the top left corner.

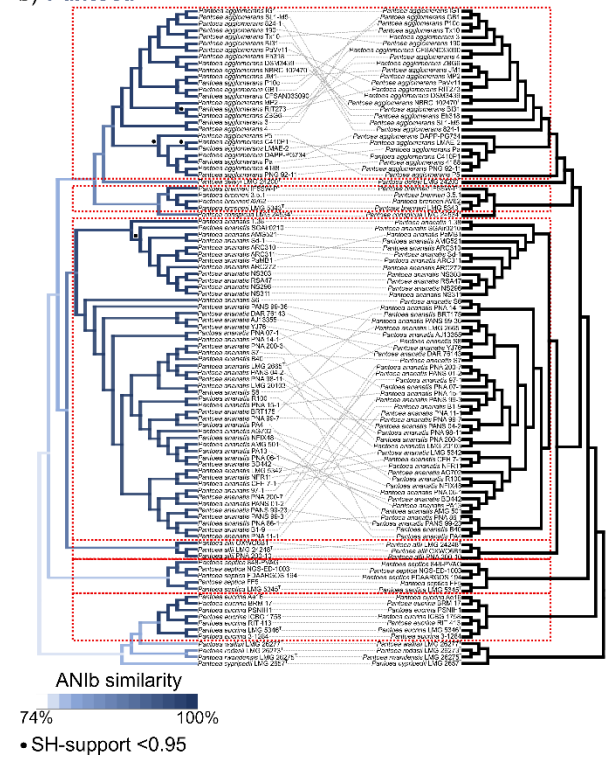


**Figure S2.** Approximate maximum-likelihood (AML) phylogenies of a) *Bradyrhizobium*, b) *Pantoea* and c) *Paraburkholderia*, constructed from a concatenated supermatrix of 92 shared protein sequences using UBCG (Na et al., 2018). Shimodaira-Hasegawa (SH) branch support values and gene support index (GSI) are indicated on the nodes (SH/GSI) and GSI depict how many individual gene trees of the 92 conserved protein-coding genes recovered specific groupings. A GSI of 92 thus means that all 92 gene trees recovered that node. The scale bar indicates the number of changes per site.

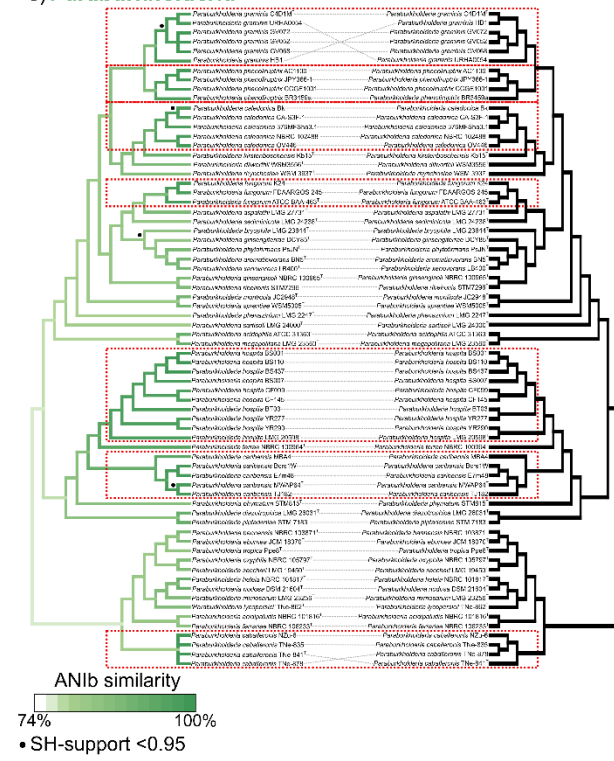
a) *Bradyrhizobium*



b) *Pantoea*



c) *Paraburkholderia*



**Figure S3.** Comparison of cladograms inferred from phylogenomic approximate maximum-likelihood (AML) and ANI-based neighbour joining (NJ; indicated in black) phylogenies for a) *Bradyrhizobium*, b) *Pantoea* and c) *Paraburkholderia*. AML analyses were performed with the concatenated supermatrix of 92 shared protein sequences using UBCG. NJ trees were inferred using distances inferred from ANiB values. Similarity values obtained with ANiB are indicated as a colour range on branches of the corresponding AML cladograms, with all pair-wise comparisons between members (inter- and intraspecies) having an ANiB value of at least 76% within a genus. Multi-strain species are indicated in red dotted lines, while specific taxa are connected with grey dotted lines.

## OrthoANI (OAT) Python Script

### The following script was written to run OrthoANI analyses with all query sequences to a single reference in a sequential manner. The script was written in Python 2.7.12 in Linux, but can be adapted for any python including windows by changing the forward slashes to back slashes and ensuring that the correct EOL characters are used in fasta files and scripts. In all instances “.” refers to the folder in which the script is located and any following information navigates toward the directory where specific genomes are located. Thus, replace the paths to the files with the appropriate path and filenames of your files before running this script. These paths and filenames are those referring to the data used in this study. Download the necessary blast directory and files from NCBI according to the OAT instructions. As a suggestion, first test the script with a single pair of genomes. Be sure to complete all the steps outlined below. ###

```
import sys, os, re
```

```
for fname in os.listdir("./Intraspecies_calculations/Genomes/Paraburkholderia_terrae//"):
```

```
    #####STEP 1 - SET FILE EXTENSION .FAS or .FASTA ###
```

```
if fname[fname.rfind("."):].upper() == ".FAS":
```

```
    ### STEP 2 - NB!!!! SPECIFY HERE WHICH GENOME IS GOING TO BE USED FOR THE -FASTA2  
    OPTION; IF THIS PART IS LEFT EMPTY ONE CANNOT SEE WHICH COMPARISONS WERE PERFORMED  
    AND ONLY RESULTS ARE GIVEN ###
```

```
    print "OAT_calculation: " + fname + " and " + "P_terrae_YR290"
```

```
    ### STEP 3 - THE COMMANDS NECESSARY TO RUN OAT IS THE JAR CALL, THE PATH TO THE  
    BLAST+ DIRECTORY (-blastplus_dir), THE FIRST GENOME TO BE COMPARED (-fasta1) AND THE  
    SECOND GENOME TO BE COMPARED (-fasta2) --- THE FOLLOWING COMMAND IS TO USE A SINGLE -  
    fasta2 AGAINST ALL FASTA (.fas or .fasta) GENOMES IN THE FOLDER ###
```

```
    os.system('java -jar OAT_cmd.jar' + ' ' + '-blastplus_dir' + ' ' + './ncbi-blast-2.7.1+/bin/' + ' ' + '-  
fasta1' + ' ' + './' + 'Intraspecies_calculations/Genomes/Paraburkholderia_terrae//' + fname + ' ' + '-  
fasta2' + ' ' + './' + 'Intraspecies_calculations/Genomes/Paraburkholderia_terrae/' +  
'Paraburkholderia_A_terrae_YR290.fas')
```

## ANICalculator (gANI) Python Script

### The following script was written to run the ANICalculator analyses with all query sequences to a single reference in a sequential manner. The script was written in Python 2.7.12 in Linux, but can be adapted for any python including windows by changing the forward slashes to back slashes and ensuring that the correct EOL characters are used in fasta files and scripts. In all instances “./” refers to the folder in which the script is located and any following information navigates toward the directory where specific genomes are located. Thus replace the paths to the files with the appropriate path and filenames of your files before running this script. These paths and filenames are those referring to the data used in this study. Download the necessary programs and dependencies as per ANICalculator instructions. As a suggestion, first test the script with a single pair of genomes. Be sure to complete all the steps outlined below. ###

```
import sys, os, re

for fname in os.listdir("./Intraspecies_calculations/Nucleotide_CDSs/Paraburkholderia_terrae/"):

    ##### STEP 1 - SET FILE EXTENSION .FAS, .FASTA or .FAA #####

    if fname[fname.rfind("."):].upper() == ".FAS":

        ### STEP 2 – ENSURE THAT THE REFERENCE NAME IS SPECIFIED AT THIS STEP TO MAKE CLEAR WHICH GENOME IS BEING ANALYSED AS REFERENCE ###

        print "gANI_calculation: " + fname + " and " + "P_terrae_YR290"

        ### STEP 3 - CALL PROGRAM "ANICalculator" WITH ASSOCIATED OPTIONS (-genome1fna -genome2fna -outfile) AS PER PROGRAM INSTRUCTIONS. ###

        os.system('./ANICalculator_v1/ANICalculator' + ' ' + '-genome1fna' + ' ' + './' + 'Intraspecies_calculations/Nucleotide_CDSs/Paraburkholderia_terrae/' + fname + ' ' + '-genome2fna' + ' ' + './' + 'Intraspecies_calculations/Nucleotide_CDSs/Paraburkholderia_terrae/' + 'Paraburkholderia_A_terrae_YR290.fas' + ' ' + '-outfile' + ' ' + './ANICalculator_v1/Intraspecies/' + 'gANI_' + fname + 'vs_P_terrae_YR290')
```