

THE LANCET Microbe

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Nimmo C, Millard J, van Dorp L, et al. Population-level emergence of bedaquiline and clofazimine resistance-associated variants among patients with drug-resistant tuberculosis in southern Africa: a phenotypic and phylogenetic analysis. *Lancet Microbe* 2020; **1**: e165–74.

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Research in Context

The following studies were identified during a search of PubMed and Google Scholar for articles reporting clinical data in English published since 2000 using the terms “bedaquiline resistance” and “clofazimine resistance” in combination with country names for the Southern African Development Community conducted on 13th May 2020. Studies reporting only *in vitro* results and review articles were excluded.

Dheda K, Limberis JD, Pietersen E, Phelan J, Esmail A, Lesosky M, et al. Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *Lancet Respir Med.* 2017 Apr;5(4):269-281.

Makhado NA, Matabane E, Faccin M, Pinçon C, Jouet A, Boutachkourt F, et al. Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study. *Lancet Infect Dis.* 2018;18(12):1350–9.

de Vos M, Ley SD, Wiggins KB, Derendinger B, Dippenaar A, Grobbelaar M, et al. Bedaquiline Microheteroresistance after Cessation of Tuberculosis Treatment. *N Engl J Med.* 2019 May 30;380(22):2178-2180.

Klopper M, Heupink TH, Hill-Cawthorne G, Streicher EM, Dippenaar A, De Vos M, et al. A landscape of genomic alterations at the root of a near-untreatable tuberculosis epidemic. *BMC Med.* 2020;18(1).

Supplementary Methods

Whole genome sequencing bioinformatics pipeline

Adapters were removed using Trim Galore v0.3.7. Reads were mapped to H37Rv (NC_000962.3) with BMap v38.32 specifying a 98% identity threshold, de-duplicated with Picard Tools v2.20 and then mean genome coverage measured with Qualimap v2.21. FreeBayes v1.2 was used to call variants with supported by ≥ 4 reads, including ≥ 1 on each strand, with mapping quality ≥ 20 and base quality ≥ 30 . Functional consequences of the identified variants were annotated with snpEff.

Additional studies included in phylogenies

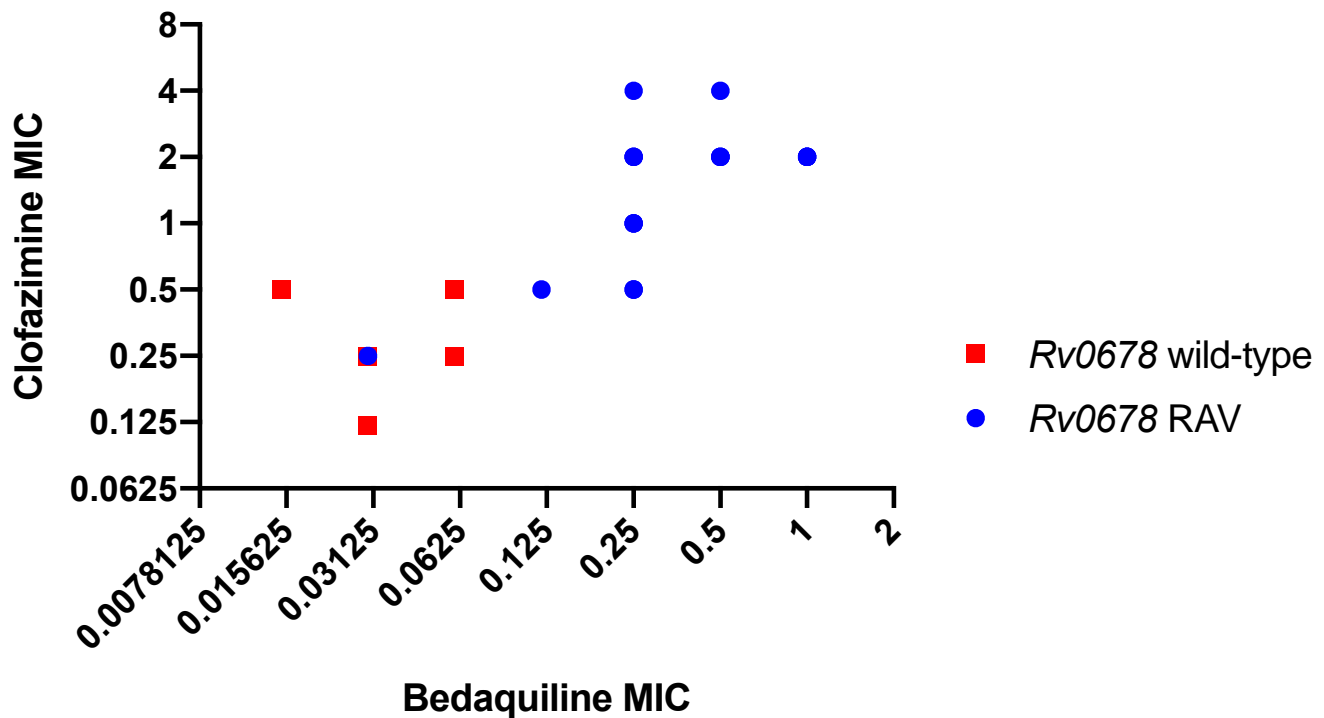
A selection of additional whole genome sequences from studies in southern Africa were included to provide phylogenetic context. Where isolates with *Rv0678* resistance-associated variants had been identified by BIGSI or PYGSI from a published study, other whole genome sequences from that study were also included.

Additional lineage 2 genomes from southern Africa were identified by screening the National Center for Biotechnology Information (NCBI) Sequencing Read Archive with BIGSI for *Mycobacterium tuberculosis* whole genome sequences collected from Southern African Development Community countries with the phylogenetically definitive nucleotide change in *rpsA* 636 A→C.(1)

Additional lineage 4 genomes from Southern African Development Community countries were included from a comprehensive global study of lineage 4.(2)

Supplementary Figures

Supplementary Figure 1. Correlation between bedaquiline and clofazimine MICs in isolates with a wild-type (red squares) and mutated (blue circles) *Rv0678* genes.

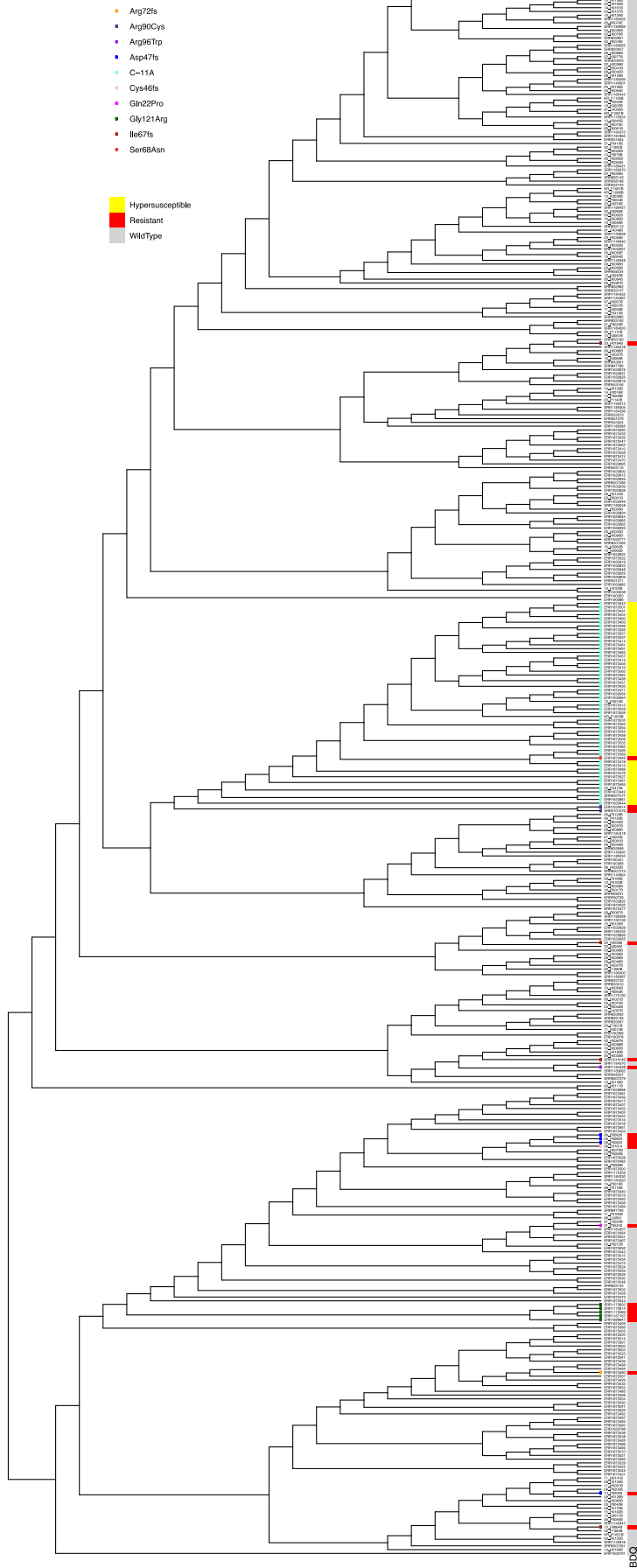


Supplementary Figure 2 (page 5). Linear phylogenetic tree of lineage 2 sequences, indicating study identification number (format XX_X0000X) for samples sequenced for this study, or NCBI Sequencing Read Archive / European Nucleotide Archive accession number for publicly available samples (starting SRR/ERR). Annotations indicate resistance-associated variants and previously observed resistance profile associated with the variant.

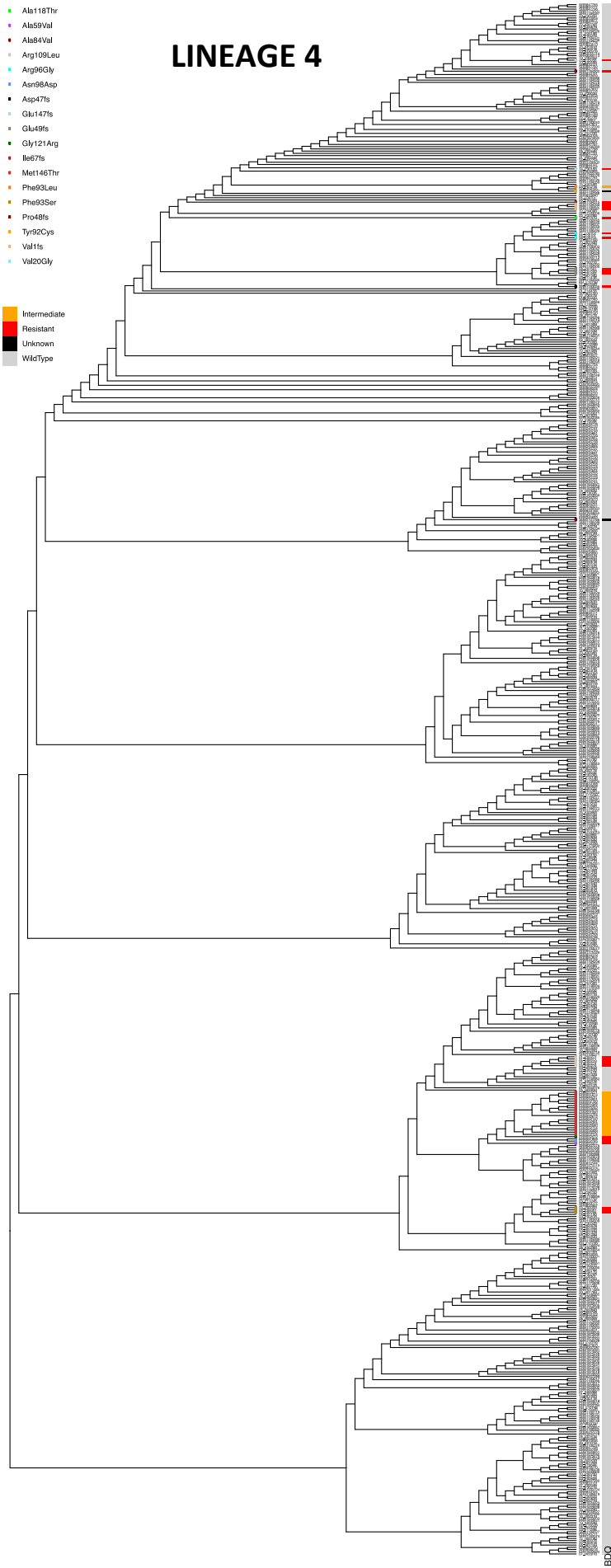
Supplementary Figure 3 (page 6). Linear phylogenetic tree of lineage 4 sequences, indicating study identification number (format XX_X0000X) for samples sequenced for this study, or NCBI Sequencing Read Archive / European Nucleotide Archive accession number for publicly available samples (starting SRR/ERR). Annotations indicate resistance-associated variants and previously observed resistance profile associated with the variant.

Supplementary Figure 2

LINEAGE 2



Supplementary Figure 3



Supplementary Tables

Supplementary Table 1. Previously observed bedaquiline resistance associated with *Rv0678* variants included in the extended datasets used for phylogenetic reconstruction. int = associated with intermediate MIC, comb = only seen in combination with other *Rv0678* variants.

Observed resistance	Variant	Associated with MIC	
		Resistant / intermediate	Susceptible
Resistant	Val1Ala	Somoskovi (3), this study	
	Gln22Pro	This study	
	Ala59Val	Villellas (4)	
	Arg90Cys	Yang (5)	
	Arg94Gln	Andries (6)	
	Asn98Asp	Yang (5)	
	Ala118Thr	This study	
	Leu136Arg	This study	
	Met146Thr	Xu (7)	Yang (5)
	<i>Frameshift variants</i> Val1fs Cys46fs Asp47fs Pro48fs Glu49fs Ile67fs Arg156fs	This study This study, Andres (8) Andres (8) Andres (8) This study (int), Andres (8) This study This study	
Intermediate	Val20Gly	This study, Ghodousi (9)	
	Phe93Ser	This study	
	Arg109Leu	This study	
	Gly121Arg	This study	
	<i>Frameshift variants</i> Glu147fs	This study	
Susceptible	Phe93Leu		This study
Hypersusceptible	C-11A		Andries (6)
Unknown	Ala62Thr		
	Ser68Asn		
	Ala84Val		
	Tyr92Cys		
	Arg96Gly		
	Arg96Thr		
	<i>Frameshift variants</i> Arg72fs		

Supplementary Table 2. Isolates from our centre with *Rv0678* variants, and preceding isolates from the same patient if available. Patients previously reported in Nimmo et al (10) are denoted with the corresponding patient identifier in brackets. All variants in *Rv0678* and *Rv1979c* are shown. No resistance-associated variants were found in *pepQ* or *atpE*. Drug resistance classification was determined by phenotypic testing. Bedaquiline and clofazimine MICs are shown where tested. Patients are referred to by five-character study identifiers. *There was discordance with the same sample tested separately at a critical concentration of 0.25µg/mL appearing phenotypically resistant.

Study ID (Key to Nimmo et al) (10)	Time-point	Sequencing ID	Rv0678 variant (frequency)	Variants in Rv1979c	Drug resistance classification (phenotypic DST)	Bedaquiline MIC	Clofazimine MIC
P0027 (A)	Baseline	06_P0027B	Arg109Leu (67.9%) Arg156fs (25.8%)		Pre-XDR (F)	0.25	1.0
	Month 1	01_P00271	Arg109Leu (86.8%) Arg156fs 16.5%)				
	Month 3	01_P00273	Arg109Leu (86.7%) Arg156fs (9.5%)				
	Month 4	01_P00274	Arg109Leu (29.1%) Arg156fs (69.6%)				
	Month 5	07_P00275	Arg109Leu (27.8%) Arg156fs (69.2%)				
	Month 6	04_P00276	Arg109Leu (23.9%) Arg156fs (57.1%)		Pre-XDR (F)	0.25	2.0
P0150 (B)	Baseline	08_P0150B	Glu49fs (72.0%)	Rv1979c Glu38Asp (100.0%)	Pre-XDR (F)	0.03	0.25
	Month 1	08_P01501	Glu49fs (94.2%)	Rv1979c Glu38Asp (100.0%)			
	Month 2	08_P01502	Glu49fs (96.7%)	Rv1979c Glu38Asp (100.0%)		0.25	2.0
P3016 (C)	Baseline	14_P3016B	Val20Gly (100.0%)	Rv1979c Glu38Asp (100.0%)	Pre-XDR (SLI)	0.25	4.0
P0005 (D)	Baseline	03_P0005B	Phe93Ser (100.0%)		Pre-XDR (F)	0.25	1.0
	Month 1	09_P00051	Phe93Ser (100.0%)				
	Month 5	06_P00055	Phe93Ser (100.0%)				
	Month 6	03_P00056	Phe93Ser (100.0%)			0.25	1.0
P0121 (E)	Baseline	04_P0121B	Phe93Leu (100.0%)	Rv1979c Glu38Asp (100.0%)	XDR	0.12	Failed x3

P0082 (F)	Baseline	03_P0082B	Wild type	Rv1979c Arg409Gln (100.0%)	XDR	0.06	0.25
	Month 4	05_P00824	Asp47fs (93.4%)	Rv1979c Arg409Gln (100.0%)		0.5	2.0
	Month 5	06_P00825	Cys46fs (35.9%) Asp47fs (53.3%)	Rv1979c Arg409Gln (100.0%)		1.0	2.0
	Month 6	06_P00826	Cys46fs (23.1%) Asp47fs (69.8%)	Rv1979c Arg409Gln (100.0%)	XDR	1.0	2.0
P0101 (G)	Baseline	04_P0101B	Wild type	Rv1979c Glu38Asp (100.0%)	XDR	0.03	0.12
	Month 4	06_P01014	Cys46fs (97.7%)	Rv1979c Arg409Gln (100.0%)		1.0	2.0
P0200 (H)	Baseline	09_P0200B	Wild type	Rv1979c Arg409Gln (100.0%)	XDR	0.06	0.25
	Month 6	14_P02006	Asp47fs (92.3%)	Rv1979c Arg409Gln (100.0%)	XDR	0.5	2.0
P3505 (I)	Baseline	13_P3505B	Wild type	Rv1979c Glu38Asp (100.0%)	XDR	0.03	0.25
	Month 6	25_P35056	Glu147fs (100.0%)	Rv1979c Glu38Asp (100.0%)		0.25	0.5
P3524 (J)	Baseline	21_P3524B	Wild type	Rv1979c Arg409Gln (100.0%)	XDR	<0.03	0.25
	Month 2	21_P35242	Gln22Pro (85.1%) Asp47fs (12.6%)	Rv1979c Arg409Gln (100.0%)		1.0	2.0
	Month 6	25_P35246	Glu22Pro (9.8%) Asp47fs (45.2%) Ala57Glu (10.6%) Arg72Thr (9.2%) Asp88fs (12.0%) Asp88Ala (13.0%) Glu121Arg (6.0%) Leu122Pro (7.4%)	Rv1979c Arg409Gln (100.0%)	XDR	0.5	2.0
L001	Baseline	06_L00101	Wild type	Rv1979c Arg409Gln (100.0%)			
	Month 12	07_L00102	Arg96Gly (8.8%)	Rv1979c Arg409Gln (100.0%)			
	Month 19	06_L00103	Arg96Gly (100.0%)	Rv1979c Arg409Gln (100.0%)			
H0147	Baseline	12_H0147B	Ala118Thr (100.0%)	Rv1979c Glu38Asp (100.0%)	MDR	0.12*	0.5
H0184	Baseline	23_H0184B	Ile67fs (8.3%)		MDR	0.25	2.0
F8064	Month 20	13_F8064B	Asp47fs (7.1%) Ile67fs (54.3%) Leu136Arg (22.6%)	Rv1979c Arg409Gln (100.0%)	Pre-XDR	1.0	2.0
K0056	Week 2	22_K00562	Wild type		Rifampicin mono-resistant	0.06	0.5
	Week 6	22_K00566	Ile67fs (95.1%)			0.5	4.0
K0084	Baseline	24_K0084B	Wild type	Rv1979c Asp286Gly (100.0%)		<0.03	0.5
	Week 2	24_K00842	Wild type	Rv1979c Asp286Gly (100.0%)			
	Month 6	26_K0084K	Val1Ala (62.8%) Gly37fs (8.1%) Arg89Trp (11.8%)	Rv1979c Asp286Gly (100.0%)		0.25	0.5

Supplementary Table 3. List of lineage 2 samples included in the extended Southern Africa dataset.

Lineage	Sample ID	Accession	Country	Region	Study	Day	Month	Year
2	01_C4006B	SRR9971318	South Africa	Southern Africa	Other AHRI KZN study	13	11	2014
2	01_C4048B	SRR9971120	South Africa	Southern Africa	Other AHRI KZN study	26	3	2014
2	01_C4067B	SRR9971317	South Africa	Southern Africa	Other AHRI KZN study	11	4	2014
2	02_F1069B	SRR11349175	South Africa	Southern Africa	Other AHRI KZN study	14	12	2015
2	02_F1083B	SRR11349164	South Africa	Southern Africa	Other AHRI KZN study	25	1	2016
2	02_F1090B	SRR11349131	South Africa	Southern Africa	Other AHRI KZN study	3	2	2015
2	02_F1110B	SRR9971270	South Africa	Southern Africa	Other AHRI KZN study	4	4	2016
2	02_H0041B	SRR9971081	South Africa	Southern Africa	Other AHRI KZN study	26	10	2016
2	02_H0059B	SRR9971065	South Africa	Southern Africa	Other AHRI KZN study	8	2	2017
2	02_H0067B	SRR9971110	South Africa	Southern Africa	Other AHRI KZN study	30	3	2017
2	02_H0076B	SRR9971307	South Africa	Southern Africa	Other AHRI KZN study	24	5	2017
2	02_H0083B	SRR9971009	South Africa	Southern Africa	Other AHRI KZN study	19	6	2017
2	03_F1021B	SRR11349208	South Africa	Southern Africa	Other AHRI KZN study	24	8	2015
2	03_F1144B	SRR9971271	South Africa	Southern Africa	Other AHRI KZN study	25	7	2016
2	03_H0107B	SRR9971217	South Africa	Southern Africa	Other AHRI KZN study	29	9	2017
2	03_P0040B	SRR9971305	South Africa	Southern Africa	PRAXIS (this study)	4	5	2017
2	03_P0050B	SRR9971087	South Africa	Southern Africa	PRAXIS (this study)	30	5	2017
2	03_P0072B	SRR9971011	South Africa	Southern Africa	PRAXIS (this study)	29	6	2017
2	03_P0082B	SRR9971019	South Africa	Southern Africa	PRAXIS (this study)	12	7	2017
2	03_R0001B	SRR9971076	South Africa	Southern Africa	Other AHRI KZN study	20	12	2016
2	04_H0098B	SRR9971018	South Africa	Southern Africa	Other AHRI KZN study	25	8	2017
2	04_H0106B	SRR9971214	South Africa	Southern Africa	Other AHRI KZN study	21	9	2017
2	04_R0009B	SRR9971064	South Africa	Southern Africa	Other AHRI KZN study	13	2	2017
2	04_R0026B	SRR9971109	South Africa	Southern Africa	Other AHRI KZN study	27	3	2017
2	04_R0030B	SRR9971107	South Africa	Southern Africa	Other AHRI KZN study	7	4	2017
2	04_R0036B	SRR9971108	South Africa	Southern Africa	Other AHRI KZN study	24	4	2017
2	04_R0042B	SRR9971054	South Africa	Southern Africa	Other AHRI KZN study	8	5	2017
2	04_R0043B	SRR9971304	South Africa	Southern Africa	Other AHRI KZN study	8	5	2017
2	04_R0045B	SRR9971037	South Africa	Southern Africa	Other AHRI KZN study	16	5	2017
2	04_R0055B	SRR9971313	South Africa	Southern Africa	Other AHRI KZN study	13	6	2017
2	05_H0027B	SRR9971266	South Africa	Southern Africa	Other AHRI KZN study	4	8	2016
2	05_H0030B	SRR9971268	South Africa	Southern Africa	Other AHRI KZN study	29	8	2016
2	05_H0074B	SRR9971309	South Africa	Southern Africa	Other AHRI KZN study	17	5	2017
2	05_H0111B	SRR9971144	South Africa	Southern Africa	Other AHRI KZN study	2	11	2017
2	05_P0082A	SRR9971143	South Africa	Southern Africa	PRAXIS (this study)	7	11	2017
2	05_R0056E	SRR9971016	South Africa	Southern Africa	Other AHRI KZN study	15	6	2017
2	05_R0057B	SRR9971015	South Africa	Southern Africa	Other AHRI KZN study	15	6	2017
2	05_R0059B	SRR9971010	South Africa	Southern Africa	Other AHRI KZN study	21	6	2017
2	05_R0060B	SRR9971012	South Africa	Southern Africa	Other AHRI KZN study	30	6	2017
2	05_R0061B	SRR9971005	South Africa	Southern Africa	Other AHRI KZN study	5	7	2017
2	05_R0064B	SRR9971022	South Africa	Southern Africa	Other AHRI KZN study	14	7	2017
2	05_R0074E	SRR9971025	South Africa	Southern Africa	Other AHRI KZN study	23	8	2017
2	05_R0076E	SRR9971223	South Africa	Southern Africa	Other AHRI KZN study	29	8	2017
2	05_R0087B	SRR9971218	South Africa	Southern Africa	Other AHRI KZN study	9	10	2017
2	05_R0091B	SRR9971208	South Africa	Southern Africa	Other AHRI KZN study	20	10	2017
2	05_R0092B	SRR9971149	South Africa	Southern Africa	Other AHRI KZN study	23	10	2017
2	05_R0094E	SRR11349190	South Africa	Southern Africa	Other AHRI KZN study	31	10	2017
2	05_R0095B	SRR9971145	South Africa	Southern Africa	Other AHRI KZN study	31	10	2017
2	05_R0098B	SRR9971142	South Africa	Southern Africa	Other AHRI KZN study	8	11	2017
2	06_H0032B	SRR9971265	South Africa	Southern Africa	Other AHRI KZN study	29	8	2016
2	06_H0035B	SRR9971264	South Africa	Southern Africa	Other AHRI KZN study	23	9	2016
2	06_H0037B	SRR9971267	South Africa	Southern Africa	Other AHRI KZN study	23	9	2016
2	06_H0042B	SRR9971078	South Africa	Southern Africa	Other AHRI KZN study	26	10	2016
2	06_H0070B	SRR9971116	South Africa	Southern Africa	Other AHRI KZN study	3	5	2017
2	06_H0080B	SRR9971306	South Africa	Southern Africa	Other AHRI KZN study	26	5	2017
2	06_H0086B	SRR9971020	South Africa	Southern Africa	Other AHRI KZN study	6	7	2017
2	06_L00501	SRR9971263	South Africa	Southern Africa	Other AHRI KZN study	6	10	2016
2	06_P0063B	SRR9971085	South Africa	Southern Africa	PRAXIS (this study)	8	6	2017
2	06_P0066B	SRR9971013	South Africa	Southern Africa	PRAXIS (this study)	13	6	2017
2	06_P00825	SRR9971254	South Africa	Southern Africa	PRAXIS (this study)	5	12	2017
2	06_P00826	SRR9971329	South Africa	Southern Africa	PRAXIS (this study)	5	1	2018
2	06_P0085B	SRR9971021	South Africa	Southern Africa	PRAXIS (this study)	19	7	2017
2	06_P01014	SRR9971255	South Africa	Southern Africa	PRAXIS (this study)	5	12	2017
2	06_P0128B	SRR9971220	South Africa	Southern Africa	PRAXIS (this study)	29	9	2017
2	06_P0129B	SRR9971219	South Africa	Southern Africa	PRAXIS (this study)	29	9	2017

2	07_K0005B	SRR9971330	South Africa	Southern Africa	Other AHRI KZN study	19	10	2017
2	07_K0007B	SRR9971148	South Africa	Southern Africa	Other AHRI KZN study	26	10	2017
2	08_H0006B	SRR9971262	South Africa	Southern Africa	Other AHRI KZN study	17	2	2016
2	08_H0021B	SRR9971269	South Africa	Southern Africa	Other AHRI KZN study	9	5	2016
2	08_H0046B	SRR9971079	South Africa	Southern Africa	Other AHRI KZN study	16	11	2016
2	08_H0056B	SRR9971249	South Africa	Southern Africa	Other AHRI KZN study	27	1	2017
2	08_H0088B	SRR9971006	South Africa	Southern Africa	Other AHRI KZN study	6	7	2017
2	08_H0123B	SRR9971324	South Africa	Southern Africa	Other AHRI KZN study	23	1	2018
2	08_H0124B	SRR9971321	South Africa	Southern Africa	Other AHRI KZN study	25	1	2018
2	08_H0125B	SRR9971322	South Africa	Southern Africa	Other AHRI KZN study	25	1	2018
2	08_P0169B	SRR9971257	South Africa	Southern Africa	PRAXIS (this study)	6	12	2017
2	08_P0176B	SRR9971327	South Africa	Southern Africa	PRAXIS (this study)	12	1	2018
2	08_P0179B	SRR9971325	South Africa	Southern Africa	PRAXIS (this study)	19	1	2018
2	08_R0025B	SRR9971112	South Africa	Southern Africa	Other AHRI KZN study	24	3	2017
2	08_R0066B	SRR9971024	South Africa	Southern Africa	Other AHRI KZN study	21	7	2017
2	08_R0067B	SRR9971023	South Africa	Southern Africa	Other AHRI KZN study	25	7	2017
2	09_H0096B	SRR9971222	South Africa	Southern Africa	Other AHRI KZN study	25	8	2017
2	09_H0122B	SRR9971323	South Africa	Southern Africa	Other AHRI KZN study	23	1	2018
2	09_H0129B	SRR9971029	South Africa	Southern Africa	Other AHRI KZN study	5	3	2018
2	09_P0200B	SRR9971028	South Africa	Southern Africa	PRAXIS (this study)	20	2	2018
2	09_R0040B	SRR9971115	South Africa	Southern Africa	Other AHRI KZN study	2	5	2017
2	09_R0069E	SRR9971154	South Africa	Southern Africa	Other AHRI KZN study	11	8	2017
2	09_R0075E	SRR9971017	South Africa	Southern Africa	Other AHRI KZN study	25	8	2017
2	10_H0054B	SRR9971058	South Africa	Southern Africa	Other AHRI KZN study	24	1	2017
2	10_H0065B	SRR9971111	South Africa	Southern Africa	Other AHRI KZN study	24	3	2017
2	10_H0133B	SRR9971034	South Africa	Southern Africa	Other AHRI KZN study	14	3	2018
2	10_H0134B	SRR9971035	South Africa	Southern Africa	Other AHRI KZN study	22	3	2018
2	10_H0136B	SRR9971088	South Africa	Southern Africa	Other AHRI KZN study	16	4	2018
2	10_H0138B	SRR9971089	South Africa	Southern Africa	Other AHRI KZN study	16	4	2018
2	10_K0025B	SRR9971030	South Africa	Southern Africa	Other AHRI KZN study	9	2	2018
2	10_K0026B	SRR9971031	South Africa	Southern Africa	Other AHRI KZN study	14	2	2018
2	10_R0099B	SRR9971156	South Africa	Southern Africa	Other AHRI KZN study	10	11	2017
2	10_R0100B	SRR9971155	South Africa	Southern Africa	Other AHRI KZN study	20	11	2017
2	11_H0001B	SRR9971260	South Africa	Southern Africa	Other AHRI KZN study	21	1	2016
2	11_H0119B	SRR9971259	South Africa	Southern Africa	Other AHRI KZN study	7	12	2017
2	11_H0141B	SRR9971353	South Africa	Southern Africa	Other AHRI KZN study	14	5	2018
2	11_K0009B	SRR9971256	South Africa	Southern Africa	Other AHRI KZN study	5	12	2017
2	11_K0010B	SRR11349182	South Africa	Southern Africa	Other AHRI KZN study	7	12	2017
2	11_K0013B	SRR9971328	South Africa	Southern Africa	Other AHRI KZN study	10	1	2018
2	11_K0021B	SRR11349180	South Africa	Southern Africa	Other AHRI KZN study	31	1	2018
2	11_P0155B	SRR9971147	South Africa	Southern Africa	PRAXIS (this study)	31	10	2017
2	11_P0165B	SRR9971253	South Africa	Southern Africa	PRAXIS (this study)	27	11	2017
2	12_F8103B	SRR9971354	South Africa	Southern Africa	Other AHRI KZN study	10	5	2018
2	12_K0057B	SRR9971356	South Africa	Southern Africa	Other AHRI KZN study	6	6	2018
2	12_P3004B	SRR9971355	South Africa	Southern Africa	PRAXIS (this study)	7	6	2018
2	12_P3008B	SRR9971357	South Africa	Southern Africa	PRAXIS (this study)	19	6	2018
2	12_P3010B	SRR9971350	South Africa	Southern Africa	PRAXIS (this study)	27	6	2018
2	13_F8064B	SRR9971308	South Africa	Southern Africa	Other AHRI KZN study	23	5	2017
2	13_F8078B	SRR9971213	South Africa	Southern Africa	Other AHRI KZN study	31	8	2017
2	13_K0036B	SRR9971032	South Africa	Southern Africa	Other AHRI KZN study	27	3	2018
2	13_K0040B	SRR9971033	South Africa	Southern Africa	Other AHRI KZN study	10	4	2018
2	13_K0045B	SRR9971352	South Africa	Southern Africa	Other AHRI KZN study	8	5	2018
2	13_K0060B	SRR9971349	South Africa	Southern Africa	Other AHRI KZN study	27	6	2018
2	13_K0062B	SRR9971140	South Africa	Southern Africa	Other AHRI KZN study	9	7	2018
2	14_F8102B	SRR9971351	South Africa	Southern Africa	Other AHRI KZN study	8	5	2018
2	14_H0161B	SRR11349173	South Africa	Southern Africa	Other AHRI KZN study	8	8	2018
2	14_H0166B	SRR9971211	South Africa	Southern Africa	Other AHRI KZN study	29	8	2018
2	14_K0016B	SRR9971326	South Africa	Southern Africa	Other AHRI KZN study	16	1	2018
2	14_P02006	SRR11349170	South Africa	Southern Africa	PRAXIS (this study)	3	8	2018
2	14_P3019B	SRR9971205	South Africa	Southern Africa	PRAXIS (this study)	24	8	2018
2	14_P3410B	SRR9971135	South Africa	Southern Africa	PRAXIS (this study)	18	7	2018
2	14_P3510B	SRR9971138	South Africa	Southern Africa	PRAXIS (this study)	28	6	2018
2	15_H0157B	SRR11349169	South Africa	Southern Africa	Other AHRI KZN study	24	7	2018
2	15_H0163B	SRR11349168	South Africa	Southern Africa	Other AHRI KZN study	16	8	2018
2	15_K0061B	SRR9971139	South Africa	Southern Africa	Other AHRI KZN study	3	7	2018
2	15_K0063B	SRR9971134	South Africa	Southern Africa	Other AHRI KZN study	17	7	2018

2	15_K0064B	SRR9971136	South Africa	Southern Africa	Other AHRI KZN study	18	7	2018
2	15_K0065B	SRR9971137	South Africa	Southern Africa	Other AHRI KZN study	19	7	2018
2	15_K0071B	SRR11349159	South Africa	Southern Africa	Other AHRI KZN study	24	8	2018
2	16_R0017B	SRR9971113	South Africa	Southern Africa	Other AHRI KZN study	10	3	2017
2	16_R0020B	SRR9971114	South Africa	Southern Africa	Other AHRI KZN study	15	3	2017
2	21_P3029B	SRR9971060	South Africa	Southern Africa	PRAXIS (this study)	5	10	2018
2	21_P3418B	SRR9971216	South Africa	Southern Africa	PRAXIS (this study)	25	9	2018
2	21_P35242	SRR9971062	South Africa	Southern Africa	PRAXIS (this study)	6	11	2018
2	21_P3524B	SRR9971210	South Africa	Southern Africa	PRAXIS (this study)	5	9	2018
2	22_H0039B	SRR9971080	South Africa	Southern Africa	Other AHRI KZN study	12	10	2016
2	22_K00562	SRR9971358	South Africa	Southern Africa	Other AHRI KZN study	18	6	2018
2	22_K00566	SRR9971141	South Africa	Southern Africa	Other AHRI KZN study	16	7	2018
2	22_K0077B	SRR9971209	South Africa	Southern Africa	Other AHRI KZN study	20	9	2018
2	22_P3415B	SRR9971206	South Africa	Southern Africa	PRAXIS (this study)	17	8	2018
2	23_H0183B	SRR11349151	South Africa	Southern Africa	Other AHRI KZN study	29	11	2018
2	23_H0184B	SRR9971248	South Africa	Southern Africa	Other AHRI KZN study	29	11	2018
2	23_H0190B	SRR9971247	South Africa	Southern Africa	Other AHRI KZN study	12	12	2018
2	23_K0079B	SRR9971215	South Africa	Southern Africa	Other AHRI KZN study	2	10	2018
2	23_K0085B	SRR9971059	South Africa	Southern Africa	Other AHRI KZN study	15	10	2018
2	ERR1034914	ERR1034914	South Africa	Southern Africa	Publicly available	Missing	Missing	Missing
2	ERR1633777	ERR1633777	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633791	ERR1633791	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633796	ERR1633796	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633800	ERR1633800	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633809	ERR1633809	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633816	ERR1633816	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633819	ERR1633819	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633822	ERR1633822	South Africa	Southern Africa	Publicly available	1	7	2010
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2	ERR1633831	ERR1633831	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633835	ERR1633835	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633844	ERR1633844	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633868	ERR1633868	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633872	ERR1633872	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633881	ERR1633881	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633882	ERR1633882	South Africa	Southern Africa	Publicly available	1	7	2010
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2	ERR1633895	ERR1633895	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1633896	ERR1633896	South Africa	Southern Africa	Publicly available	1	7	2010
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2	ERR1873389	ERR1873389	South Africa	Southern Africa	Publicly available	1	7	2009
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2	ERR1873394	ERR1873394	South Africa	Southern Africa	Publicly available	1	7	2009
2	ERR1873395	ERR1873395	South Africa	Southern Africa	Publicly available	1	7	2008
2	ERR1873397	ERR1873397	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1873398	ERR1873398	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1873399	ERR1873399	South Africa	Southern Africa	Publicly available	1	7	2010
2	ERR1873402	ERR1873402	South Africa	Southern Africa	Publicly available	1	7	2011
2	ERR1873403	ERR1873403	South Africa	Southern Africa	Publicly available	1	7	2011

2	ERR550739	ERR550739	South Africa	Southern Africa	Publicly available	1	7	2004
2	ERR551071	ERR551071	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR551079	ERR551079	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR551854	ERR551854	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR552743	ERR552743	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR553116	ERR553116	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR553156	ERR553156	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR553313	ERR553313	eSwatini	Southern Africa	Publicly available	1	7	2009
2	ERR553324	ERR553324	eSwatini	Southern Africa	Publicly available	1	7	2009
2	KR_F1051B	SRR9971075	South Africa	Southern Africa	Other AHRI KZN study	9	11	2015
2	KR_F1056B	SRR11349132	South Africa	Southern Africa	Other AHRI KZN study	16	11	2015
2	KR_F1061B	SRR11349207	South Africa	Southern Africa	Other AHRI KZN study	30	11	2015
2	KR_F1066B	SRR11349205	South Africa	Southern Africa	Other AHRI KZN study	9	12	2015
2	KR_F1067B	SRR11349204	South Africa	Southern Africa	Other AHRI KZN study	9	12	2015
2	KR_F1126B	SRR11349196	South Africa	Southern Africa	Other AHRI KZN study	30	5	2016
2	SRR1019146	SRR1019146	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1140900	SRR1140900	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1140901	SRR1140901	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1140916	SRR1140916	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1140928	SRR1140928	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1140940	SRR1140940	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1140947	SRR1140947	South Africa	Southern Africa	Publicly available	1	7	2013
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2	SRR1140964	SRR1140964	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1167167	SRR1167167	South Africa	Southern Africa	Publicly available	Missing	Missing	Missing
2	SRR1173130	SRR1173130	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1173289	SRR1173289	South Africa	Southern Africa	Publicly available	Missing	Missing	Missing
2	SRR1173814	SRR1173814	South Africa	Southern Africa	Publicly available	Missing	Missing	Missing
2	SRR1173850	SRR1173850	South Africa	Southern Africa	Publicly available	Missing	Missing	Missing
2	SRR1175054	SRR1175054	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1175358	SRR1175358	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180352	SRR1180352	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180357	SRR1180357	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180378	SRR1180378	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180385	SRR1180385	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180396	SRR1180396	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180398	SRR1180398	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180400	SRR1180400	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180404	SRR1180404	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180421	SRR1180421	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180427	SRR1180427	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180432	SRR1180432	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180434	SRR1180434	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1180442	SRR1180442	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1182970	SRR1182970	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1182987	SRR1182987	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1183012	SRR1183012	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1183048	SRR1183048	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1183082	SRR1183082	South Africa	Southern Africa	Publicly available	1	7	2011
2	SRR1183089	SRR1183089	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1183095	SRR1183095	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1183120	SRR1183120	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1183136	SRR1183136	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184296	SRR1184296	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184303	SRR1184303	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184307	SRR1184307	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184311	SRR1184311	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184318	SRR1184318	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184323	SRR1184323	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184330	SRR1184330	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184332	SRR1184332	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184338	SRR1184338	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184344	SRR1184344	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184364	SRR1184364	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184365	SRR1184365	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184370	SRR1184370	South Africa	Southern Africa	Publicly available	1	7	2013

2	SRR1184373	SRR1184373	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184376	SRR1184376	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184386	SRR1184386	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR1184387	SRR1184387	South Africa	Southern Africa	Publicly available	1	7	2013
2	SRR2101676	SRR2101676	South Africa	Southern Africa	Publicly available	18	5	2013
2	SRR6207273	SRR6207273	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR6207277	SRR6207277	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR6207279	SRR6207279	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR6207281	SRR6207281	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR6207285	SRR6207285	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR6207286	SRR6207286	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR832982	SRR832982	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR832985	SRR832985	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR832998	SRR832998	South Africa	Southern Africa	Publicly available	1	7	2008
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2	SRR833007	SRR833007	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR833010	SRR833010	South Africa	Southern Africa	Publicly available	1	7	2010
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2	SRR833057	SRR833057	South Africa	Southern Africa	Publicly available	1	7	2010
2	SRR833061	SRR833061	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR833092	SRR833092	South Africa	Southern Africa	Publicly available	1	7	2010
2	SRR833112	SRR833112	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR833133	SRR833133	South Africa	Southern Africa	Publicly available	1	7	2009
2	SRR833142	SRR833142	South Africa	Southern Africa	Publicly available	1	7	2010
2	SRR833143	SRR833143	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR833145	SRR833145	South Africa	Southern Africa	Publicly available	1	7	2010
2	SRR833147	SRR833147	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR833190	SRR833190	South Africa	Southern Africa	Publicly available	1	7	2010
2	SRR833192	SRR833192	South Africa	Southern Africa	Publicly available	1	7	2010
2	SRR847786	SRR847786	South Africa	Southern Africa	Publicly available	1	7	2012
2	SRR847799	SRR847799	South Africa	Southern Africa	Publicly available	1	7	2011
2	SRR958198	SRR958198	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR958231	SRR958231	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR958234	SRR958234	South Africa	Southern Africa	Publicly available	1	7	2008
2	SRR958237	SRR958237	South Africa	Southern Africa	Publicly available	1	7	2008

Supplementary Table 4. List of lineage 4 samples included in the extended Southern Africa dataset.

Lineage	Sample ID	Accession	Country	Study	Region	Day	Month	Year
4	01_C0067B	SRR9971303	South Africa	Other AHRI KZN study	Southern Africa	22	10	2013
4	01_C4027B	SRR9971302	South Africa	Other AHRI KZN study	Southern Africa	26	2	2014
4	01_F1045B	SRR11349211	South Africa	Other AHRI KZN study	Southern Africa	10	7	2015
4	01_F1049B	SRR11349210	South Africa	Other AHRI KZN study	Southern Africa	19	10	2015
4	01_F1064B	SRR9971298	South Africa	Other AHRI KZN study	Southern Africa	12	2	2015
4	01_F1098B	SRR11349186	South Africa	Other AHRI KZN study	Southern Africa	29	2	2016
4	01_P00271	SRR9971044	South Africa	PRAXIS (this study)	Southern Africa	22	3	2017
4	01_P00273	SRR9971043	South Africa	PRAXIS (this study)	Southern Africa	9	6	2017
4	01_P00274	SRR9971042	South Africa	PRAXIS (this study)	Southern Africa	21	6	2017
4	01_P0033B	SRR9971041	South Africa	PRAXIS (this study)	Southern Africa	16	3	2017
4	01_P0034B	SRR9971040	South Africa	PRAXIS (this study)	Southern Africa	29	3	2017
4	01_P0037B	SRR9971039	South Africa	PRAXIS (this study)	Southern Africa	12	4	2017
4	02_F1086B	SRR11349153	South Africa	Other AHRI KZN study	Southern Africa	2	1	2016
4	02_F1091B	SRR9971008	South Africa	Other AHRI KZN study	Southern Africa	2	8	2016
4	02_F1092B	SRR9971310	South Africa	Other AHRI KZN study	Southern Africa	2	10	2016
4	02_F1093B	SRR11349197	South Africa	Other AHRI KZN study	Southern Africa	2	10	2016
4	02_F1097B	SRR9971204	South Africa	Other AHRI KZN study	Southern Africa	24	2	2016
4	02_H0007B	SRR11349195	South Africa	Other AHRI KZN study	Southern Africa	17	2	2016
4	02_H0045B	SRR9971196	South Africa	Other AHRI KZN study	Southern Africa	11	10	2016
4	02_H0052B	SRR9971195	South Africa	Other AHRI KZN study	Southern Africa	19	12	2016
4	02_H0066B	SRR9971038	South Africa	Other AHRI KZN study	Southern Africa	29	3	2017
4	02_H0069B	SRR9971360	South Africa	Other AHRI KZN study	Southern Africa	21	4	2017
4	02_H0075B	SRR9971361	South Africa	Other AHRI KZN study	Southern Africa	24	5	2017
4	02_H0077B	SRR9971362	South Africa	Other AHRI KZN study	Southern Africa	24	5	2017
4	03_F1003B	SRR9971301	South Africa	Other AHRI KZN study	Southern Africa	22	6	2014
4	03_F1004B	SRR11349209	South Africa	Other AHRI KZN study	Southern Africa	20	6	2015
4	03_F1082B	SRR11349194	South Africa	Other AHRI KZN study	Southern Africa	25	1	2016
4	03_F1095B	SRR9971197	South Africa	Other AHRI KZN study	Southern Africa	22	2	2016
4	03_F1100B	SRR9971200	South Africa	Other AHRI KZN study	Southern Africa	29	2	2016
4	03_F1124B	SRR9971199	South Africa	Other AHRI KZN study	Southern Africa	25	5	2016
4	03_F1139B	SRR11349193	South Africa	Other AHRI KZN study	Southern Africa	11	7	2016
4	03_F1141B	SRR9971201	South Africa	Other AHRI KZN study	Southern Africa	20	7	2016
4	03_F1143B	SRR11349192	South Africa	Other AHRI KZN study	Southern Africa	20	7	2016
4	03_H0085B	SRR9971363	South Africa	Other AHRI KZN study	Southern Africa	21	6	2017
4	03_H0104B	SRR9971364	South Africa	Other AHRI KZN study	Southern Africa	21	9	2017
4	03_P00056	SRR9971365	South Africa	PRAXIS (this study)	Southern Africa	10	7	2017
4	03_P0005B	SRR9971366	South Africa	PRAXIS (this study)	Southern Africa	23	1	2017
4	03_P0017B	SRR9971367	South Africa	PRAXIS (this study)	Southern Africa	7	2	2017
4	03_P0051B	SRR9971368	South Africa	PRAXIS (this study)	Southern Africa	30	5	2017
4	03_P0061B	SRR9971369	South Africa	PRAXIS (this study)	Southern Africa	8	6	2017
4	03_P0078B	SRR9971186	South Africa	PRAXIS (this study)	Southern Africa	11	7	2017
4	03_P0080B	SRR9971345	South Africa	PRAXIS (this study)	Southern Africa	10	7	2017
4	03_P0081B	SRR9971188	South Africa	PRAXIS (this study)	Southern Africa	12	7	2017
4	03_P0090B	SRR9971187	South Africa	PRAXIS (this study)	Southern Africa	1	8	2017
4	03_P0106B	SRR9971190	South Africa	PRAXIS (this study)	Southern Africa	30	8	2017
4	03_P0117B	SRR9971189	South Africa	PRAXIS (this study)	Southern Africa	21	9	2017
4	04_H0102B	SRR9971192	South Africa	Other AHRI KZN study	Southern Africa	7	9	2017
4	04_H0109B	SRR9971191	South Africa	Other AHRI KZN study	Southern Africa	11	10	2017
4	04_H0110B	SRR9971194	South Africa	Other AHRI KZN study	Southern Africa	24	10	2017
4	04_P00276	SRR9971193	South Africa	PRAXIS (this study)	Southern Africa	18	8	2017
4	04_P0097B	SRR11349191	South Africa	PRAXIS (this study)	Southern Africa	16	8	2017
4	04_P0098B	SRR9971056	South Africa	PRAXIS (this study)	Southern Africa	21	8	2017
4	04_P0101B	SRR9970995	South Africa	PRAXIS (this study)	Southern Africa	22	8	2017
4	04_P0119B	SRR9970996	South Africa	PRAXIS (this study)	Southern Africa	22	9	2017
4	04_P0121B	SRR9971057	South Africa	PRAXIS (this study)	Southern Africa	27	9	2017
4	04_P0133B	SRR9970994	South Africa	PRAXIS (this study)	Southern Africa	5	10	2017
4	04_P0139B	SRR9970999	South Africa	PRAXIS (this study)	Southern Africa	16	10	2017
4	04_P0141B	SRR9971000	South Africa	PRAXIS (this study)	Southern Africa	18	10	2017
4	04_P0152B	SRR9970997	South Africa	PRAXIS (this study)	Southern Africa	31	10	2017
4	04_P0156B	SRR9970998	South Africa	PRAXIS (this study)	Southern Africa	31	10	2017

4	04_R0003B	SRR9971168	South Africa	Other AHRI KZN study	Southern Africa	1	2	2017
4	04_R0005B	SRR9971167	South Africa	Other AHRI KZN study	Southern Africa	3	2	2017
4	04_R0041B	SRR9971162	South Africa	Other AHRI KZN study	Southern Africa	4	5	2017
4	04_R0051B	SRR9971161	South Africa	Other AHRI KZN study	Southern Africa	30	5	2017
4	04_R0052B	SRR9971160	South Africa	Other AHRI KZN study	Southern Africa	5	6	2017
4	05_H0015B	SRR9971125	South Africa	Other AHRI KZN study	Southern Africa	4	5	2016
4	05_H0033B	SRR9971130	South Africa	Other AHRI KZN study	Southern Africa	15	9	2016
4	05_H0079B	SRR9971150	South Africa	Other AHRI KZN study	Southern Africa	26	5	2017
4	05_H0115B	SRR9971319	South Africa	Other AHRI KZN study	Southern Africa	23	11	2017
4	05_P0002B	SRR9971131	South Africa	PRAXIS (this study)	Southern Africa	13	12	2016
4	05_P0044B	SRR9971315	South Africa	PRAXIS (this study)	Southern Africa	19	5	2017
4	05_R0062B	SRR9971071	South Africa	Other AHRI KZN study	Southern Africa	7	7	2017
4	05_R0071B	SRR9971069	South Africa	Other AHRI KZN study	Southern Africa	15	8	2017
4	05_R0073B	SRR9971388	South Africa	Other AHRI KZN study	Southern Africa	21	8	2017
4	05_R0077B	SRR9971389	South Africa	Other AHRI KZN study	Southern Africa	30	8	2017
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