



Mosquitoes provide a transmission route between possums and humans for Buruli ulcer in southeastern Australia

In the format provided by the authors and unedited

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Supplementary Table 1 | Male mosquitoes collected and screened for *M. ulcerans* on the Mornington Peninsula.

Species	No. collected/No. screened (No. positive for <i>M. ulcerans</i>)					Total
	Dec 16- Apr 17	Nov 17- May 18	Dec 18- May 19	Nov 19- Mar 20	Feb 21- Mar 21	
<i>Ae. notoscriptus</i>		2/2	7/7	44/24	5/5	58/38
<i>Ae. rubrithorax</i>			1/1			1/1
<i>Cq. linealis</i>	9/9	24/24		67/17		100/50
<i>Cx. australicus</i>		11/11	2/2	303/16		316/29
<i>Cx. globocoxitus</i>				619/175		619/175
<i>Cx. molestus</i>	1/1		7/7	69/14	2/2	79/24
<i>Cx. quinquefasciatus</i>		32/32	1/1	108/76		141/109
<i>Tp. tasmaniensis</i>				3/3		3/3
Total	10/10	69/69	18/18	1,213/325	7/7	1,317/429

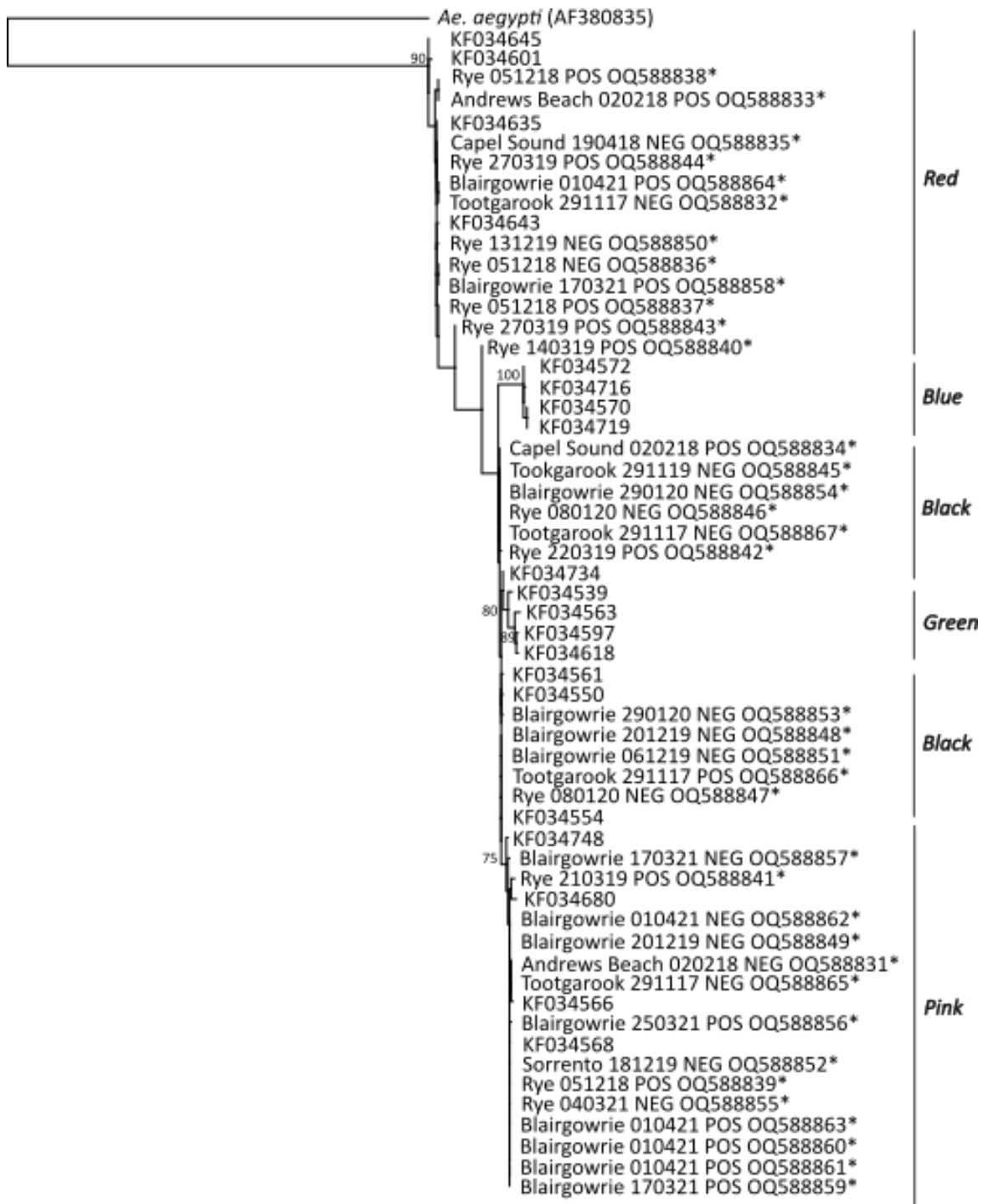
Supplementary Table 2 | *Mycobacterium ulcerans* qPCR testing results for positive IS2404, IS2606 and KR in *Ae. notoscriptus*, *Ae. camptorhynchus* and *C. hilli*.

Species (season collected)	Location	qPCR assay			<i>M. ulcerans</i> genome equivalents/mosquito ¹
		IS2404	IS2606	KR	
<i>Ae. notoscriptus</i> (16/17)	Sorrento	33.64	35.18	34.94	401
<i>Ae. notoscriptus</i> (16/17)	Tootgarook	36.41	-	-	76
<i>Ae. notoscriptus</i> (17/18)	Capel Sound	38.21	-	-	26
<i>Ae. notoscriptus</i> (17/18)	Capel Sound	36.34	NT ²	-	79
<i>Ae. notoscriptus</i> (17/18)	Capel Sound	38.27	-	-	25
<i>Ae. notoscriptus</i> (17/18)	Capel Sound	36.89	-	-	57
<i>Ae. notoscriptus</i> (17/18)	St Andrews Beach	35.02	-	-	175
<i>Ae. notoscriptus</i> (17/18)	Tootgarook	32.62	NT	32.18	740
<i>Ae. notoscriptus</i> (17/18)	Tootgarook	38.22	NT	-	25
<i>Ae. notoscriptus</i> (17/18)	Tootgarook	33.40	-	34.76	463
<i>Ae. notoscriptus</i> (18/19)	Rye	36.79	NT	32.31	60
<i>Ae. notoscriptus</i> (18/19)	Rye	36.67	NT	35.91	65
<i>Ae. notoscriptus</i> (18/19)	Rye	36.06	NT	-	93
<i>Ae. notoscriptus</i> (18/19)	Rye	35.56	39.371	34.95	126
<i>Ae. notoscriptus</i> (18/19)	Rye	36.34	NT	35.61	79
<i>Ae. notoscriptus</i> (18/19)	Rye	36.07	38.01	35.41	93
<i>Ae. notoscriptus</i> (18/19)	Rye	33.70	32.49	31.62	386
<i>Ae. notoscriptus</i> (18/19)	Rye	29.74	38.36	33.36	4183
<i>Ae. notoscriptus</i> (18/19)	Rye	31.51	38.90	33.67	1443
<i>Ae. notoscriptus</i> (18/19)	Rye	36.89	NT	-	57
<i>Ae. notoscriptus</i> (18/19)	Rye	33.32	39.00	35.92	486
<i>Ae. notoscriptus</i> (18/19)	Rye	37.25	-	35.80	46
<i>Ae. notoscriptus</i> (18/19)	Rye	35.11	-	34.19	165
<i>Ae. notoscriptus</i> (18/19)	Rye	36.44	40.07	35.02	74
<i>Ae. notoscriptus</i> (18/19)	Rye	35.27	45.00	35.49	150
<i>Ae. notoscriptus</i> (19/20)	Blairgowrie	35.60	37.90	33.02	123
<i>Ae. notoscriptus</i> (19/20)	Blairgowrie	38.56	39.89	-	21
<i>Ae. notoscriptus</i> (19/20)	Blairgowrie	37.45	38.58	35.23	40
<i>Ae. notoscriptus</i> (19/20)	Rye	38.11	39.96	38.86	27
<i>Ae. notoscriptus</i> (19/20)	Rye	35.14	39.55	-	163
<i>Ae. notoscriptus</i> (19/20)	Rye	39.15	-	-	15
<i>Ae. notoscriptus</i> (19/20)	Rye	33.02	35.18	32.44	582
<i>Ae. notoscriptus</i> (19/20)	Rye	36.76	37.03	35.15	61
<i>Ae. notoscriptus</i> (19/20)	Rye	37.96	38.67	33.48	30
<i>Ae. notoscriptus</i> (19/20)	Rye	36.09	34.62	32.86	92
<i>Ae. notoscriptus</i> (19/20)	Rye	37.86	39.25	39.26	32
<i>Ae. notoscriptus</i> (19/20)	Rye	36.51	36.79	34.07	71
<i>Ae. notoscriptus</i> (19/20)	Sorrento	37.73	37.08	34.86	34
<i>Ae. notoscriptus</i> (19/20)	Sorrento	38.51	38.80	36.34	21
<i>Ae. notoscriptus</i> (20/21)	Blairgowrie	33.47	34.52	29.36	489
<i>Ae. notoscriptus</i> (20/21)	Blairgowrie	34.15	35.05	30.10	295
<i>Ae. notoscriptus</i> (20/21)	Blairgowrie	38.64	40.21	35.24	20
<i>Ae. notoscriptus</i> (20/21)	Blairgowrie	31.20	33.26	28.52	1738
<i>Ae. notoscriptus</i> (20/21)	Blairgowrie	39.65	-	-	11
<i>Ae. notoscriptus</i> (20/21)	Rye	37.50	-	NT	39
<i>Ae. notoscriptus</i> (20/21)	Rye	37.48	-	-	40
<i>Ae. camptorhynchus</i> (19/20)	Sorrento	41.21	43.10	35.25	
<i>C. hilli</i> (18/19)	Rye	35.9215	34.69	-	
<i>C. hilli</i> (18/19)	Rye	37.541	-	38.26	

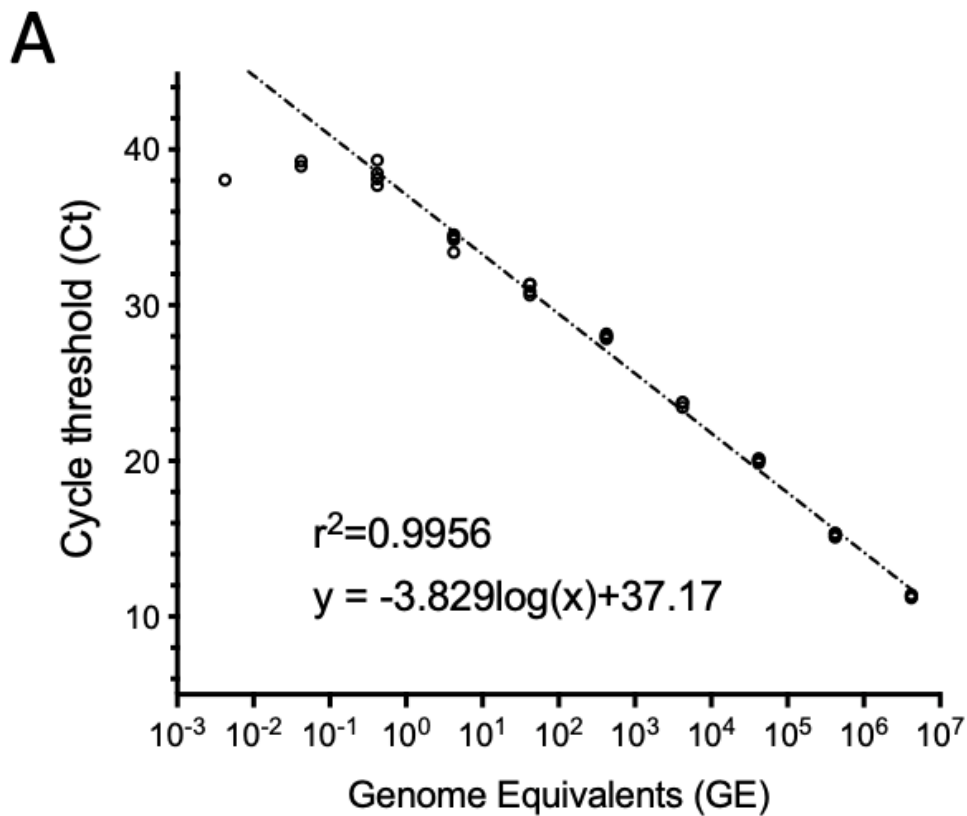
¹ Note that insect DNA was eluted in 100uL and 2uL was used for qPCR; ² NT = Not tested

Supplementary Table 3 | Breakdown of the number of individual or pools of female *Ae. notoscriptus* that were screened each year.

Year	Screening size	No. individuals or pools	Number positive
2016-17	Individuals screened	4	1
	Pools <15 (total no <i>Ae. notoscriptus</i> screened in pools 170)	23	1
2017-18	Individuals screened	367	8
	Pools <15	0	0
2018-19	Individuals screened	112	3
	Pools <15 (total no <i>Ae. notoscriptus</i> screened in pools 1667)	341	12
2019-20	Individuals screened	448	3
	Pools <15 (total no <i>Ae. notoscriptus</i> screened in pools 3882)	383	11
2021	Individuals screened	1247	7
	Pools <15	0	0



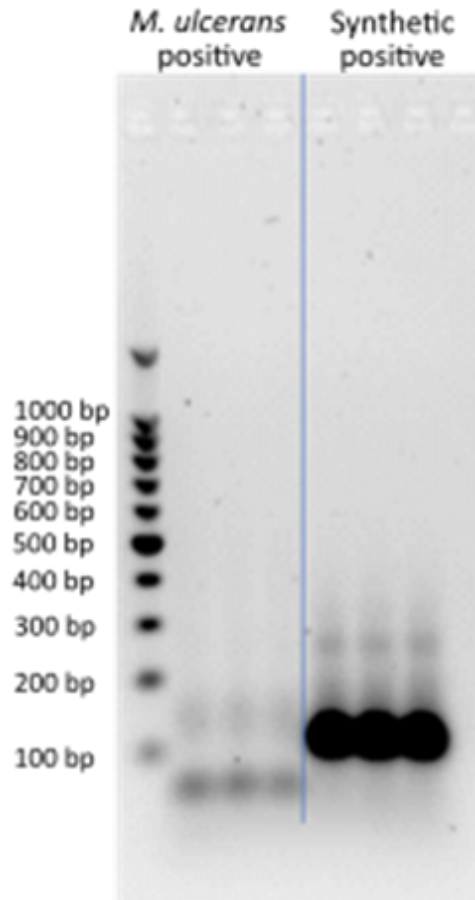
Supplementary Fig. 1 | Maximum likelihood phylogenetic tree based on an 871 bp region of the COI gene. Labelling indicates capture location, collection date, *M. ulcerans* infection status and accession number. Clades are labelled based on Endersby et al. 2013, with four well-supported clades (blue, red, green and pink) and a fifth poorly supported clade (black). Insects from the blue clade had previously been collected in the subtropics of Queensland (QLD), green in the tropics of QLD, pink in temperate southern Australia, red from a broad distribution along the east coast and black from New Zealand and south-eastern Australia. The Hasegawa-Kishino-Yano (HKY) substitution model was used with 1,000 bootstrap replicates. Bootstrap proportions (BSP \geq 70%) are indicated alongside the nodes. The number of nucleotide substitutions per site is represented by the scale bar. *Aedes aegypti* was used as an outgroup. * DNA Sequences generated in this study.



B

gDNA conc. (ng/ μ L)	Genome Equivalents	Rep 1	Rep 2	Rep 3	Rep 4
12.1	4200000	11.34	11.2	11.4	11.27
1.2	420000	15.09	15.17	15.35	15.35
0.12	42000	19.87	20.02	20.05	20.15
0.012	4200	23.75	23.74	23.44	-
0.0012	420	27.95	28.02	27.84	28.16
0.00012	42	30.89	31.34	31.35	30.66
0.000012	4.2	34.38	33.4	34.53	34.21
0.0000012	0.42	39.32	37.68	38.47	38.12

Supplementary Fig. 2 | IS2404 qPCR standard curve. (A) Plot of 10-fold dilution series of *M. ulcerans* genomic DNA tested by IS2404 Taqman real-time PCR (each dilution tested in quadruplicate). The x-axis is \log_{10} scale. (B) Cycle-threshold (Ct) values for each genomic DNA dilution used to prepare the plot in (A). Results for four independent qPCR reactions are shown.



Supplementary Fig. 3 | IS2404 qPCR synthetic positive control. Comparison of the size of the qPCR amplicon (electrophoresis on 1% agarose gel) between a real *M. ulcerans* positive control using genomic DNA and the synthetic DNA positive control used for the screening assays.