

**Table SM1:** *Culicoides* vector collection sites, province and geographical location with regards to interface and animals associated as well as sampling period.

<b>Site</b>	<b>Province</b>	<b>Animals near light trap</b>	<b>Sampling period</b>
<b>Hluvukani (Mnisi)</b>	Mpumalanga	Humans, dogs, pigs	2015-2017
<b>Hlalahle/ Ludlow (Mnisi)</b>		Cattle, donkeys, small livestock	2015/ 2016-2017
<b>Manyeleti/ HHWS (KNP)</b>		Wildlife, humans	2015/2016-2017
<b>Lapalala Wilderness</b>	Limpopo	Wildlife, humans	Sep 2011-2017
<b>Marakele Natural Park</b>		Wildlife, humans	Nov 2011-2017

HHWS: Hans Hoheisen Wildlife Research Station; KNP: Kruger National Park

**Table SM2:** Summary of *Culicoides* collections per site, including identifications inferred from molecular samples. ( $\bar{x}$ ): mean catch per trap per night; # spp.: number of species present per site from morphologically identified subsamples; #BF: number of bloodfed females collected per site; #CytB (%): number of successful CytB sequences (success rate); # Host ID: number of different hosts identified from CytB sequences; # COI (%): number of successful COI sequences (success rate); # Culi ID: Number of *Culicoides* species molecularly identified (number of unidentified specimens).

Site	( $\bar{x}$ )	# spp.	# BF	# CytB (%)	# Host ID	# COI (%)	# Culi ID
<b>Mpumulanga</b>							
<b>KNP</b>	580	20	49	12 (24,5%)	5	9 (18,4%)	3 (1*)
<b>Mnisi</b>	836	12	30	25 (83,3%)	4	10 (33,3%)	7 (2)
<b>Limpopo</b>							
<b>Marekele</b>	567	9	30	16 (53,3%)	6	15 (50%)	6 (1)
<b>Lapalala</b>	285	2	33	21 (63,6%)	5	20 (60,6%)	3 (2)
		<b>25</b>	<b>142</b>	<b>74 (52,1%)</b>	<b>15</b>	<b>54 (38%)</b>	<b>12 (5)</b>
<b>*non-biting midge</b>							

**Table SM 3:** Taxonomic and locality information of sequences used in phylogenetic analysis. Bold accession numbers are sequences generated for this study.

Species (lab_ref)	Acc_Number	Origin_specimen_locality	Country
C. bedfordi (MN71.1)	<b>MN329648</b>	Mpumalanga, Hluvukani	RSA
C. bedfordi (ref_1)	<b>MN329588</b>	Gauteng, Onderstepoort	RSA
C. bedfordi (ref_2)	<b>MN329589</b>	Gauteng, Onderstepoort	RSA
C. bedfordi (ref_3)	<b>MN329590</b>	Gauteng, Onderstepoort	RSA
C. bedfordi (ref_4)	<b>MN329591</b>	Gauteng, Onderstepoort	RSA
C. bolitinos	KJ162961.1	Western Cape, Hoekwil	RSA
C. bolitinos	KJ162962.1	Western Cape, Hoekwil	RSA
C. bolitinos	KT339686.1	Uncertain	Uncertain
C. bolitinos	MF399809.1	Kwazulu-Natal	RSA
C. coarctatus	KY933274.1	Eastern Cape, Grahamstown	RSA
C. coarctatus	KY933275.1	Eastern Cape, Grahamstown	RSA
C. coarctatus (MN92.2)	<b>MN329660</b>	Mpumalanga, Hluvukani	RSA
C. cornutus	KY933281.1	Eastern Cape, Grahamstown	RSA
C. cornutus	KY933282.1	Eastern Cape, Grahamstown	RSA
C. cornutus	KY933283.1	Eastern Cape, Grahamstown	RSA
C. enderleini (KNP2)	<b>MN329609</b>	Manyeleti Game Reserve	RSA
C. enderleini (KNP5)	<b>MN329610</b>	Manyeleti Game Reserve	RSA
C. enderleini (KNP6)	<b>MN329611</b>	Manyeleti Game Reserve	RSA
C. enderleini (MAR395.3)	<b>MN329647</b>	Marekele National Park	RSA
C. enderleini (MN79.2)	<b>MN329653</b>	Mpumalanga, Hluvukani	RSA
C. enderleini (MN83.1)	<b>MN329655</b>	Mpumalanga, Hluvukani	RSA
C. enderleini (MN83.5)	<b>MN329656</b>	Mpumalanga, Hluvukani	RSA
C. enderleini (MN93.1)	<b>MN329661</b>	Mpumalanga, Hluvukani	RSA
C. enderleini (MN93.4)	<b>MN329662</b>	Mpumalanga, Hluvukani	RSA
C. gulbenkiani	KJ162978.1	Western Cape, Hoekwil	RSA
C. gulbenkiani	MF399760.1	Kitui, Munukathi	Kenya
C. gulbenkiani	MK760129.1	Western Cape, Hoekwil	RSA
C. gulbenkiani	MK760132.1	Western Cape, Hoekwil	RSA
C. imicola	KJ162982.1	Mpumalanga, Skukuza	RSA
C. imicola	KJ162983.1	Mpumalanga, Skukuza	RSA
C. imicola	KT339739.1	Uncertain	Uncertain
C. imicola (GAU110)	<b>MN329608</b>	Gauteng, Boschkop	RSA
C. imicola (LAP276.1)	<b>MN329613</b>	Lapalala Wilderness	RSA
C. imicola (LAP276.2)	<b>MN329614</b>	Lapalala Wilderness	RSA
C. imicola (LAP277.3)	<b>MN329617</b>	Lapalala Wilderness	RSA
C. imicola (LAP277.5)	<b>MN329618</b>	Lapalala Wilderness	RSA
C. imicola (LAP288.1)	<b>MN329619</b>	Lapalala Wilderness	RSA
C. imicola (LAP288.5)	<b>MN329621</b>	Lapalala Wilderness	RSA
C. imicola (LAP288.6)	<b>MN329622</b>	Lapalala Wilderness	RSA
C. imicola (LAP288.7)	<b>MN329623</b>	Lapalala Wilderness	RSA
C. imicola (LAP288.8)	<b>MN329624</b>	Lapalala Wilderness	RSA
C. imicola (LAP293.2)	<b>MN329626</b>	Lapalala Wilderness	RSA
C. imicola (LAP299)	<b>MN329627</b>	Lapalala Wilderness	RSA
C. imicola (LAP304.2)	<b>MN329629</b>	Lapalala Wilderness	RSA
C. imicola (LAP306)	<b>MN329630</b>	Lapalala Wilderness	RSA
C. imicola (LAP311.2)	<b>MN329631</b>	Lapalala Wilderness	RSA
C. imicola (MAR216.1)	<b>MN329633</b>	Marekele National Park	RSA
C. imicola (MAR216.2)	<b>MN329634</b>	Marekele National Park	RSA
C. imicola (MAR216.3)	<b>MN329635</b>	Marekele National Park	RSA
C. imicola (MAR285)	<b>MN329638</b>	Marekele National Park	RSA

C. imicola (MAR299)	<b>MN329639</b>	Marekele National Park	RSA
C. imicola (MAR395.1)	<b>MN329646</b>	Marekele National Park	RSA
C. imicola (MN86)	<b>MN329658</b>	Mpumalanga, Hluvukani	RSA
C. imicola (MN87.1)	<b>MN329659</b>	Mpumalanga, Hluvukani	RSA
C. imicola (ref_1)	<b>MN329592</b>	Gauteng, Onderstepoort	RSA
C. imicola (ref_2)	<b>MN329593</b>	Gauteng, Onderstepoort	RSA
C. imicola (ref_4)	<b>MN329594</b>	Gauteng, Onderstepoort	RSA
C. imicola (ref_5)	<b>MN329595</b>	Gauteng, Onderstepoort	RSA
C. imicola (ref_6)	<b>MN329596</b>	Gauteng, Onderstepoort	RSA
C. isioloensis	MF399799.1	Eastern Cape, Port Elizabeth	RSA
C. isioloensis	MF399800.1	Eastern Cape, Port Elizabeth	RSA
C. kanagai	MF399811.1	Mpumalanga, Kruger NP	RSA
C. kanagai	MK760180.1	Mpumalanga, Berg-en-Dal	RSA
C. leucosticus (MAR356.1)	<b>MN329640</b>	Marekele National Park	RSA
C. leucosticus (ref_1)	<b>MN329597</b>	Gauteng, Onderstepoort	RSA
C. leucosticus (ref_2)	<b>MN329598</b>	Gauteng, Onderstepoort	RSA
C. leucosticus (ref_3)	<b>MN329599</b>	Gauteng, Onderstepoort	RSA
C. leucosticus (ref_4)	<b>MN329600</b>	Gauteng, Onderstepoort	RSA
C. leucosticus (ref_5)	<b>MN329601</b>	Gauteng, Onderstepoort	RSA
C. loxodontis	AF069236.2	Limpopo Province, Phalaborwa	RSA
C. loxodontis	KJ162988.1	Mpumalanga, Skukuza	RSA
C. loxodontis	MF399807.1	Mpumalanga, Kruger NP	RSA
C. loxodontis	MF399808.1	Mpumalanga, Kruger NP	RSA
C. loxodontis	MK760191.1	Mpumalanga, Berg-en-Dal	RSA
C. loxodontis (MN79.3)	<b>MN329654</b>	Mpumalanga, Hluvukani	RSA
C. magnus (ref_1)	<b>MN329602</b>	Gauteng, Onderstepoort	RSA
C. magnus (ref_2)	<b>MN329603</b>	Gauteng, Onderstepoort	RSA
C. magnus (ref_3)	<b>MN329604</b>	Gauteng, Onderstepoort	RSA
C. neavei	MF399805.1	Gauteng, Pretoria	RSA
C. ovalis	MF399806.1	Mpumalanga, Nelspruit	RSA
C. pycnostictus (MAR258.2)	<b>MN329636</b>	Marekele National Park	RSA
C. pycnostictus (MAR368)	<b>MN329642</b>	Marekele National Park	RSA
C. pycnostictus (MN71.2)	<b>MN329649</b>	Mpumalanga, Hluvukani	RSA
C. pycnostictus (ref_1)	<b>MN329605</b>	Gauteng, Onderstepoort	RSA
C. pycnostictus (ref_2)	<b>MN329606</b>	Gauteng, Onderstepoort	RSA
C. pycnostictus (ref_3)	<b>MN329607</b>	Gauteng, Onderstepoort	RSA
C. ravus	MF399678.1	Harare, Malipati	Zimbabwe
C. ravus	MF399679.1	Harare, Malipati	Zimbabwe
C. schultzei	MF399697.1	Maputo, Maputo	Mozambique
C. schultzei	MF399699.1	Maputo, Maputo	Mozambique
C. similis	MF399801.1	Eastern Cape, Port Elizabeth	RSA
C. similis	MF399802.1	Eastern Cape, Port Elizabeth	RSA
C. sp (MAR356.2)	<b>MN329641</b>	Marekele National Park	RSA
C. sp.	KY933263.1	Eastern Cape, Grahamstown	RSA
C. sp.	KY933264.1	Eastern Cape, Grahamstown	RSA
C. sp.	KY933266.1	Eastern Cape, Grahamstown	RSA
C. sp.	KY933269.1	Eastern Cape, Grahamstown	RSA
C. sp. #107	AF069237.2	Limpopo Province, Ellisras	RSA
C. sp. #107	AF069240.2	Limpopo Province, Ellisras	RSA
C. sp. #107	KJ162986.1	KwaZulu-Natal, Hluhluwe	RSA
C. sp. #107	KJ162987.1	KwaZulu-Natal, Hluhluwe	RSA
C. sp. #107	MF399803.1	Gauteng, Pretoria	RSA
C. sp. #107	MF399804.1	Gauteng, Pretoria	RSA
C. sp. #107 (LAP288.4)	<b>MN329620</b>	Lapalala Wilderness	RSA

C. sp. #107 (LAP304.1)	<b>MN329628</b>	Lapalala Wilderness	RSA
C. sp. #54 d/f (MAR371.3)	<b>MN329643</b>	Marekele National Park	RSA
C. sp. #54 df	MF399674.1	Harare, Swimuwini	Zimbabwe
C. sp. #54 df	MF399795.1	Eastern Cape, Port Elizabeth	RSA
C. sp. (LAP277.1)	<b>MN329615</b>	Lapalala Wilderness	RSA
C. sp. (LAP293.1)	<b>MN329625</b>	Lapalala Wilderness	RSA
C. sp. (MAR277.2)	<b>MN329637</b>	Marekele National Park	RSA
C. sp. (MN71.3)	<b>MN329650</b>	Mpumalanga, Hluvukani	RSA
C. sp. (MN76.1)	<b>MN329651</b>	Mpumalanga, Hluvukani	RSA
C. subschultzei	MF399704.1	Maputo, Maputo	Mozambique
C. subschultzei	MF399705.1	Maputo, Maputo	Mozambique
C. subschultzei (LAP277.2)	<b>MN329616</b>	Lapalala Wilderness	RSA
C. subschultzei (LAP457.6)	<b>MN329632</b>	Lapalala Wilderness	RSA
C. subschultzei (MAR372.1)	<b>MN329644</b>	Marekele National Park	RSA
C. subschultzei (MAR372.3)	<b>MN329645</b>	Marekele National Park	RSA
C. tororoensis	MK760248.1	Mpumalanga, Berg-en-Dal	RSA
C. tororoensis	MK760249.1	Mpumalanga, Berg-en-Dal	RSA
C. tororoensis	MK760250.1	Mpumalanga, Berg-en-Dal	RSA
C. tropicalis	MF399797.1	Eastern Cape, Port Elizabeth	RSA
C. tropicalis	MF399798.1	Eastern Cape, Port Elizabeth	RSA
C. tropicalis (MN78.2)	<b>MN329652</b>	Mpumalanga, Hluvukani	RSA
C. tuttifrutti	AF069243.2	Northern Cape, Kimberley	RSA
C. tuttifrutti	AF069244.1	Northern Cape, Kimberley	RSA
C. tuttifrutti	AF069246.2	Northern Cape, Kimberley	RSA
C. tuttifrutti	KJ162998.1	KwaZulu-Natal, Ramsgate	RSA
C. tuttifrutti	KJ162999.1	KwaZulu-Natal, Ramsgate	RSA
C. tuttifrutti	MF399675.1	Harare, Malipati	Zimbabwe
C. tuttifrutti	MF399676.1	Harare, Malipati	Zimbabwe
C. tuttifrutti	MF399677.1	Harare, Malipati	Zimbabwe
C. tuttifrutti	MF399810.1	Gauteng, Pretoria	RSA
C. tuttifrutti	MK760251.1	Gauteng, Onderstepoort	RSA
C. tuttifrutti (MN84.9)	<b>MN329657</b>	Mpumalanga, Hluvukani	RSA
C. zuluensis	MF399793.1	Eastern Cape, Port Elizabeth	RSA
C. zuluensis	MF399794.1	Eastern Cape, Port Elizabeth	RSA
Chironomidae (KNP8)	<b>MN329612</b>	Manyeleti Game Reserve	RSA

**Table SM4:** *Culicoides* species identification, accession number, collection date and phylogenetic analyses data and BLAST results. Bloodmeal host results are also indicated.

ID	Location	Collection date	Species	DDN (bs)	ML (bs)	MrB (pp)	BLAST (%_ID)	Host blood BLAST	Notes
MN 71.1	Mnisi	2016 Oct	<i>C. bedfordi</i>	100	100	1	98.84	Kurrichane thrush ( <i>Turdus libonyana</i> )	
MN 92.2	Mnisi	2017 May	<i>C. coarctatus</i>	100	100	1	99.68	Cattle ( <i>Bos taurus</i> )	
KNP 5	KNP	2016 Apr	<i>C. enderleini</i>	100	96	1	99.84	Buffalo ( <i>Syncerus caffer</i> )	
MN 83.5	KNP	2017 Feb	<i>C. enderleini</i>	90	96	1	-	Buffalo ( <i>Syncerus caffer</i> )	
MN 93.1	Mnisi	2017 May	<i>C. enderleini</i>	100	96	1	99.68	Cattle ( <i>Bos taurus</i> )	
MN 93.4	Mnisi	2017 May	<i>C. enderleini</i>	100	96	1	99.52	Cattle ( <i>Bos taurus</i> )	
MN 83.1	KNP	2017 Feb	<i>C. enderleini</i>	90	96	1	-	Elephant ( <i>Loxodonta africana</i> )	
KNP 2	KNP	2016 Apr	<i>C. enderleini</i>	100	96	1	99.52	Hippo ( <i>Hippopotamus amphibius</i> )	
KNP 6	KNP	2016 Apr	<i>C. enderleini</i>	100	96	1	99.36	Hippo ( <i>Hippopotamus amphibius</i> )	
MN 79.2	KNP	2017 Jan	<i>C. enderleini</i>	100	96	1	98.87	Hippo ( <i>Hippopotamus amphibius</i> )	
MAR 395.3	Marekele	2015 Feb	<i>C. enderleini</i>	100	96	1	98.87	Hippopotamus ( <i>Hippopotamus amphibius</i> )	
LAP 229	Lapalala	2014 Jan	<i>C. imicola</i>	99	94	1	99.84	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
LAP 288.5	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	100.00	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
LAP 288.6	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	100.00	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
LAP 293.2	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	100.00	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
LAP 304.2	Lapalala	2014 Apr	<i>C. imicola</i>	99	94	1	99.84	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
MAR 285	Marekele	2014 Apr	<i>C. imicola</i>	99	94	1	99.52	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
MAR 299	Marekele	2014 May	<i>C. imicola</i>	99	94	1	98.23	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
MN 86	KNP	2017 Mar	<i>C. imicola</i>	99	94	1	-	Buffalo ( <i>Syncerus caffer</i> )	
MN 87.1	Mnisi	2017 Apr	<i>C. imicola</i>	99	94	1	99.84	Cattle ( <i>Bos taurus</i> )	
MAR 216.1	Marekele	2014 Feb	<i>C. imicola</i>	99	94	1	99.52	Hippo ( <i>Hippopotamus amphibius</i> )	
MAR 216.2	Marekele	2014 Feb	<i>C. imicola</i>	99	94	1	99.52	Hippo ( <i>Hippopotamus amphibius</i> )	
MAR 216.3	Marekele	2014 Feb	<i>C. imicola</i>	99	94	1	98.60	Hippo ( <i>Hippopotamus amphibius</i> )	
LAP 288.7	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	99.84	Human ( <i>Homo sapiens</i> )	
LAP 311.2	Lapalala	2014 May	<i>C. imicola</i>	99	94	1	99.18	Human ( <i>Homo sapiens</i> )	
MAR 395.1	Marekele	2015 Feb	<i>C. imicola</i>	99	94	1	100.00	Impala ( <i>Aepyceros melampus</i> )	

LAP 276.1	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	100.00	Kudu ( <i>Tragelaphus strepsiceros</i> )	
LAP 277.5	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	100.00	Kudu ( <i>Tragelaphus strepsiceros</i> )	
LAP 288.1	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	99.36	Kudu ( <i>Tragelaphus strepsiceros</i> )	
LAP 288.8	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	100.00	White rhino ( <i>Ceratotherium simum</i> )	
LAP 306	Lapalala	2014 Apr	<i>C. imicola</i>	99	94	1	99.84	White rhino ( <i>Ceratotherium simum</i> )	
LAP 276.2	Lapalala	2014 Mar	<i>C. imicola</i>	99	94	1	-	White rhinoceros ( <i>Ceratotherium simum</i> )	
LAP 277.3	Lapalala	2014 Mar	<i>C. imicola</i>	87	94	1	-	White rhinoceros ( <i>Ceratotherium simum</i> )	
LAP 288.4	Lapalala	2014 Mar	<i>C. kwagga</i>	67	91	1	-	Human ( <i>Homo sapiens</i> )	
LAP 304.1	Lapalala	2014 Apr	<i>C. kwagga</i>	67	91	1	-	Human ( <i>Homo sapiens</i> )	
MAR 356.1	Marekele	2014 Sep	<i>C. leucosticus</i>	100	100	1	99.29	Zebra ( <i>Equus burchellii</i> )	
MAR 356.2	Marekele	2014 Sep	<i>C. leucosticus</i>	100	100	1	-	Blue wildebeest ( <i>Connochaetes taurinus</i> )	
MN 79.3	KNP	2017 Jan	<i>C. loxodontis</i>	100	100	1	98.39	White rhino ( <i>Ceratotherium simum</i> )	
MN 71.2	Mnisi	2016 Oct	<i>C. pycnostictus</i>	100	100	1	99.12	Cisticola spp ( <i>Cisticola</i> spp)	
MAR 258.2	Marekele	2014 Sep	<i>C. pycnostictus</i>	100	100	1	98.59	Human ( <i>Homo sapiens</i> )	
MAR 368	Marekele	2014 Oct	<i>C. pycnostictus</i>	100	100	1	98.59	Human ( <i>Homo sapiens</i> )	
LAP 277.2	Lapalala	2014 Mar	<i>C. subschulzei</i>	100	100	1	98.23	Human ( <i>Homo sapiens</i> )	
LAP 457.6	Lapalala	2015 Feb	<i>C. subschulzei</i>	100	83	1	-	Kudu ( <i>Tragelaphus strepsiceros</i> )	
MAR 372.1	Marekele	2014 Oct	<i>C. subschulzei</i>	100	100	1	97.27	Zebra ( <i>Equus burchellii</i> )	
MAR 372.3	Marekele	2014 Oct	<i>C. subschulzei</i>	100	100	1	98.23	Zebra ( <i>Equus burchellii</i> )	
MN 78.2	Mnisi	2017 Jan	<i>C. tropicalis</i>	98	84	1	-	Cattle ( <i>Bos taurus</i> )	
MN 84.9	Mnisi	2017 Mar	<i>C. tuttifrutti</i>	98	99	1	100*; 99.84	Cattle ( <i>Bos taurus</i> )	* <i>C. imicola</i> (KT339740)
KNP 8	KNP	2016 Apr	<i>Cladontanytarsus</i> sp.?	-	-	-	87.28	Southern yellow-billed Hornbill ( <i>Tockus leucomelas</i> )	Closest mach <i>Cladontanytarsus vanderwulpi</i> Monophyly lacks support when including MF399674 (ID as <i>C. sp. #54</i> df)
MAR 371.3	Marekele	2014 Oct	<i>C. sp. # 54</i> df	100	100	1	97.91	Elephant ( <i>Loxodonta africana</i> )	
MN 71.3	Mnisi	2016 Oct	Undet sp.	100	-	-	-	Cisticola spp ( <i>Cisticola</i> spp)	
MN 76.1	Mnisi	2017 Jan	Undet sp.	100	-	-	-	Rat ( <i>Steatomys</i> spp)	
LAP 293.1	Lapalala	2014 Mar	Undet sp.	-	100	1	-	Buffalo ( <i>Syncerus caffer</i> )	
LAP 277.1	Lapalala	2014 Mar	Undet. sp. 1	-	100	1	-	White rhinoceros ( <i>Ceratotherium simum</i> )	
MAR 277.2	Marekele	2014 Apr	Undet sp.	-	-	-	-	Impala ( <i>Aepyceros melampus</i> )	

