

In-silico DNA barcoding surpasses whole genome sequencing for species identification from vector surveillance pools

Supplementary Materials

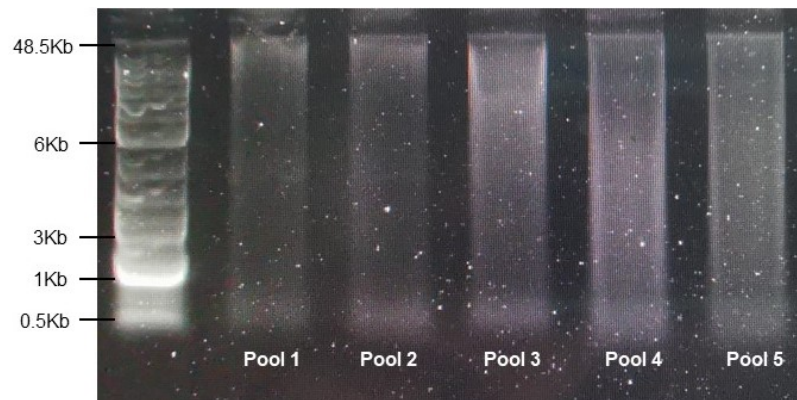


Figure S1. Gel electrophoresis of pooled samples (1-5) for determination of DNA fragment length.

DNA extractions from pooled mosquitoes, in 0.7% agarose gel. Quick-LoadPurple Extended 1 kb Plus DNA Ladder NEB. Samples subjected to current for 2h 100V.

Table S1. Mapped reads to species individual chromosomes using CLT

Species	Number of mapped reads						Total
	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr	Undefined	
Pool 1							
<i>Ae. aegypti</i>	105932	162324	141911	-	166	18062	428395
<i>An. arabiensis</i>	-	815893	757577	43317	31357	70445	1718589
<i>An. coluzzii</i>	-	2235567	1900857	667417	21332	531590	5356763
<i>Cx. quinquefasciatus</i>	25638	44496	39701	-	618	5	110458
Pool 2							
<i>Ae. aegypti</i>	196130	301521	261961	-	287	32903	792802
<i>An. arabiensis</i>	-	722268	670374	41364	29989	65250	1529245
<i>An. coluzzii</i>	-	1936198	1648901	590723	15957	457026	4648805
<i>Cx. quinquefasciatus</i>	48402	82134	74393	-	1226	13	206168
Pool 3							
<i>Ae. aegypti</i>	356713	553317	477084	-	244	57480	1444838
<i>An. arabiensis</i>	-	701001	661333	45351	18754	79810	1506249
<i>An. coluzzii</i>	-	1839471	1584678	518135	10474	441006	4393764
<i>Cx. quinquefasciatus</i>	208657	348913	327084	-	2115	45	886814
Pool 4							
<i>Ae. aegypti</i>	655603	976108	851527	-	671	108865	2592774
<i>An. arabiensis</i>	-	709124	605621	85714	7049	148661	1556169
<i>An. coluzzii</i>	-	725333	627221	201479	4293	152046	1710372
<i>B.malayi</i>	-	-	-	-	-	19939	19939
<i>Cx. quinquefasciatus</i>	1412592	2386656	2248807	-	7813	369	6056237
<i>D.immitis</i>	-	-	-	-	-	4376	4376
<i>Pfalciptarum</i>	-	-	-	-	-	249	249
Pool 5							
<i>Ae. aegypti</i>	551018	832852	723594	-	385	87273	219522
<i>An. arabiensis</i>	-	482879	421985	57920	6260	113714	1082758
<i>An. coluzzii</i>	-	512548	451243	143104	3705	109328	1219928
<i>B.malayi</i>	-	-	-	-	-	32201	32201
<i>Cx. quinquefasciatus</i>	1188104	2021193	1904514	-	6665	304	5120780
<i>D.immitis</i>	-	-	-	-	-	8110	8110
<i>Pfalciptarum</i>	-	-	-	-	-	382	382

Table S2. Mapped reads to species individual chromosomes using CLTq20

Species	Number of mapped reads					Undefined	Total
	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr		
Pool 1							
<i>Ae. aegypti</i>	61821	100475	85657	-	9	4761	2527723
<i>An. arabiensis</i>	-	472584	450925	13395	12400	4079	953383
<i>An. coluzzii</i>	-	1723129	1426296	567613	4625	49401	3771064
<i>Cx. quinquefasciatus</i>	14935	25372	23496	-	534	5	64342
Pool 2							
<i>Ae. aegypti</i>	113774	187252	158669	-	8	8983	468686
<i>An. arabiensis</i>	-	413601	394563	13653	12523	3540	837880
<i>An. coluzzii</i>	-	1478594	1226124	495081	3040	42085	3244924
<i>Cx. quinquefasciatus</i>	28490	47029	43611	-	1061	11	120202
Pool 3							
<i>Ae. aegypti</i>	207768	342553	287743	-	9	16717	854790
<i>An. arabiensis</i>	-	435747	417928	19054	8801	5448	886978
<i>An. coluzzii</i>	-	1448654	1215930	445138	2312	42122	3154156
<i>Cx. quinquefasciatus</i>	123928	210040	195263	-	1929	33	531193
Pool 4							
<i>Ae. aegypti</i>	375796	615389	523322	-	11	23561	1538079
<i>An. arabiensis</i>	-	450957	393171	61082	2362	6797	914369
<i>An. coluzzii</i>	-	456765	394101	163838	717	8118	1023539
<i>B.malayi</i>	-	-	-	-	-	16204	16204
<i>Cx. quinquefasciatus</i>	882710	1531851	1418975	-	7033	321	3840890
<i>D.immitis</i>	-	-	-	-	-	3043	3043
<i>P.falciplarum</i>	-	-	-	-	-	60	60
Pool 5							
<i>Ae. aegypti</i>	325077	529482	450837	-	4	22688	1328088
<i>An. arabiensis</i>	-	342919	302143	43165	2758	6836	697821
<i>An. coluzzii</i>	-	362226	315904	119940	857	8626	807553
<i>B.malayi</i>	-	-	-	-	-	26905	26905
<i>Cx. quinquefasciatus</i>	744957	1296088	1204687	-	6161	264	3252157
<i>D.immitis</i>	-	-	-	-	-	6583	6583
<i>P.falciplarum</i>	-	-	-	-	-	154	154

Table S3. Mapped reads to species individual chromosomes using CLTq20prim

Species	Number of mapped reads					Undefined	Total
	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr		
Pool 1							
<i>Ae. aegypti</i>	45749	75625	64532	-	5	3382	189293
<i>An. arabiensis</i>	-	437019	416448	8821	11783	3193	877264
<i>An. coluzzii</i>	-	1610496	1326379	525045	3850	45560	3511330
<i>Cx. quinquefasciatus</i>	11561	19186	17857	-	394	3	49001
Pool 2							
<i>Ae. aegypti</i>	83766	139084	118224	-	6	6374	347454
<i>An. arabiensis</i>	-	379057	361355	9537	11692	2755	764396
<i>An. coluzzii</i>	-	1372075	1131218	454017	2517	38268	2998095
<i>Cx. quinquefasciatus</i>	21825	35279	32721	-	770	9	90604
Pool 3							
<i>Ae. aegypti</i>	153116	256315	213917	-	9	12043	635400
<i>An. arabiensis</i>	-	400740	384084	14130	8306	4397	811657
<i>An. coluzzii</i>	-	1349018	1126193	409038	1907	38383	2924539
<i>Cx. quinquefasciatus</i>	89912	151975	142917	-	1351	19	386174
Pool 4							
<i>Ae. aegypti</i>	332532	544729	463291	-	8	20513	1361073
<i>An. arabiensis</i>	-	428872	372983	57385	2255	6215	867710
<i>An. coluzzii</i>	-	433884	373269	154735	620	770	970208
<i>B.malayi</i>	-	-	-	-	-	14281	14281
<i>Cx. quinquefasciatus</i>	787676	1372007	1271859	-	5945	276	3437763
<i>D.immitis</i>	-	-	-	-	-	2788	2788
<i>P.falciptarum</i>	-	-	-	-	-	36	36
Pool 5							
<i>Ae. aegypti</i>	138798	226303	192994	-	0	9593	567688
<i>An. arabiensis</i>	-	162458	143584	20062	1353	3168	330625
<i>An. coluzzii</i>	-	171580	149004	56315	359	4209	381467
<i>B.malayi</i>	-	-	-	-	-	12000	12000
<i>Cx. quinquefasciatus</i>	319684	561135	522659	-	2647	104	1406229
<i>D.immitis</i>	-	-	-	-	-	3049	3049
<i>P.falciptarum</i>	-	-	-	-	-	48	48

Table S4. Mapped reads to species individual chromosomes using Epi2me Agent

Species	Number of mapped reads						Total
	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr	Undefined	
Pool 1							
<i>Ae. aegypti</i>	67188	103459	90915	-	114	11727	273403
<i>An. arabiensis</i>	-	764569	691652	28201	28509	92180	1605111
<i>An. coluzzii</i>	-	1985846	1685066	592191	16501	497237	4776841
<i>Cx. quinquefasciatus</i>	17551	29991	26842	-	454	4	74842
Pool 2							
<i>Ae. aegypti</i>	123126	189690	165822	-	211	21198	500047
<i>An. arabiensis</i>	-	665783	602958	27310	25956	58451	1380458
<i>An. coluzzii</i>	-	1702115	1445371	517531	11841	422530	4099388
<i>Cx. quinquefasciatus</i>	32726	54522	49194	-	885	10	137337
Pool 3							
<i>Ae. aegypti</i>	21875	343585	294622	-	226	63663	920851
<i>An. arabiensis</i>	-	651874	599736	29795	17157	45330	1343892
<i>An. coluzzii</i>	-	1616331	1390315	454476	7584	410245	3878951
<i>Cx. quinquefasciatus</i>	125971	209317	198058	-	1515	24	534885
Pool 4							
<i>Ae. aegypti</i>	540958	801355	699411	-	550	97527	2139801
<i>An. arabiensis</i>	-	663061	563618	78264	6499	142203	1453645
<i>An. coluzzii</i>	-	674459	581706	185914	3563	136754	1582396
<i>B.malayi</i>	-	-	-	-	-	17294	17294
<i>Cx. quinquefasciatus</i>	1173790	1992580	1977580	-	6624	12	5050586
<i>D.immitis</i>	-	-	-	-	-	3894	3894
<i>P.falciptarum</i>	-	-	-	-	-	102	102
Pool 5							
<i>Ae. aegypti</i>	422045	635058	553232	-	305	66852	1677492
<i>An. arabiensis</i>	-	448645	389478	51719	5931	108627	1004400
<i>An. coluzzii</i>	-	471360	414083	130166	2993	103364	1121966
<i>B.malayi</i>	-	-	-	-	-	28379	28379
<i>Cx. quinquefasciatus</i>	918420	1574482	1483454	-	5672	231	3982259
<i>D.immitis</i>	-	-	-	-	-	7343	7343
<i>P.falciptarum</i>	-	-	-	-	-	168	168

Table S5. Classified reads to species using Centrifuge

Species	Read number	Unique read number
Pool 1		
<i>Ae. aegypti</i>	333637	288320
<i>An. arabiensis</i>	2349294	1929441
<i>An. coluzzii</i>	4596297	4196524
<i>Cx. quinquefasciatus</i>	600044	261892
Pool 2		
<i>Ae. aegypti</i>	591298	519333
<i>An. arabiensis</i>	2129728	1703181
<i>An. coluzzii</i>	3964552	3600674
<i>Cx. quinquefasciatus</i>	866192	415041
Pool 3		
<i>Ae. aegypti</i>	960129	887641
<i>An. arabiensis</i>	1843612	1618903
<i>An. coluzzii</i>	3704163	3436075
<i>Cx. quinquefasciatus</i>	648841	571595
Pool 4		
<i>Ae. aegypti</i>	2461755	2043539
<i>An. arabiensis</i>	1831406	1446947
<i>An. coluzzii</i>	1684545	1351648
<i>B.malayi</i>	24622	20069
<i>Cx. quinquefasciatus</i>	5661889	5017438
<i>D.immitis</i>	32914	14600
<i>P.falciparum</i>	383	59
Pool 5		
<i>Ae. aegypti</i>	1846576	1633678
<i>An. arabiensis</i>	1210322	1035423
<i>An. coluzzii</i>	1140208	990866
<i>B.malayi</i>	33887	30311
<i>Cx. quinquefasciatus</i>	4306379	3988363
<i>D.immitis</i>	31042	15426
<i>P.falciparum</i>	290	36

Table S6. Sequencing depth and coverage per species from pools 1-5

Species	Sequencing coverage and (I) depth					Mitochondrial Chr	Unplaced
	Chr 1	Chr 2	Chr 3	Chr X			
Pool 1							
<i>Ae. aegypti</i>	19.07/0.23	20.37/0.25	20.24/0.24	-	16.69/0.20	12.16/0.17	
<i>An. arabiensis</i>	-	68.04/4.34	71.92/5.32	17.00/0.35	100.00/1184.60	6.88/0.17	
<i>An. coluzzii</i>	-	96.51/23.29	96.17/21.46	97.32/27.37	100.00/201.07	37.26/3.64	
<i>Cx. quinquefasciatus</i>	10.12/0.12	9.93/0.11	10.09/0.11	-	100.00/29.32	20.53/0.26	
Pool 2							
<i>Ae. aegypti</i>	30.00/0.41	32.52/0.45	32.45/0.45	-	27.74/0.31	17.22/0.26	
<i>An. arabiensis</i>	-	68.92/3.79	72.68/4.59	22.41/0.39	100.00/1191.33	8.07/0.16	
<i>An. coluzzii</i>	-	96.19/19.21	95.85/17.68	97.11/22.84	98.23/133.76	35.68/3.19	
<i>Cx. quinquefasciatus</i>	18.54/0.22	17.45/0.21	17.27/0.21	-	100.00/65.74	8.81/0.09	
Pool 3							
<i>Ae. aegypti</i>	44.32/0.77	47.79/0.85	46.86/0.81	-	63.24/1.10	21.17/0.38	
<i>An. arabiensis</i>	-	80.29/4.63	82.21/5.51	39.10/0.67	100.00/1011.76	11.94/0.30	
<i>An. coluzzii</i>	-	96.01/19.85	95.40/18.72	97.00/22.56	99.94/115.98	34.49/3.05	
<i>Cx. quinquefasciatus</i>	51.79/1.02	51.71/1.00	54.74/1.07	-	100.00/136.61	19.50/0.31	
Pool 4							
<i>Ae. aegypti</i>	38.53/0.59	40.75/0.63	40.20/0.62	-	18.35/0.22	14.55/0.23	
<i>An. arabiensis</i>	-	75.68/1.88	75.61/1.90	57.74/0.97	100.00/152.95	11.22/0.25	
<i>An. coluzzii</i>	-	74.92/1.97	74.36/1.95	85.73/2.68	95.88/28.54	14.70/0.36	
<i>B. malayi</i>	-	-	-	-	-	16.52/0.26	
<i>Cx. quinquefasciatus</i>	75.55/2.99	76.88/3.10	78.60/3.24	-	100.00/264.14	21.34/1.02	
<i>D. immitis</i>	-	-	-	-	-	5.01/0.06	
<i>P. falciparum</i>	-	-	-	-	-	0.49/0.00	
Pool 5							
<i>Ae. aegypti</i>	26.95/0.35	28.63/0.37	28.32/0.37	-	0.00/0.00	12.13/0.17	
<i>An. arabiensis</i>	-	58.96/1.02	59.51/1.05	37.60/0.50	100.00/120.10	8.93/0.15	
<i>An. coluzzii</i>	-	60.50/1.11	60.11/1.11	71.51/1.43	996.36/17.35	11.31/0.22	
<i>B. malayi</i>	-	-	-	-	-	24.38/0.43	
<i>Cx. quinquefasciatus</i>	67.48/1.73	69.24/1.79	71.04/1.89	-	100.00/166.54	17.80/0.39	
<i>D. immitis</i>	-	-	-	-	-	8.72/0.09	
<i>P. falciparum</i>	-	-	-	-	-	0.62/0.01	

Table S7. Mapped reads to control reference genome using CLTq20prim

Species	Number of mapped reads					Total
	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr	
Pool 1						
<i>Ae. aegypti</i>	45652	75521	64477	-	5	3352
<i>An. arabiensis</i>	-	519409	201068	4957	2845	2892
<i>An. coluzzii</i>	-	682076	611514	180650	2635	15643
<i>An. Gambian</i>	-	535260	527960	130500	2015	130816
<i>B.malayi</i>	-	-	-	-	-	4
<i>Cx. quinquefasciatus</i>	11524	19114	17791	-	393	3
<i>D.immitis</i>	-	-	-	-	-	191
<i>E.asinus</i>	-	-	-	-	-	405
<i>P.falciparum</i>	-	-	-	-	-	0
Pool 2						
<i>Ae. aegypti</i>	83625	138905	118069	-	6	6307
<i>An. arabiensis</i>	-	452366	177315	5966	2396	2592
<i>An. coluzzii</i>	-	577722	521057	155658	2023	12854
<i>An. Gambian</i>	-	452143	447114	111353	1524	111496
<i>B.malayi</i>	-	-	-	-	-	23
<i>Cx. quinquefasciatus</i>	21752	35171	32619	-	766	9
<i>D.immitis</i>	-	-	-	-	-	268
<i>E.asinus</i>	-	-	-	-	-	605
<i>P.falciparum</i>	-	-	-	-	-	0
Pool 3						
<i>Ae. aegypti</i>	152805	256036	213631	-	8	11949
<i>An. arabiensis</i>	-	481023	204858	11092	1674	4087
<i>An. coluzzii</i>	-	580282	529489	150829	1549	14321
<i>An. Gambian</i>	-	450427	455313	103437	1172	110188
<i>B.malayi</i>	-	-	-	-	-	28
<i>Cx. quinquefasciatus</i>	89700	151617	142472	-	1347	18
<i>D.immitis</i>	-	-	-	-	-	80
<i>E.asinus</i>	-	-	-	-	-	62
<i>P.falciparum</i>	-	-	-	-	-	0
Pool 4						
<i>Ae. aegypti</i>	332197	544371	462960	-	8	20447
<i>An. arabiensis</i>	-	433099	271777	55414	454	5227
<i>An. coluzzii</i>	-	159327	158134	41510	471	1899
<i>An. Gambian</i>	-	134902	141298	30641	282	32141
<i>B.malayi</i>	-	-	-	-	-	14262
<i>Cx. quinquefasciatus</i>	786425	1369671	1269591	-	5938	276
<i>D.immitis</i>	-	-	-	-	-	3023
<i>E.asinus</i>	-	-	-	-	-	27
<i>P.falciparum</i>	-	-	-	-	-	35
Pool 5						
<i>Ae. aegypti</i>	276883	451237	384831	-	3	18949
<i>An. arabiensis</i>	-	332839	214413	38991	679	5257
<i>An. coluzzii</i>	-	137392	136976	37196	582	277007
<i>An. Gambian</i>	-	115510	121434	26770	387	26126
<i>B.malayi</i>	-	-	-	-	-	24082
<i>Cx. quinquefasciatus</i>	638994	1119903	1042619	-	5192	216
						2806924

Continued on next page

Table S7 – Continued from previous page

Species	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr	Undefined	Total
<i>D.immitis</i>	-	-	-	-	-	6479	6479
<i>E.asinus</i>	-	-	-	-	-	24	24
<i>P.falciparum</i>	-	-	-	-	-	107	107

Table S8. Classified reads to species with Centrifuge when using a control reference genome

Species	Read number	Unique read number
Pool 1		
<i>Ae. aegypti</i>	333637	288320
<i>An. arabiensis</i>	2349294	1929441
<i>An. coluzzii</i>	4596297	4196524
<i>An. Gambian</i>	600044	261892
<i>B.malayi</i>	2349294	1929441
<i>Cx. quinquefasciatus</i>	4596297	4196524
<i>D.immitis</i>	600044	261892
<i>E.asinus</i>	4596297	4196524
<i>P.falci-parum</i>	600044	261892
Pool 2		
emph <i>Ae. aegypti</i>	333637	288320
<i>An. arabiensis</i>	2349294	1929441
<i>An. coluzzii</i>	4596297	4196524
<i>An. Gambian</i>	600044	261892
<i>B.malayi</i>	2349294	1929441
<i>Cx. quinquefasciatus</i>	4596297	4196524
<i>D.immitis</i>	600044	261892
<i>E.asinus</i>	4596297	4196524
<i>P.falci-parum</i>	600044	261892
Pool 3		
<i>Ae. aegypti</i>	333637	288320
<i>An. arabiensis</i>	2349294	1929441
<i>An. coluzzii</i>	4596297	4196524
<i>An. Gambian</i>	600044	261892
<i>B.malayi</i>	2349294	1929441
<i>Cx. quinquefasciatus</i>	4596297	4196524
<i>D.immitis</i>	600044	261892
<i>E.asinus</i>	4596297	4196524
<i>P.falci-parum</i>	600044	261892
Pool 4		
emph <i>Ae. aegypti</i>	333637	288320
<i>An. arabiensis</i>	2349294	1929441
<i>An. coluzzii</i>	4596297	4196524
<i>An. Gambian</i>	600044	261892
<i>B.malayi</i>	2349294	1929441
<i>Cx. quinquefasciatus</i>	4596297	4196524
<i>D.immitis</i>	600044	261892
<i>E.asinus</i>	4596297	4196524
<i>P.falci-parum</i>	600044	261892
Pool 5		
emph <i>Ae. aegypti</i>	333637	288320
<i>An. arabiensis</i>	2349294	1929441
<i>An. coluzzii</i>	4596297	4196524
<i>An. Gambian</i>	600044	261892
<i>B.malayi</i>	2349294	1929441
<i>Cx. quinquefasciatus</i>	4596297	4196524
<i>D.immitis</i>	600044	261892
<i>E.asinus</i>	4596297	4196524
<i>P.falci-parum</i>	600044	261892

Table S9. Sequencing depth and coverage per species, from pools 1-5, when mapping reads to a control reference genome

Species	Sequencing coverage (%) and (/) depth (fold)				
	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr
Pool 1					
<i>Ae. aegypti</i>	19.06/0.23	20.35/0.25	20.23/0.24	-	16.69/0.20
<i>An. arabiensis</i>	-	56.04/6.67	56.85/2.58	14.33/0.22	98.59/207.60
<i>An. coluzzii</i>	-	78.74/9.56	92.89/11.14	92.74/12.08	100.00/144.04
<i>An. gambiae</i>	-	71.44/7.50	83.04/8.69	84.78/7.74	100.00/144.04
<i>B. malayi</i>	-	-	-	-	-
<i>Cx. quinquefasciatus</i>	10.42/0.12	9.92/0.11	10.08/0.11	-	100.00/29.31
<i>D. immitis</i>	-	-	-	-	-
<i>E. asinus</i>	-	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	-
Pool 2					
<i>Ae. aegypti</i>	29.99/0.41	32.48/0.45	32.42/0.45	-	27.74/0.31
<i>An. arabiensis</i>	-	58.29/5.72	58.32/2.31	20.08/0.29	99.62/181.12
<i>An. coluzzii</i>	-	77.14/7.84	91.68/9.18	91.79/9.99	100.00/110.42
<i>An. gambiae</i>	-	71.44/7.50	83.04/8.69	83.19/6.47	95.41/92.2
<i>B. malayi</i>	-	-	-	-	-
<i>Cx. quinquefasciatus</i>	18.51/0.22	17.41/0.21	17.25/0.21	-	100.00/65.35
<i>D. immitis</i>	-	-	-	-	-
<i>E. asinus</i>	-	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	-
Pool 3					
<i>Ae. aegypti</i>	44.29/0.77	47.77/0.85	46.83/0.81	-	60.85/1.06
<i>An. arabiensis</i>	-	73.71/6.53	72.54/3.01	37.39/0.56	98.35/141.72
<i>An. coluzzii</i>	-	76.33/8.11	90.93/9.68	90.44/9.77	100.00/93.92
<i>An. gambiae</i>	-	71.44/7.50	83.04/8.69	100.00/93.92	94.50/72.83
<i>B. malayi</i>	-	-	-	-	-
<i>Cx. quinquefasciatus</i>	51.68/1.02	51.66/1.00	54.72/1.07	-	100.00/136.02
<i>D. immitis</i>	-	-	-	-	-
<i>E. asinus</i>	-	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	-
Pool 4					
<i>Ae. aegypti</i>	38.51/0.59	40.73/0.63	40.18/0.62	-	18.35/0.22
<i>An. arabiensis</i>	-	72.80/1.95	66.03/1.45	57.02/0.95	91.75/28.26
<i>An. coluzzii</i>	-	38.04/0.72	49.49/0.94	46.51/0.90	98.54/22.66
<i>An. gambiae</i>	-	35.82/0.66	43.96/0.82	39.31/0.70	87.53/14.99
<i>B. malayi</i>	-	-	-	-	-
<i>Cx. quinquefasciatus</i>	75.50/2.98	76.82/3.09	78.57/3.24	-	100.00/263.89
<i>D. immitis</i>	-	-	-	-	-
<i>E. asinus</i>	-	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	-

Continued on next page

Table S9 – Continued from previous page

Species	Chr 1	Chr 2	Chr 3	Chr X	Mitochondrial Chr	Unplaced
Pool 5						
<i>Ae. aegypti</i>	42.88/0.70	45.13/0.74	44.57/0.74	-	12.65/0.16	17.02/0.29
<i>An. arabiensis</i>	-	76.03/2.15	69.74/1.63	57.72/0.97	87.24/44.95	11.58/0.26
<i>An. coluzzii</i>	-	43.57/0.87	54.76/1.13	52.45/1.10	99.67/29.82	8.96/0.50
<i>An. gambiae</i>	-	79.10/1.57	48.63/0.98	43.57/0.84	93.53/21.32	45.69/1.07
<i>B. malayi</i>	-	-	-	-	-	29.64/0.66
<i>Cx. quinquefasciatus</i>	78.54/3.45	79.41/3.58	81.00/3.78	-	100.00/325.17	21.05/1.01
<i>D. immitis</i>	-	-	-	-	-	19.67/0.30
<i>E. asinus</i>	-	-	-	-	-	0.00/0.00
<i>P. falciparum</i>	-	-	-	-	-	1.46/0.01

Table S10. Number of mapped reads per species to target identification loci

Target loci	<i>Ae. aegypti</i>	<i>An. arabiensis</i>	<i>An. coluzzii</i>	<i>B. malayi</i>	<i>Cx. quinquefasciatus</i>	<i>D. immitis</i>	<i>Pf. falciparum</i>
Pool 1							
<i>COX1</i>	192	1026	404	-	38	-	-
<i>ITS2</i>	443	70	28336	-	29	-	-
Combo 0	635	1096	28737	-	657	-	-
Combo 1	192	1080	27829	-	38	-	-
Combo 2	635	1096	72034	-	67	-	-
Combo 3	635	1142	46690	-	67	-	-
Combo 4	635	1142	89977	-	67	-	-
Pool 2							
<i>COX1</i>	340	1048	188	-	87	-	-
<i>ITS2</i>	910	116	23726	-	70	-	-
Combo 0	1143	1163	23472	-	140	-	-
Combo 1	340	1140	55540	-	87	-	-
Combo 2	1143	1164	59964	-	140	-	-
Combo 3	1143	1238	39052	-	140	-	-
Combo 4	1191	3953	92115	-	144	-	-
Pool 3							
<i>COX1</i>	523	789	213	-	192	-	-
<i>ITS2</i>	1196	248	21942	-	279	-	-
Combo 0	1719	1037	22155	-	470	-	-
Combo 1	523	1030	5667	-	192	-	-
Combo 2	1719	1039	59549	-	470	-	-
Combo 3	1719	1268	38560	-	470	-	-
Combo 4	1719	1270	75956	-	470	-	-
Pool 4							
<i>COX1</i>	639	140	82	22	638	3	0
<i>ITS2</i>	1901	688	3619	0	2763	0	0
Combo 5	2540	828	3701	22	3401	3	0
Combo 6	639	661	8273	22	638	3	0
Combo 7	2540	830	9532	22	3401	3	0
Combo 8	2540	1343	6048	22	3401	3	0
Combo 9	2540	1344	11879	22	3401	3	0
Pool 5							
<i>COX1</i>	476	166	76	32	599	2	-
<i>ITS2</i>	1804	544	2966	-	2308	-	-
Combo 5	2280	709	3043	32	2907	2	0
Combo 6	476	667	8835	32	599	2	0
Combo 7	2280	713	9655	32	2907	2	0
Combo 8	2280	1200	5168	32	2907	2	0
Combo 9	2280	1204	11780	32	2907	2	0

Table S11. Sequencing coverage and depth when mapping WGS reads to individual target genes

Species	COXI	Sequencing coverage (%) / and depth (fold -x)		
		ITS2	SINEs200	IGS
Pool 1				
<i>Ae. aegypti</i>	100.00/91.34	100.00/391.23	-	<i>An. arabiensis</i>
100.00/57.94	0.00	100.00/44.91	<i>An. coluzzii</i>	100.00/225.04
100.00/17075.90	<i>Cx. quinquefasciatus</i>	100.00/26.30	100.00/22.49	-
Pool 2				
<i>Ae. aegypti</i>	100.00/183.53	100.00/817.56	-	<i>An. arabiensis</i>
100.00/99.40	100.00/1.00	100.00/83.70	<i>An. coluzzii</i>	100.00/104.91
100.00/15460.90	<i>Cx. quinquefasciatus</i>	100.00/61.95	100.00/60.37	-
Pool 3				
<i>Ae. aegypti</i>	100.00/271.63	100.00/1052.72	-	98.90/0.99
100.00/571.64	100.00/203.69	100.00/1.93	<i>An. coluzzii</i>	100.00/129.80
100.00/16669.90	100.00/15681.30	<i>Cx. quinquefasciatus</i>	100.00/135.04	100.00/17973.60
100.00/1.80			100.00/221.61	-
Pool 4				
<i>Ae. aegypti</i>	100.00/246.22	100.00/1367.92	-	<i>An. arabiensis</i>
100.00/397.59	90.78/1.69	100.00/479.75	<i>An. coluzzii</i>	100.00/44.27
100.00/2144.06	<i>B. malayi</i>	100.00/15.51	-	81.66/2383.73
100.00/320.27	100.00/1641.70	-	<i>D. immitis</i>	<i>Cx. quinquefasciatus</i>
-	-		-	100.00/2.18
Pool 5				
<i>Ae. aegypti</i>	100.00/227.01	100.00/1443.06	-	<i>An. arabiensis</i>
100.00/385.37	100.00/3.73	100.00/465.38	<i>An. coluzzii</i>	100.00/1987.22
100.00/1997.65	<i>B. malayi</i>	100.00/23.84	-	100.00/2813.45
100.00/355.10	100.00/1591.18	-	<i>D. immitis</i>	<i>Cx. quinquefasciatus</i>
-	-		-	100.00/1.96
-	-		-	-

Table S12. Number of mapped reads per species to control reference loci

Species	COX1	ITS2	IGS	SINES200	18srRNA
Pool 1					
<i>Ae. aegypti</i>	382	886	-	-	-
<i>An. arabiensis</i>	206	140	92	0	-
<i>An. coluzzii</i>	628	28690	30	86596	-
<i>An. funestus</i>	0	0	-	-	-
<i>An. gambiae</i>	10	6	0	0	-
<i>B. malayi</i>	0	-	-	-	-
<i>Cx. quinquefasciatus</i>	76	58	-	-	-
<i>D. immitis</i>	0	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	0
<i>P. vivax</i>	-	-	-	-	0
Pool 2					
<i>Ae. aegypti</i>	680	1606	-	-	-
<i>An. arabiensis</i>	236	230	146	2	-
<i>An. coluzzii</i>	316	23646	36	72974	-
<i>An. funestus</i>	0	0	-	-	-
<i>An. gambiae</i>	2	0	0	0	-
<i>B. malayi</i>	0	-	-	-	-
<i>Cx. quinquefasciatus</i>	174	106	-	-	-
<i>D. immitis</i>	0	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	0
<i>P. vivax</i>	-	-	-	-	0
Pool 3					
<i>Ae. aegypti</i>	1046	2392	-	-	-
<i>An. arabiensis</i>	220	492	462	4	-
<i>An. coluzzii</i>	336	21704	42	74790	-
<i>An. funestus</i>	0	0	-	-	-
<i>An. gambiae</i>	0	0	2	0	-
<i>B. malayi</i>	0	-	-	-	-
<i>Cx. quinquefasciatus</i>	382	558	-	-	-
<i>D. immitis</i>	0	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	0
<i>P. vivax</i>	-	-	-	-	0
Pool 4					
<i>Ae. aegypti</i>	1278	3802	-	-	-
<i>An. arabiensis</i>	22	1366	1018	4	-
<i>An. coluzzii</i>	120	2882	8	11662	-
<i>An. funestus</i>	0	0	-	-	-
<i>An. gambiae</i>	2	0	0	0	-
<i>B. malayi</i>	44	-	-	-	-
<i>Cx. quinquefasciatus</i>	1276	5526	-	-	-
<i>D. immitis</i>	6	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	0
<i>P. vivax</i>	-	-	-	-	0
Pool 5					
<i>Ae. aegypti</i>	952	3608	-	-	-
<i>An. arabiensis</i>	22	1080	980	8	-
<i>An. coluzzii</i>	118	2726	10	13224	-
<i>An. funestus</i>	0	0	-	-	-
<i>An. gambiae</i>	2	0	0	0	-
<i>B. malayi</i>	64	-	-	-	-

Continued on next page

Table S12 – *Continued from previous page*

Species	<i>COX1</i>	<i>ITS2</i>	<i>IGS</i>	<i>SINES200</i>	<i>18srRNA</i>
<i>Cx. quinquefasciatus</i>	1198	4616	-	-	-
<i>D. immitis</i>	4	-	-	-	-
<i>P. falciparum</i>	-	-	-	-	0
<i>P. vivax</i>	-	-	-	-	0

Table S13. Sequencing coverage and depth when mapping extracted WGS reads to control target loci for species identification and abundance estimation

Species	Sequencing coverage (%) and depth (fold -x)			
	COXI	ITS2	SINEs200	IGS
Pool 1				
<i>Ae. aegypti</i>	100/182.39	100/782.46	-	-
<i>An. arabiensis</i>	100/191.56	100/115.87	100/0.00	100/89.81
<i>An. coluzzii</i>	100/355.90	100/24547.3	100/38910.80	100/24.39
<i>An. gambiae</i>	74.53/6.99	100/4.56	0.00/0.00	0.00/0.00
<i>B. malayi</i>	0.00/0.00	-	-	-
<i>Cx. quinquefasciatus</i>	100/52.59	100/44.95	-	-
<i>D. immitis</i>	0.00/0.00	-	-	-
Pool 2				
<i>Ae. aegypti</i>	100/367.05	100/1445.01	-	-
<i>An. arabiensis</i>	100/213.76	100/196.85	100/2.00	100/141.69
<i>An. coluzzii</i>	100/175.95	100/20192.90	100/32728.70	100/31.06
<i>An. gambiae</i>	71.01/1.42	0.00/0.00	0.00/0.00	0.00/0.00
<i>B. malayi</i>	0.00/0.00	-	-	-
<i>Cx. quinquefasciatus</i>	100/123.90	100/88.36	-	-
<i>D. immitis</i>	0.00/0.00	-	-	-
Pool 3				
<i>Ae. aegypti</i>	100/543.26	100/2105.45	-	-
<i>An. arabiensis</i>	100/209.14	100/405.20	100/3.85	100/446.53
<i>An. coluzzii</i>	100/202.65	100/18628.50	100/33339.40	100/37.64
<i>An. gambiae</i>	0.00/0.00	0.00/0.00	0.00/0.00	96.09/1.92
<i>B. malayi</i>	0.00/0.00	-	-	-
<i>Cx. quinquefasciatus</i>	100/268.64	100/443.22	-	-
<i>D. immitis</i>	0.00/0.00	-	-	-
Pool 4				
<i>Ae. aegypti</i>	100/492.43	100/2735.84	-	-
<i>An. arabiensis</i>	100/18.66	100/790.09	98.78/3.38	100/950.61
<i>An. coluzzii</i>	100/69.64	100/1790.69	81.67/4767.45	100/6.69
<i>An. gambiae</i>	75.25/1.50	0.00/0.00	0.00/0.00	0.00/0.00
<i>B. malayi</i>	100/31.02	-	-	-
<i>Cx. quinquefasciatus</i>	100/640.55	100/3283.41	-	-
<i>D. immitis</i>	99.85/4.36	-	-	-
Pool 5				
<i>Ae. aegypti</i>	100/454.02	100/2886.12	-	-
<i>An. arabiensis</i>	100/18.61	100/766.62	100/7.46	100/928.81
<i>An. coluzzii</i>	99.85/64.22	100/1979.90	100/5626.90	100/8.29
<i>An. gambiae</i>	67.20/1.34	0.00/0.00	0.00/0.00	0.00/0.00
<i>B. malayi</i>	100/47.67	-	-	-
<i>Cx. quinquefasciatus</i>	100/710.19	100/3182.37	-	-
<i>D. immitis</i>	99.85/3.91	-	-	-