Supplementary Material:

Species identification of Swedish mosquitoes through DNA metabarcoding

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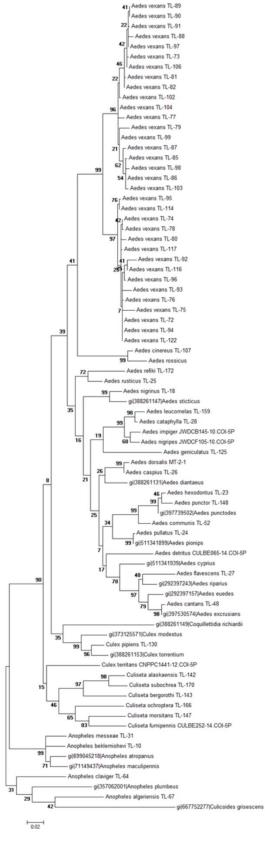


Figure 1 (Supplementary Material): Supplementary Material, Figure 1: Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-11361.0261) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 80 nucleotide sequences. Codon positions included were 1st+2nd+3rd+noncoding. There were a total of 1,539 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Species/Abbrv	Group Name															*	
1. Anopheles algeriensis		TT	ГC	ΑÆ	C	A	AΑ	T	A	T.	AΑ	G	G Z	I	A :	Τ	GG
2. Anopheles claviger		CA	A	C A	A	T	A T	T	A	Α	ΤA	A	G Z	I	A :	Τ	GG
3. Aedes cinereus		TT	ГC	ΑÆ	C	A Z	AΑ	T	CA	T.	AΑ	A	G Z	I	A :	Τ	GG
4. Aedes rusticus		GG															
5. Aedes geniculatus		A C	AC	ΑI	Α	T	A T	A.	ΓΑ	A	AΑ	С	T	ΑA	T	T	GG
6. Aedes hexodontus		CC	AΑ	C A	A	A	ΑΤ	T	CA	Α	ΤA	Α	G Z	I	A :	ГΤ	GG
7. Aedes leucomelas		TT	ГС	ΑZ	ıс	A	AΑ	T	CA	T	AΑ	Α	G Z	I	A	ľΤ	GG
8. Aedes nigrinus		AC	ΔC	ΑC	Α	T	A T	A.	ΓΑ	T	ΑC	Α	G Z	I	A	ľΤ	- G
9. Aedes pullatus		TT	ГС	ΑŞ	c	A	AΑ	T	CA	T	AΑ	A	G Z	I	A	ľΤ	GG
10. Culiseta annulata		CA	AΑ	C A	A	Α	ГΤ	T	СС	Α	ΤA	A	G Z	I	A	ľΤ	GG
11. Culiseta bergrothi		TT	ГС	T	C	A	AΑ	T	A	T	AΑ	A	G Z	I	A	Τ	GG
12. Aedes atropalpus		TT	ГС	T	C	T	AΑ	T	A	T	AΑ	A	G Z	I	A	ΓΤ	GG
13. Aedes aegypti		TT	ГС	ΑZ	C	A	AΑ	T	A	T	AΑ	A	G Z	I	A	ΓΤ	GG
14. gi 401879655 Lucilia sericata		TT	ГС	A A	C	T	AΑ	T	CΑ	T.	AΑ	A	G Z	T	A I	ľΤ	GG
15. gi 374093166 Bombyx mori		A T	ГС	T Z	C	A	AΑ	T	CA	T.	AΑ	A	G Z	T	A I	ľΤ	GG
16. Primer LCO1490		GG	ГС	A A	C	A	AΑ	T	CA	T.	AΑ	A	G Z	T	A I	ľΤ	GG

Species/Abbry	Group Name	
1. Aedes cinereus		ACTCAAGAAAGAGGGAAAAAGGAAAC
2. Aedes geniculatus		ACTCAAGAAAGAGGTAAAAAGGAAAC
3. Aedes hexodontus		ACTCAAGAAAGAGGGTAAAAGGAAAC
4. Aedes leucomelas		ACTCAAGAAAGAGGTAAAAAGGAAAC
5. Aedes nigrinus		ACTCAAGAGAGAGAGTAAGAAAC
6. Aedes pullatus		ACTCAAGAAAGTGGTAAAAAGGAAAC
7. Culiseta annulata		ACTCAAGAAAGAGGTAGAAAGGAAAC
8. Culiseta bergrothi		ACTCAAGAAAGAGGAAAAAGGAAAC
9. Aedes atropalpus		ACTCAAGAAAGAGGAAAAAAGGAAAC
10. Aedes aegypti		ACTCAAGAAAGCGGAAAAAAGGAAAC
11. Anopheles atroparvus		ACACAAGAAAGTGGTAAAAAGGAAAC
12. Aedes caspius		ACTCAAGAAAGTGGTAAAAAGGAAAC
13. Aedes rossicus		ACTCAAGAAAGAGGGAAAAAGGAAAC
14. Aedes vexans		ACTCAAGAAAGTGGTAAAAAGGAAAC
15. Aedes annulipes		ACTCAAGAAAGAGGTAAAAAGGAAAC
16. Aedes cantans		ACTCAAGAAAGAGGTAAAAAGGAAAC
17. Aedes cataphylla		ACTCAAGAAAGAGGTAAAAAGGAAAC
18. Aedes communis		ACTCAAGAAAGTGGAAAAAAGGAAAC
19. Aedes diantaeus		ACTCAAGAAAGAGGTAAAAAGGAAAC
20. Aedes intrudens		ACTCAAGAAAGAGGTAAAAAGGAAAC
21. Aedes punctor		ACTCAAGAAAGTGGAAAAAAGGAAAC
22. Aedes sticticus		ACTCAAGAAAGTGGTAAAAAGGAAAC
23. Coquillettidia richiardii		ACTCAAGAAAGAGGAAAAAAAGAAAC
24. Culex pipiens		ACTCAAGAAAGAGGAAAAAAGGAAAC
25. Culex torrentium		ACTCAAGAAAGAGGTAAAAAGGAAAC
26. Culiseta morsitans		ACACAAGAAGAGGAAAAAGGAAAC
27. Culiseta ochroptera		ACACAAGAAAGAGGTAAAAAGGAAAC
28. Aedes albopictus		A C A C A A G A A G A G G A A A A A A G G A A A C
29. Primer GB 1358 83F		ACTCAAGAAAGAGGTAAAAAGGAAAC

 $\label{eq:comparisons} Figure~2(A) (Supplementary~material): LCO1490~(above)~\&~GB_1358_83F~(below).~Sequence~comparisons~of~the~primer~binding~region~from~those~mosquito~species~where~this~region~has~been~independently~sequenced.$

Species/Abbry	Group Name ** * ** *
1. Anopheles algeriensis	TTCGGAGCTTGGGCGGAATAGT
2. Anopheles claviger	TTTGGGGCATGGGCAGGAATAGT
3. Aedes cinereus	TTTGGAGTTTGATCCGGAATAGT
4. Aedes rusticus	TTTGGAGTATGATCAGGAATAGT
5. Aedes geniculatus	TTTGGGGTATGATCAGGAATAGT
6. Aedes hexodontus	TTCGGAGTTTGATCTGGAATAGT
7. Aedes leucomelas	TTTGGAGTGTGATCAGGAATAGT
8. Aedes nigrinus	TTTGGTGTTTGATCAGGAATAGT
9. Aedes pullatus	TTCGGAGTTTGATCAGGAATAGT
10. Culiseta annulata	TTTGGAGCTTGAGCTGGAATAGT
11. Culiseta bergrothi	TTCGGAGCTTGAGCTGGAATAGT
12. Aedes atropalpus	TITGGAGTTTGATCCGGAATAAT
13. Aedes aegypti	TITGGAGTATGATCTGGAATAGT
14. Anopheles messeae	TTCGGAGCTTGAGCAGGAATAGT
15. Anopheles plumbeus	TITGGAGCCTGAGCTGGAATAGT
	TTCGGAGCTTGAGCAGGAATAGT
16. Anopheles beklemishevi	GAAGGAGTITGATCAGGAATAGT
17. Aedes caspius 18. Aedes vexans	TITGGAGTITGTTCTTGAATAGG
19. Aedes dorsalis	
20. Aedes cataphylla	
21. Aedes communis	
22. Aedes diantaeus	
23. Aedes detritus	TITGGGGTTTGATCAGGAATAGT
24. Aedes excrucians	TICGGAGTITGAGCAGGAATAGT
25. Aedes flavescens	TICGGAGTITGAGCAGGAATAGT
26. Aedes impiger	TITGGAGTITGATCAGGAATAGT
27. Aedes nigripes	TTTGGAGTTTGATCAGGAATAGT
28. Aedes punctor	TTCGGAGTTTGATCTGGAATAGT
29. Aedes sticticus	TTTGGTGTTTGATCAGGAATAGT
30. Culex territans	TTTGGTGCTTGAGCAGGAATAGT
31. Culex pipiens	TTTGGGGCTTGAGCTGGAATAGT
32. Culex torrentium	TTCGGAGCTTGAGCTGGAATAAT
33. Culiseta subochrea	TTTGGAGCTTGAGCTGGAA-AGT
34. Culiseta ochroptera	TTTGGAGCTTGAGCTGGAATAGT
35. Culiseta fumipennis	TTTGGAGCTTGAGCTGGAATAGT
36. Aedes albopictus	TTCGGTATITGATCTGGAATAGT
37. Aedes koreicus	TTCGGAGTTTGATCCGGAATAGT
38. Aedes japonicus	TTTGGTGTTTGATCCGGAATAGT
39. Aedes triseriatus	TTTGGAATTTGATCAGGAATAGT
40. Culex modestus	TTCGGAGCTTGASCTGGAATAGT
41. Coquillettidia richiardii	ATTTTGGACTGATCTGGAATAGT
42. gi 667752277 Culicoides grisescens	TTTGGAGCTTGAGCCGGAATAGT
43. gi 401879655 Lucilia sericata	TTTGGAGCTTGATCCGGAATAAT
44. gi 374093166 Bombyx mori	TTTGGTATTTGATCAGGAATAAT
45. Primer GB1310 29F	GAAGGAGTTTGATCAGGAATAGT

Figure 2(B)(Supplementary material): GB1310_29F. Sequence comparisons of the primer binding region from those mosquito species where this region has been independently sequenced.

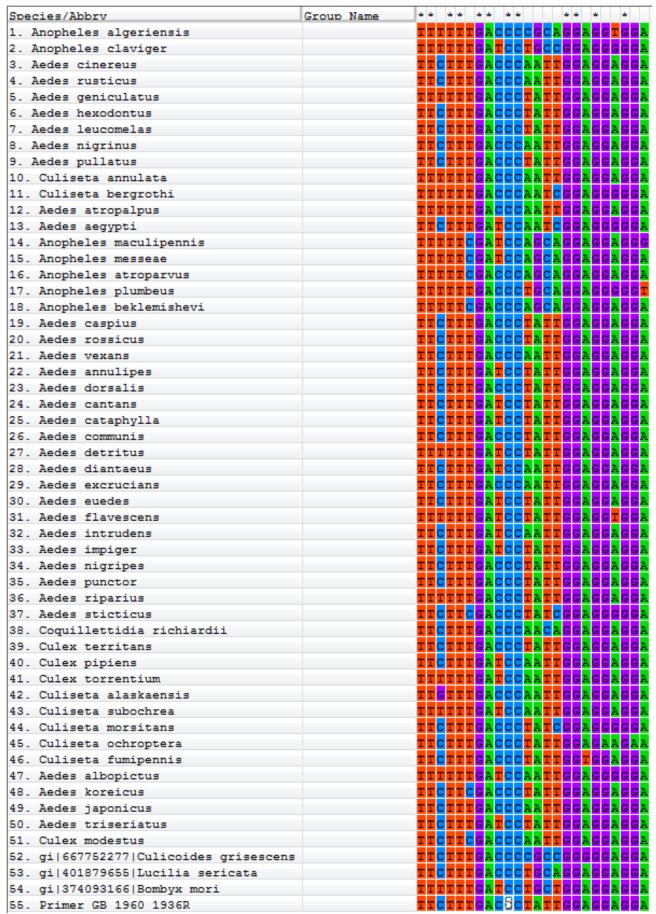


Figure 2(C)(Supplementary material): GB_1960_1936R. Sequence comparisons of the primer binding region from those mosquito species where this region has been independently sequenced.

Species/Abbrv	Group Na	ine				* *		
 Anopheles algeriensi: 	3						CCA <mark>G</mark>	
2. Anopheles claviger		I	GAT	TTTI			C C A <mark>G</mark> A	
3. Aedes cinereus		I	GAT	ICII			CCAG	
4. Anopheles maculipenn:	is	I	GAT	TTTI	TGG		CCTG	
5. Aedes leucomelas		I	GAT	TTTI	TGG.		TTAG	
6. Aedes pullatus		I	GAT	TTTI			C C A <mark>G</mark> F	
7. Culiseta bergrothi		I	GAT	TTTI			CCA <mark>G</mark>	
8. Aedes atropalpus		T	GAT	TTTI		GCAT	CCTG	AAGTTT
9. Aedes aegypti		T	GAT	TCT1	TGG	ACAC	CCAG	AAGTTT
 Anopheles atroparvu: 	3	T	GAT	TTTI	CGG	TCAC	CCTG	AAGTAT
11. Aedes caspius		T	GAT	TCT1	TGG.	ACAT	CCTG	AAGTTT
12. Aedes rossicus		T	GAT	ICTI	TGG.	ACAC	CCA <mark>G</mark>	AAGTTT
13. Aedes vexans		T	GAT	ICII	TGG.	ACAT	CCA <mark>G</mark>	AGTTT
14. Aedes annulipes		T	GAT	TTTI			C C A <mark>G</mark> A	
15. Aedes cantans		T	GAT	TTTI	TGG.	ACAT	C C A <mark>G</mark> A	AAGTTT
16. Aedes communis		T	G <mark>A</mark> T	TTTI	TGG.	ACAC	C C A <mark>G</mark> 7	AAGTTT
17. Aedes diantaeus		T	G <mark>A</mark> T	TTTI	TGG.	ACAC	C C A <mark>G</mark> A	AAGTTT
18. Aedes intrudens		T	G <mark>A</mark> T	TTTI	TGG.	ACAC	C C A <mark>G</mark> A	AAGTTT
19. Aedes punctor		T	G <mark>A</mark> T	TTTI	TGG.	ACAC	C C A <mark>G</mark> A	AAGTGT
20. Aedes sticticus		T	G <mark>A</mark> T	TCT1	TGG.	ACAC	CCTG	AAGTAT
21. Coquillettidia rich:	iardii	T	GAT	TTTI	TGG	CCAT	CCTG	A A G T A T
22. Culex pipiens		T	GAT	ICT1	TGG.	ACAT	C C A <mark>G</mark> A	AAGTTT
23. Culex torrentium		T	GAT	ICT1	TGG.	ACAT	C C A <mark>G</mark> A	AAGTTT
24. Culiseta morsitans		T	GAT	TTTI	TGG	G C A C	CCTG	AAGTTT
25. Culiseta ochroptera		T	GAT	TTTI	TGG.	ACAC	C C A <mark>G</mark> A	AAGTTT
26. Aedes albopictus		T	GAT	TTTI	TGG	TCAT	C C A <mark>G</mark> A	A A G T T T
27. Primer HCO2198		T	GAT	TTTI	TGG	TCAC	CCTG	AGTTT
Species/Abbry	Group Name							
1. Aedes cinereus		TAATA	TGG	CAG	TTA	GTGC	AATG	AATTT
2. Aedes geniculatus		TAATA		CAG		GTGC		
3. Culiseta bergrothi		TAATA				GIGC		AATTT
4. Aedes pullatus			TGG					AATTT
5. Aedes atropalpus		TAATA	TGG	CAG		GTGC	AATC	AATTT
6. Aedes hexodontus		TAATA	TGG			GIGC	ATTG	GAAGG
7. Aedes communis		TAATA		CAG		GIGC	A A T C	
8. Anopheles claviger		CTATA	TGC			TATA	TOTO	TOTAL
9. Aedes rusticus		CNAR	T C C		TTN	TOO	C T T T	CANAC
		TARAL	T C C	-7-	T I A			TOODS.
10. Culiseta annulata		TAATA	1 G G	AAA	AIT	A G III G	- G G T	1666A
11. Primer TL2-N-3014R		TAATA	IGG	C A G I	TTA	GIGC	ATTG	<u> </u>

Figure 2(D)(Supplementary Material): HCO2198 (above) & TL2-N-3014R (below). Sequence comparisons of the primer binding region from those mosquito species where this region has been independently sequenced.

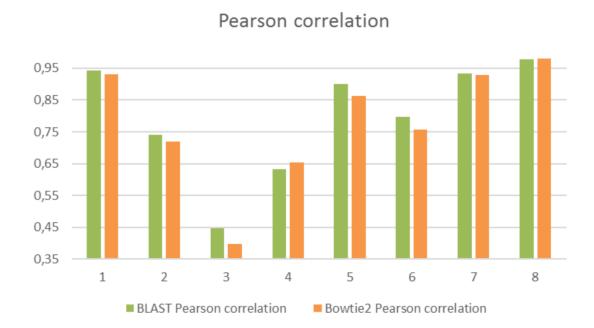


Figure 3 (Supplementary Material): Pearson correlation between input fraction for each mosquito species and resulting fractions using Blast- or Bowtie2-based identification. Mock communities 1-6 are composed of morphologically identified mosquitoes and mock communities 7 and 8 are composed of mosquitoes individually identified by COI barcoding.

Table 1(Supplementary material): Description of the mock community samples used to test the metabarcoding method.

Species	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8
Anopheles algeriensis	0	0	0	4	0	0	0	2
Anopheles atroparvus	0	0	0	0	0	0	0	0
Anopheles beklemishevi	0	0	0	0	0	0	0	0
Anopheles claviger	0	8	0	0	7	0	1	2
Anopheles maculipennis	0	0	0	0	0	15	0	0
Anopheles messeae	0	0	12	0	0	0	0	0
Anopheles plumbeus	0	0	0	0	0	0	0	0
Aedes cinereus/geminus/rossicus	40	0	1	17	0	20	0	3
Aedes vexans	5	0	0	0	12	0	0	34
Aedes refiki	0	0	0	0	0	0	0	1
Aedes geniculatus	0	10	0	0	0	0	0	3
Aedes annulipes/cantans/excrucians	0	20	6	17	0	0	22	0
Aedes caspius	0	0	0	0	0	0	0	0
Aedes cataphylla	0	20	0	15	10	0	1	0
Aedes communis	15	0	0	0	0	23	9	0
Aedes cyprius	0	0	0	0	0	0	0	0
Aedes detritus	0	0	0	0	0	0	0	0
Aedes intrudens/diantaeus	0	0	18	0	0	0	0	0
Aedes dorsalis	0	0	0	10	0	0	0	0
Aedes euedes	0	0	0	0	0	0	0	0
Aedes flavescens	0	0	0	3	0	0	0	0
Aedes hexodontus/punctor/punctodes	0	40	10	0	22	0	4	5
Aedes impiger	0	0	0	0	0	0	0	0
Aedes leucomelas	0	0	0	0	0	0	0	2
Aedes nigripes	0	0	0	0	0	0	0	0
Aedes pionips	0	0	0	0	0	0	0	0
Aedes pullatus	0	0	32	0	0	0	0	0
Aedes riparius	0	0	0	0	0	0	0	0
Aedes sticticus/rusticus/nigrinus	10	0	2	0	15	40	0	1
Coquillettidia richiardii	8	0	17	30	0	0	0	0
Culex territans	0	0	0	0	0	0	0	0
Culex pipiens	2	0	0	0	5	0	0	1
Culex torrentium	0	0	0	0	0	0	0	0
Culiseta annulata	0	1	0	0	0	2	0	0
Culiseta alaskaensis	0	1	0	0	0	0	0	1
Culiseta bergrothi	20	0	0	0	29	0	0	1
Culiseta subochrea	0	0	0	0	0	0	0	1
Culiseta fumipennis	0	0	0	0	0	0	0	0
Culiseta morsitans	0	0	2	0	0	0	0	2
Culiseta ochroptera	0	0	0	4	0	0	0	1
Aedes albopictus	0	0	0	0	0	0	0	0
Aedes koreicus	0	0	0	0	0	0	0	0
Aedes japonicus	0	0	0	0	0	0	0	0
Aedes triseratus	0	0	0	0	0	0	0	0
Aedes atropalpus	0	0	0	0	0	0	0	0
Aedes aegypti	0	0	0	0	0	0	0	0
TOTAL	100	100	100	100	100	100	37	60
IVIAL	100	100	100	100	100	100	31	00