

Supplementary Material-IV (S-IV)

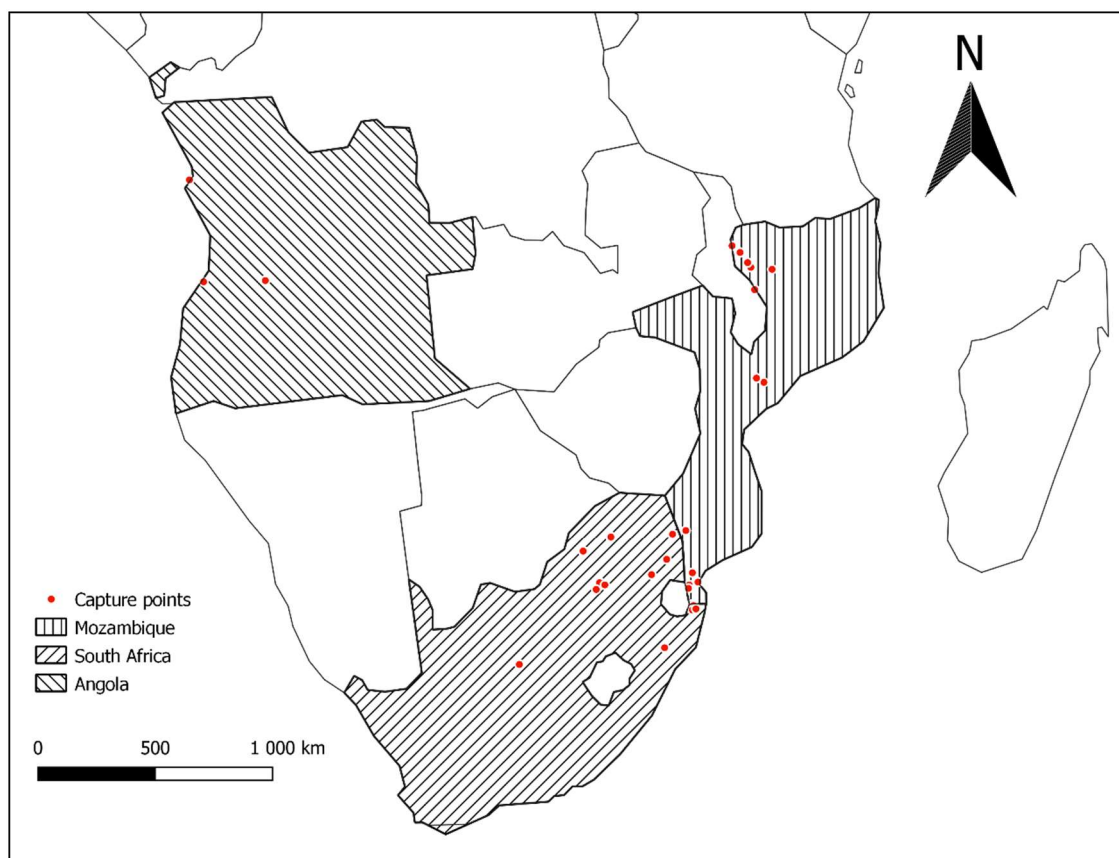


Figure S-IV 1. Map of Africa showing the three countries, and capture points, of origin for the mosquito collections analysed in this work (i.e. South Africa, Angola and Mozambique).

Table S-IV 1. Mosquitoes species for which genitalia (male or female) were analysed in the study. Their taxonomic placement within the Culicidae subfamily is indicated, as well as the number of specimens observed by country of collection [South Africa (ZA), Mozambique (MZ), Angola (AO)]. The total number os specimens analysed is also indicated.

Taxonomic level				Country			
Tribe	Genus	Subgenus	Species epithet	ZA	MZ	AO	Total
<i>Aedeomyiini</i>	<i>Aedeomyia</i>	<i>Aedeomyia</i>	<i>africana</i>	-	6	-	6
<i>Aedini</i>	<i>Aedes</i>	<i>Aedimorphus</i>	<i>dentatus</i>	4	-	-	4
			<i>durbanensis</i>	6	-	-	6
			<i>eritreae</i>	1	-	-	1
			<i>fowleri</i>	5	-	-	5
			<i>hirsutus</i>	4	-	-	4
			<i>leesoni</i>	1	-	-	1
			<i>quasiunivittatus</i>	5	-	-	5
		<i>Albuginosus</i>	<i>capensis</i>	1	-	-	1
		<i>Catageomyia</i>	<i>minutus</i>	1	-	-	1
		<i>Diceromyia</i>	<i>fascipalpis</i>	2	-	-	2
			<i>furcifer</i>	2	-	-	2
		<i>Fredwardsius</i>	<i>vittatus</i>	22	3	-	25
		<i>Mucidus</i>	<i>mucidus</i>	-	1	-	1
		<i>Neomelaniconion</i>	<i>circumluteolus</i>	14	-	-	14
			<i>mcintoshii</i>	38	-	-	38
		<i>Ochlerotatus</i>	<i>caballus</i>	2	-	-	2
		<i>Stegomyia</i>	<i>aegypti</i>	9	14	6	29
			<i>metallicus</i>	1	-	-	1
			<i>unilineatus</i>	23	-	-	23
		<i>Eretmapodites</i>	<i>quinquevittatus</i>	-	1	-	1
			<i>subsimplicipes</i>	-	4	-	4
<i>Culicini</i>	<i>Culex</i>	<i>Culex</i>	<i>andersoni</i>	1	-	-	1
			<i>argenteopunctatus</i>	1	-	-	1
			<i>decens</i>	1	-	-	1
			<i>duttoni</i>	3	1	-	4
			<i>perexiguus</i>	4	2	-	6
			<i>pipiens</i>	19	1	-	20
			<i>simpsoni</i>	3	12	-	15
			<i>telesilla</i>	18	-	-	18
			<i>theileri</i>	18	-	-	18
			<i>trifilatus</i>	8	-	-	8
			<i>tritaeniorhynchus</i>	1	-	-	1
			<i>antennatus</i>	33	1	-	34
			<i>neavei</i>	22	6	-	28
			<i>quinquefasciatus</i>	22	51	48	121
			<i>univittatus</i>	58	1	1	60
		<i>Culiciomyia</i>	<i>cinereus</i>	-	1	-	1
			<i>nebulosus</i>	-	7	-	7
		<i>Eumelanomyia</i>	<i>inconspicuus</i>	4	-	-	4

		<i>Oculomyia</i>	<i>annulioris</i>	3	8	-	11
			<i>infula</i>	-	1	-	1
			<i>bitaeniorhynchus</i>	3	2	-	5
			<i>poicilipes</i>	17	4	-	21
	<i>Lutzia</i>	<i>Metalutzia</i>	<i>tigripes</i>	4	1	9	14
<i>Ficalbiini</i>	<i>Ficalbia</i>		<i>uniformis</i>	1	-	-	1
	<i>Mimomyia</i>	<i>Mimomyia</i>	<i>hispida</i>	1	-	-	1
			<i>mimomyiaformis</i>	10	-	-	10
<i>Mansoniini</i>	<i>Coquillettidia</i>	<i>Coquillettidia</i>	<i>chrysosoma</i>	3	-	-	3
			<i>fuscopennata</i>	5	-	-	5
			<i>metallica</i>	-	1	-	1
	<i>Mansonia</i>	<i>Mansonioides</i>	<i>africana</i>	1	44	-	45
			<i>uniformis</i>	2	13	-	15
<i>Uranotaeniini</i>	<i>Uranotaenia</i>	<i>Pseudoficalbia</i>	<i>mashonaensis</i>	5	-	-	5
		<i>Uranotaenia</i>	<i>alboabdominalis</i>	1	-	-	1
Total				413	186	64	663

Table S-IV 2. Mosquito species for which partial *coxI* coding sequences were amplified by PCR. Their taxonomic position within the Culicinae subfamily, mean intraspecific diversity, number of specimens analysed per country, total number specimens analysed, and specimen code, are also indicated.

Tribe	Genus	Subgenus	Species epithet	Intraspecific Variation (% \pm DS)	ZA	MZ	AO	Total	Codes
<i>Aedeomyiini</i>	<i>Aedeomyia</i>	<i>Aedeomyia</i>	<i>africana</i>	0.1 \pm 0.1	0	4	0	4	EM245, EM246, EM247, EM248
		<i>Lepiothauma</i>	<i>furfurea</i>	0.4 \pm 0.2	2	1	0	3	EM257, EM258, EM259
<i>Aedini</i>	<i>Aedes</i>	<i>Aedimorphus</i>	<i>quasiunivittatus</i>	2.7 \pm 0.6	4	0	0	4	EM147, EM148, EM149, EM151
			<i>cumminsii</i>	- -	2	0	0	2	EM167, EM169
			<i>dentatus</i>	1.0 \pm 0.3	7	0	0	7	EM158, EM168, EM170, EM172, EM187, EM189, EM190
			<i>eritreae</i>	0.2 \pm 0.1	4	0	0	4	EM177, EM178, EM179, EM180
			<i>durbanensis</i>	0.1 \pm 0.1	3	0	0	3	EM174, EM175, EM176
			<i>fowleri</i>	0.3 \pm 0.2	2	2	0	4	EM134, EM135, EM137, EM138
			<i>hirsutus</i>	0.2 \pm 0.2	3	0	0	3	EM199, EM234, EM237
			<i>natronius</i>	0.0 \pm 0.0	0	2	0	2	EM224, EM225
			<i>ochraceus</i>	- -	1	0	0	1	EM266
		<i>Albuginosus</i>	<i>haworthi</i>	- -	1	0	0	1	EM262
			<i>capensis</i>	- -	1	0	0	1	EM146
		<i>Catageomyia</i>	<i>argenteopunctatus</i>	1.8 \pm 0.4	4	0	0	4	EM238, EM260, EM261, EM264
			<i>microstictus</i>	- -	1	0	0	1	EM 265
		<i>Diceromyia</i>	<i>fascipalpis</i>	- -	2	0	0	2	EM161, EM162
			<i>furcifer</i>	- -	1	0	0	1	EM163
		<i>Fredwardsius</i>	<i>vittatus</i>	0.7 \pm 0.3	3	1	0	4	EM083, EM085, EM087, EM198
		<i>Mucidus</i>	<i>mucidus</i>	- -	0	1	0	1	EM153
			<i>sudanensis</i>	- -	2	0	0	2	EM154, EM155
		<i>Neomelaniconion</i>	<i>circunluteolus</i>	0.9 \pm 0.3	4	2	0	6	EM128, EM129, EM130, EM131, EM132, EM133
			<i>mcintoshii</i>	0.9 \pm 0.3	8	1	0	9	EM100, EM101, EM102, EM103, EM104, EM136, EM181, EM183, EM185
			<i>unidentatus</i>	0.4 \pm 0.3	2	0	0	2	EM344, EM345
		<i>Ochlerotatus</i>	<i>caballus</i>	0.5 \pm 0.2	4	0	0	4	EM164, EM165, EM267, EM268
		<i>Stegomyia</i>	<i>aegypti</i>	0.5 \pm 0.2	2	3	2	7	EM285, EM291, EM293, EM296, EM297, EM298, EM299

Culicini	Eretmapodites		metallicus	2.1 ± 0.5	3	1	0	4	EM249, EM286, EM288, EM289	
			simpsoni	0.0 ± 0.0	3	0	0	3	EM221, EM222, EM223	
			unilineatus	1.0 ± 0.3	4	0	0	4	EM077, EM078, EM079, EM082	
			quinquevittatus	- -	0	1	0	1	EM270	
			subsimplipices	0.0 ± 0.0	0	3	0	3	EM254, EM255, EM256	
	Culex	Culex	duttoni	0.4 ± 0.3	3	1	0	4	EM139, EM140, EM141, EM145	
			andersoni	0.1 ± 0.1	4	0	0	4	EM031, EM191, EM192, EM195	
			antennatus	0.0 ± 0.0	2	3	0	5	EM060, EM061, EM062, EM063, EM064	
			decens	- -	1	0	0	1	EM074	
			sp.	- -	0	0	1	1	EM331	
			neavei	2.4 ± 0.6	0	6	1	7	EM016, EM028, EM029, EM030, EM041, EM046, EM328	
			univittatus	0.4 ± 0.1	9	0	0	9	EM012, EM013, EM017, EM021, EM036, EM038, EM045, EM094, EM274	
			perexiguus	0.2 ± 0.1	6	2	0	8	EM014, EM040, EM049, EM095, EM096, EM097, EM098, EM272	
			telesilla	0.3 ± 0.2	4	0	0	4	EM117, EM119, EM120, EM121	
			pipiens	1.5 ± 0.4	8	1	0	9	EM300, EM301, EM302, EM303, EM304, EM305, EM306, EM326, EM332	
			quinquefasciatus	0.0 ± 0.0	6	7	9	22	EM307, EM308, EM309, EM310, EM311, EM312, EM313, EM314, EM315, EM316, EM318, EM319, EM320, EM322, EM323, EM327, EM333, EM334, EM335, EM339, EM340, EM341	
			simpsoni	0.2 ± 0.1	3	3	0	6	EM024, EM025, EM026, EM032, EM033, EM160	
			argenteopunctatus	- -	1	0	0	1	EM159	
			theileri	0.0 ± 0.0	3	0	0	3	EM073, EM075, EM193	
			trifilatus	0.1 ± 0.1	4	0	0	4	EM090, EM091, EM092, EM093	
			thalassius	- -	1	0	0	1	EM263	
			tritaeniorhynchus	0.6 ± 0.3	1	1	0	2	EM157, EM269	
			Culiciomyia	cinereus	- -	0	1	0	1	EM111
				nebulosus	0.3 ± 0.2	2	1	0	3	EM112, EM113, EM115
			Eumelanomyia		inconspicuus	0.7 ± 0.3	3	0	0	3

		<i>Oculeomyia</i>	<i>annulioris</i>	0.5 ± 0.2	5	2	0	7	EM105, EM106, EM107, EM108, EM109, EM125, EM127
			<i>infula</i>	- -	0	1	0	1	EM152
			<i>poicilipes</i>	- -	1	2	0	3	EM065, EM066, EM068
			<i>bitaeniorhynchus</i>	- -	1	0	0	1	EM124
	<i>Lutzia</i>	<i>Metalutzia</i>	<i>tigripes</i>	0.6 ± 0.3	2	0	2	4	EM055, EM056, EM057, EM058
<i>Ficalbiini</i>	<i>Mimomyia</i>	<i>Mimomyia</i>	<i>hispida</i>	0.7 ± 0.3	3	0	0	3	EM276, EM277, EM278
			<i>mimomyiaformis</i>	0.5 ± 0.2	7	0	0	7	EM144, EM226, EM230, EM231, EM232, EM239, EM281
	<i>Ficalbia</i>		<i>uniformis</i>	- -	1	0	0	1	EM252
<i>Mansoniini</i>	<i>Coquillettidia</i>	<i>Coquillettidia</i>	<i>fuscopennata</i>	0.2 ± 0.1	7	0	0	7	EM201, EM202, EM203, EM204, EM205, EM218, EM219
			<i>metallica</i>	- -	0	1	0	1	EM253
			<i>chrysosoma</i>	0.0 ± 0.0	2	0	0	2	EM250, EM251
	<i>Mansonia</i>	<i>Mansonioides</i>	<i>africana</i>	0.6 ± 0.2	0	5	0	5	EM001, EM008, EM010, EM011, EM211
			<i>uniformis</i>	0.4 ± 0.2	2	1	0	3	EM206, EM209, EM210
<i>Uranotaeniini</i>	<i>Uranotaenia</i>	<i>Pseudoficalbia</i>	<i>mashonaensis</i>	4.1 ± 0.8	3	0	0	3	EM212, EM213, EM279
		<i>Uranotaenia</i>	<i>alboabdominalis</i>	0.2 ± 0.1	4	0	0	4	EM214, EM215, EM216, EM217
Total					172	60	15	247	

* South Africa (ZA), Mozambique (MZ), Angola (AO). Numbers and codes in bold indicate sequence association with genitalia. Codes in red refer to sequences that were not considered in the analysis, due to their size

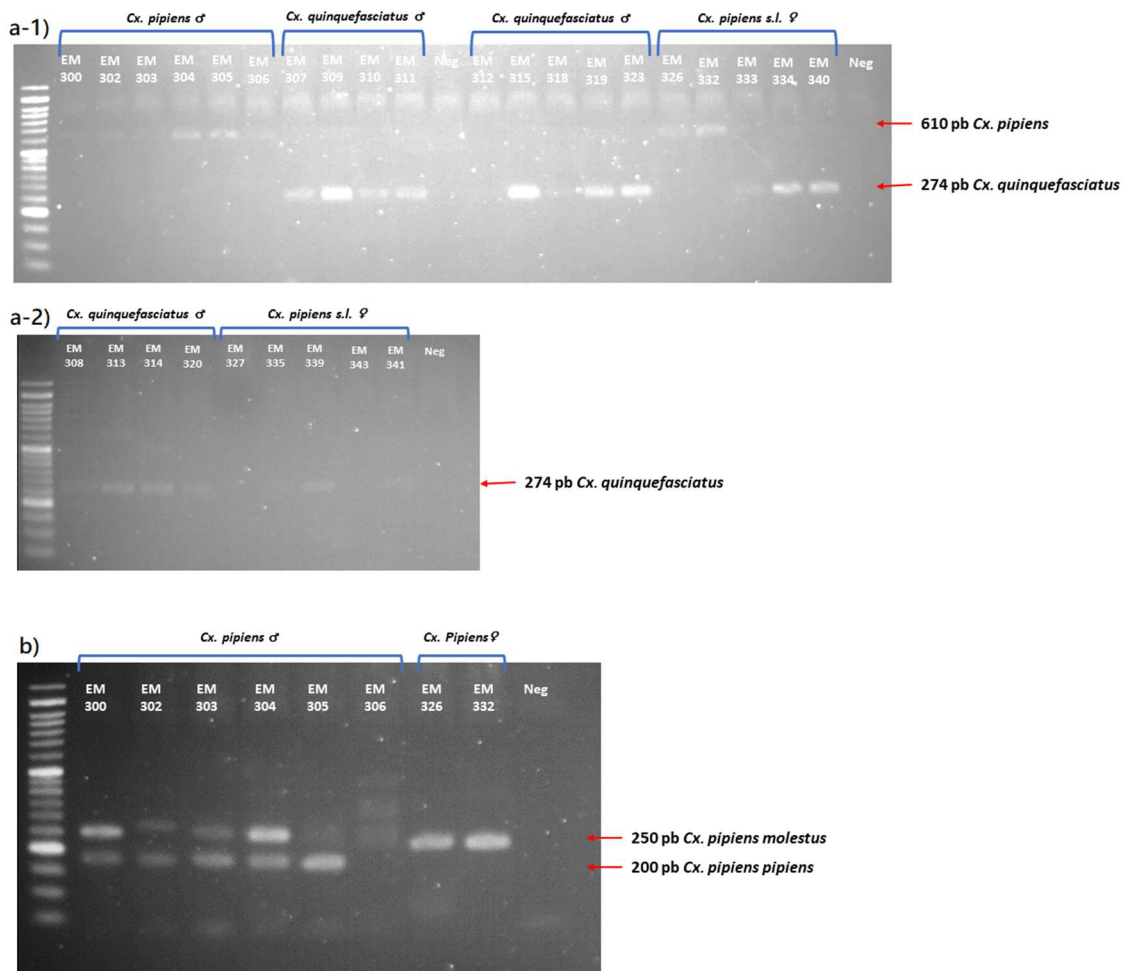
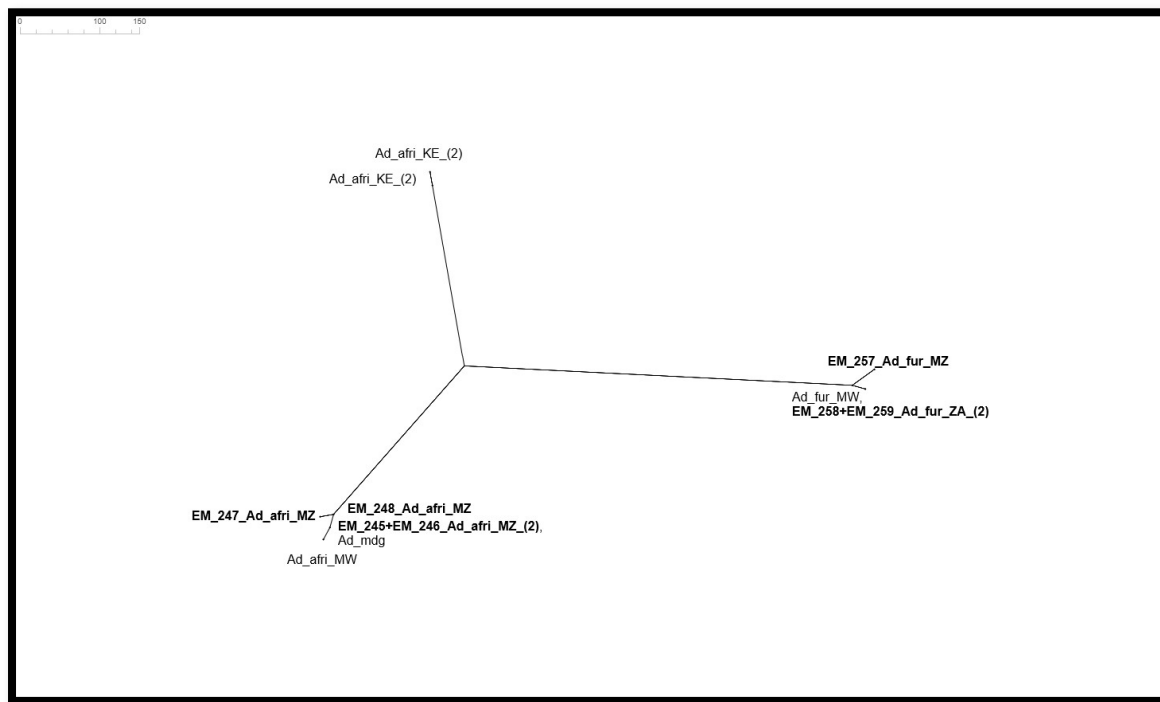


Figure S-IV 2. **a-1 and a-2)** Electrophoretic profile on a 2% agarose gel of the PCR product of amplification of the ACE2 gene from *Cx. pipiens* and *Cx. quinquefasciatus*. The bands correspond to the ACE2-specific amplicons expected for *Cx. pipiens* (610 bp) and *Cx. quinquefasciatus* (274 bp). **b)** Agarose gel (1.5%) electrophoresis of PCR products corresponding to the CQ11 microsatellite flanking region of *Cx. pipiens*. The 250 bp and 200 bp bands corresponded to those expected for *Cx. pipiens* ecotypes *molestus* and *pipiens* respectively.

Table S-IV 3. Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Ad. furfurea*, *Ad. africana* from Mozambique (MZ) and Malawi (MW), *Ad. africana* from Kenya (KE), *Ad. madagascarica* and *Ad. catasticta*. The indicated values have been calculated using the K2P model.

	Nº of sequences	Intraspecific variation (%)	Interspecific variation (%)			
			1	2	3	4
1 <i>Ad. catasticta</i>	1	-	-			
2 <i>Ad. madagascarica</i> MG	1	-	-	12.6 (± 1.8)		
3 <i>Ad. africana</i> KE	4	0.1 (± 0.1)	12.2 (± 1.8)	6.9 (± 1.3)		
4 <i>Ad. africana</i> MZ-MW	5	0.3 (± 0.2)	12.8 (± 1.8)	0.2 (± 0.1)	6.9 (± 1.3)	
5 <i>Ad. furfurea</i>	4	0.3 (± 0.2)	12.9 (± 1.8)	10.9 (± 1.6)	10.5 (± 1.6)	10.9 (± 1.6)

a)



b)

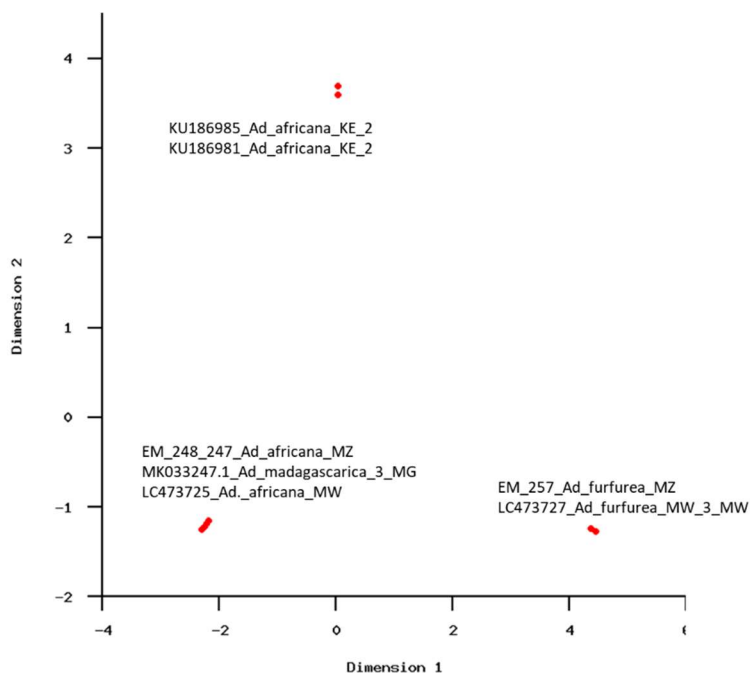
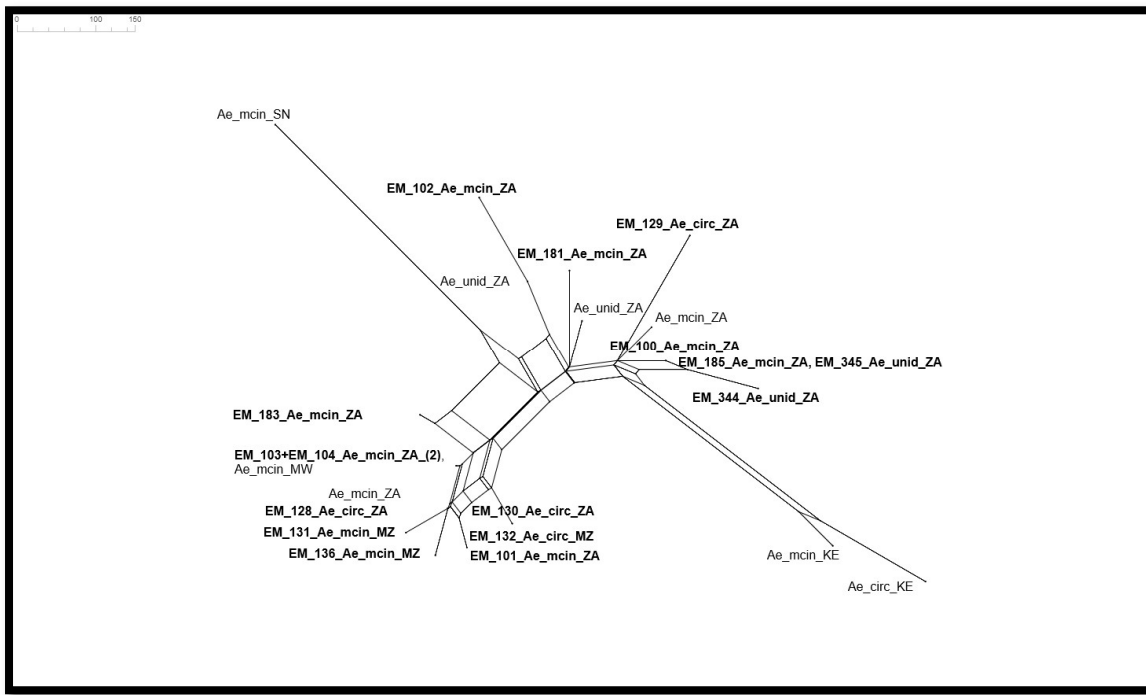


Figure S-IV 3. Networks **a)** and PCOORD **b)**, analysis of 14 partial *coxI* gene amplified from *Aedeomyia* mosquitoes. The sequences obtained in this work have been designated with the “EM” code and are boldface. Reference sequences downloaded from the public databases and are shown by their respective access code (Boldsystems) or accession number (GenBank), as well as country of origin [South Africa (ZA) , Madagascar (MG), Malawi (MW), Mozambique (MZ), Kenya (KE)] are also indicated.

Table S-IV 4. Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Ae. circumluteolus*, *Ae. mcintoshi*, *Ae. unidentatus* and *Ae. lineatopennis*. The indicated values have been calculated using the K2P model.

	N° of sequences	Intraspecific variation (%)	Interspecific variation (%)		
			1	2	3
<i>Ae. circumluteolus</i>	5	1.6 (± 0.4)			
<i>Ae. mcintoshi</i>	15	1.2 (± 0.3)	1.3 (± 0.3)		
<i>Ae. unidentatus</i>	4	0.8 (± 0.3)	1.4 (± 0.3)	1.1 (± 0.3)	
<i>Ae. lineatopennis</i>	2	0.4 (± 0.3)	5.7 (± 1.0)	5.6 (± 1.0)	5.4 (± 1.0)

a)



b)

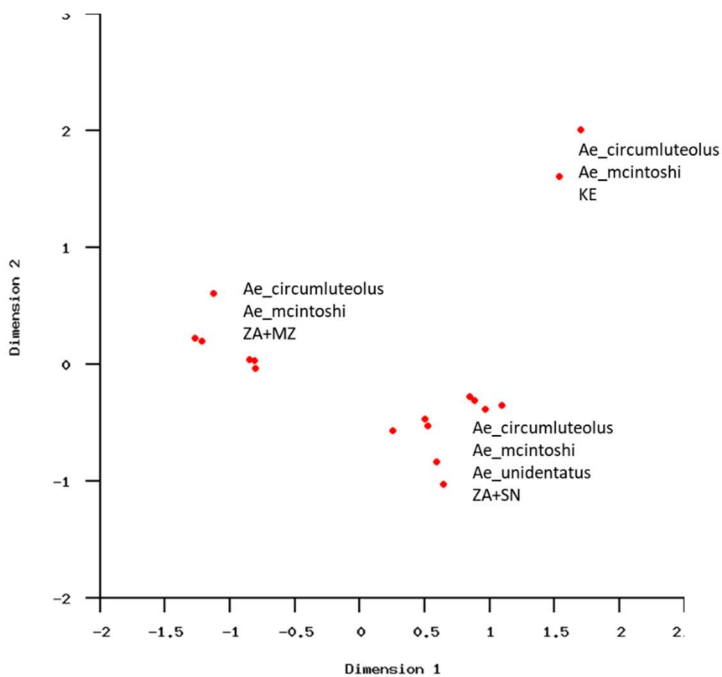
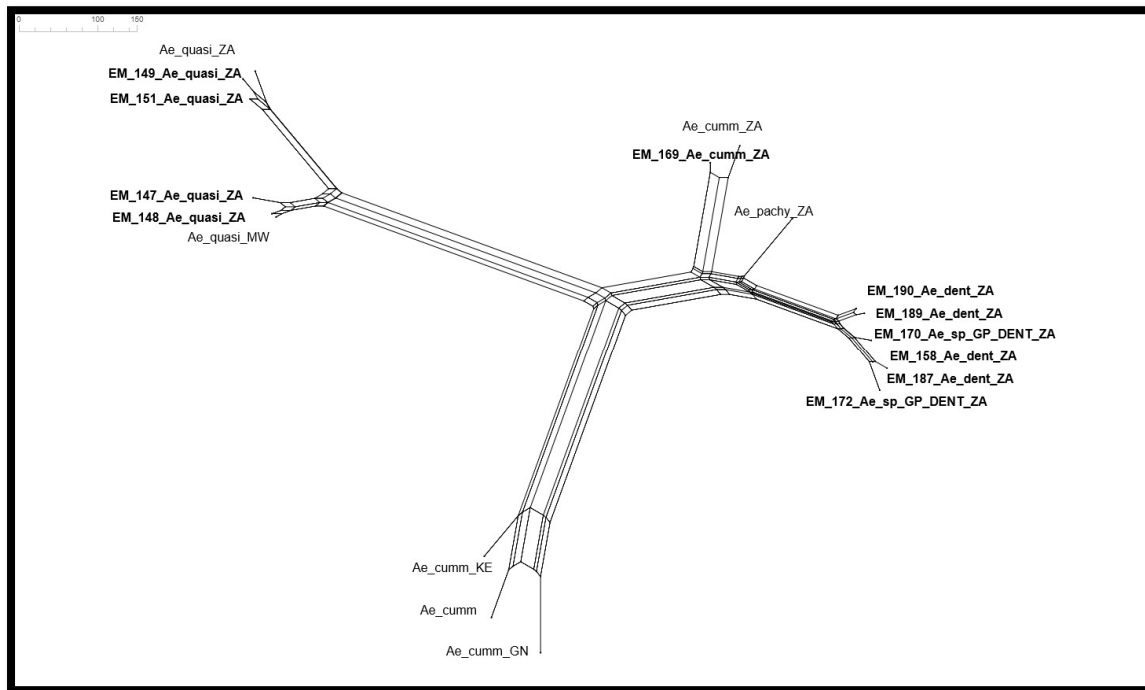


Figure S-IV 4. Networks **a)** and PCOORD **b)**, analysis of 24 partial *coxI* nucleotide sequences from mosquitoes of the genus *Aedes*, subgenus *Neomelaniconion*, except for *Ae. lineatopennis*. The sequences obtained in this work are indicated with the “EM” code and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Mozambique (MZ), Kenya (KE), Senegal (SN)] are also indicated.

Table S-IV 5. Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Ae. cumminsii* from Kenya (KE), Senegal (SN), Guinea (GN), and South Africa (ZA). The indicated values have been calculated using the K2P model.

		N° of sequences	Intraspecific variation (%)	Interspecific variation (%)		
				1	2	3
1	<i>Ae. cumminsii</i> KE	3	1.4 (± 0.4)			
2	<i>Ae. cumminsii</i> SN	1	- -	0.7 (± 0.2)		
3	<i>Ae. cumminsii</i> GN	1	- -	2.3 (± 0.6)	2.1 (± 0.7)	
4	<i>Ae. cumminsii</i> ZA	5	1.2 (± 0.4)	7.5 (± 1.2)	7.4 (± 1.2)	8.2 (± 1.3)

a)



b)

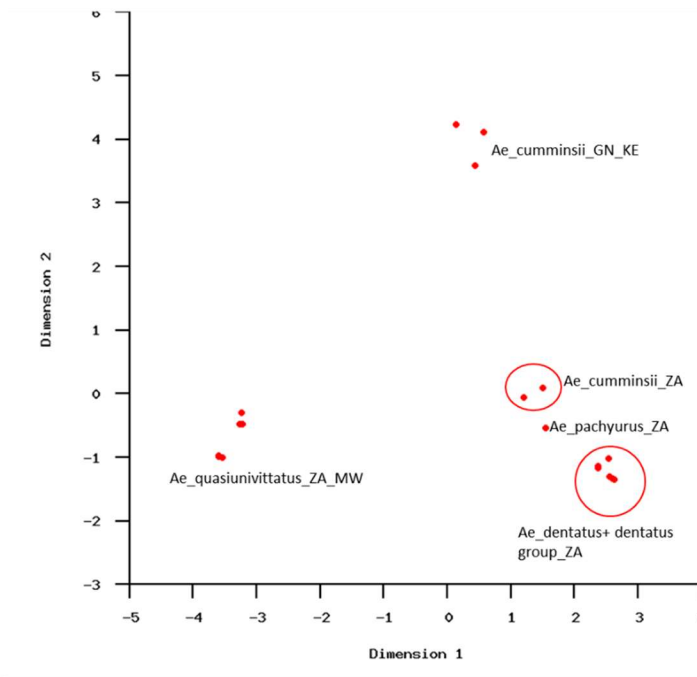
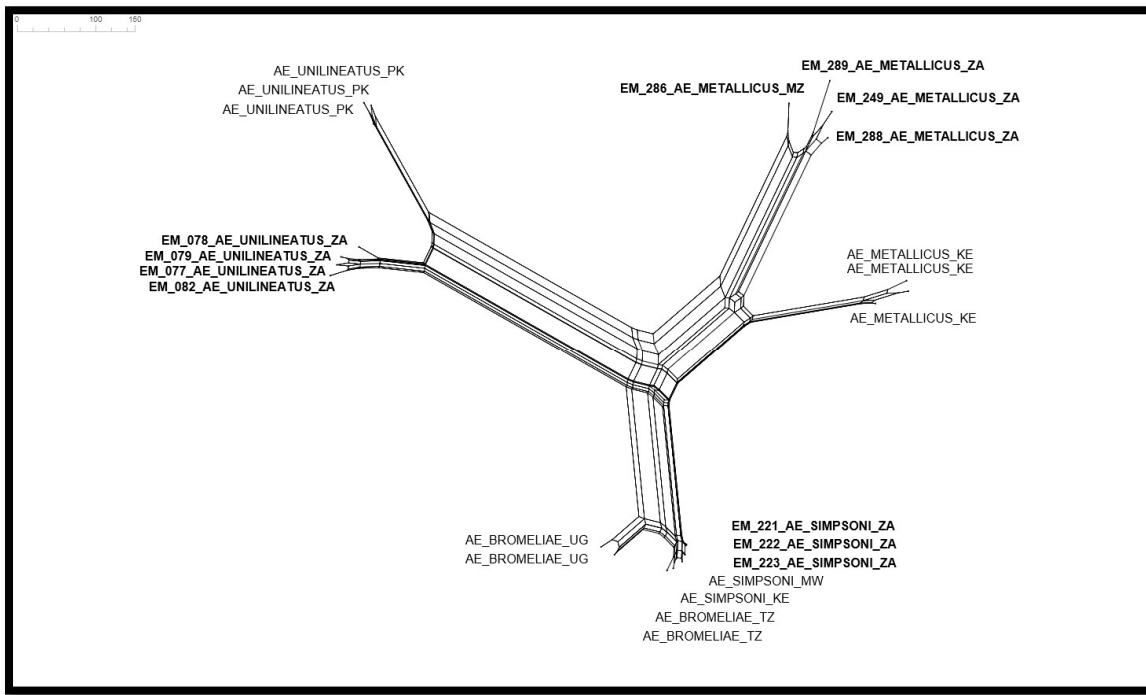


Figure S-IV 5. Networks **a)** and PCOORD **b)**, analysis of 18 partial *coxI* nucleotide sequences from mosquitoes of the genus *Aedes*, subgenus *Aedimorphus*. The sequences obtained in this work have the “EM” code and are boldface, and those with an associated genitalia photo are indicated by the horizontal arrow. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Guinea (GN), Mozambique (MZ), Malawi (MW), Kenya (KE)] are also indicated.

a)



b)

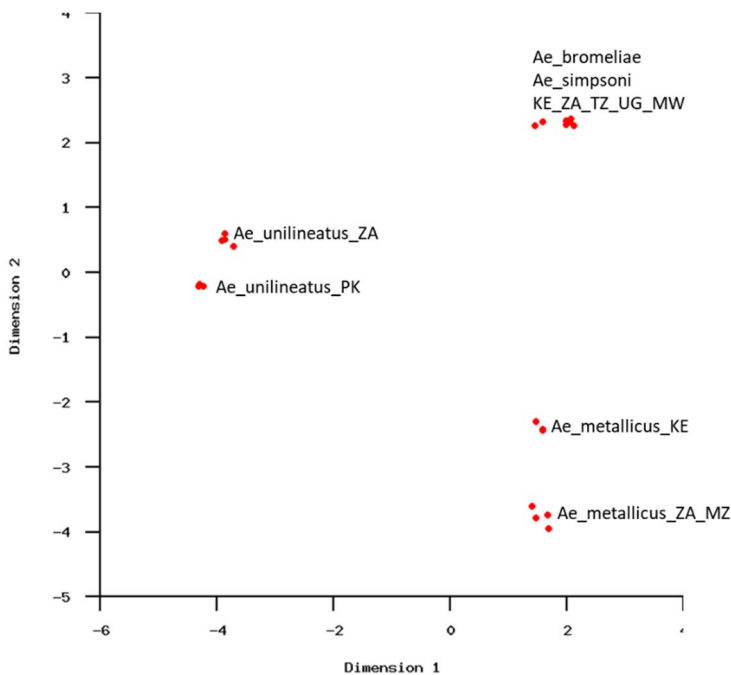
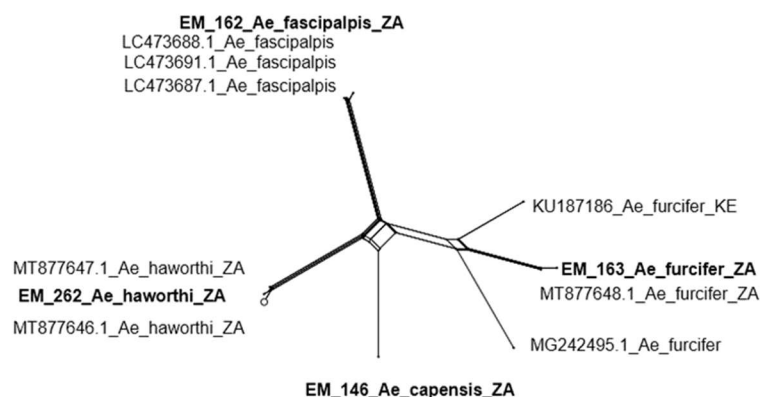


Figure S-IV 6. Networks **a)** and PCOORD **b)**, analysis of 23 partial *coxI* nucleotide sequences from mosquitoes of the genus *Aedes*, presenting the subgenus *Stegomyia*. The sequences obtained in this work are indicated with the “EM”code, and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Malawi (MW), Mozambique (MZ), Pakistan (PK), Kenya (KE), Tanzania (TZ), Uganda (UG)] are also indicated.

a)

0 0.02 0.04 0.06 0.074

Fit: 99.98



b)

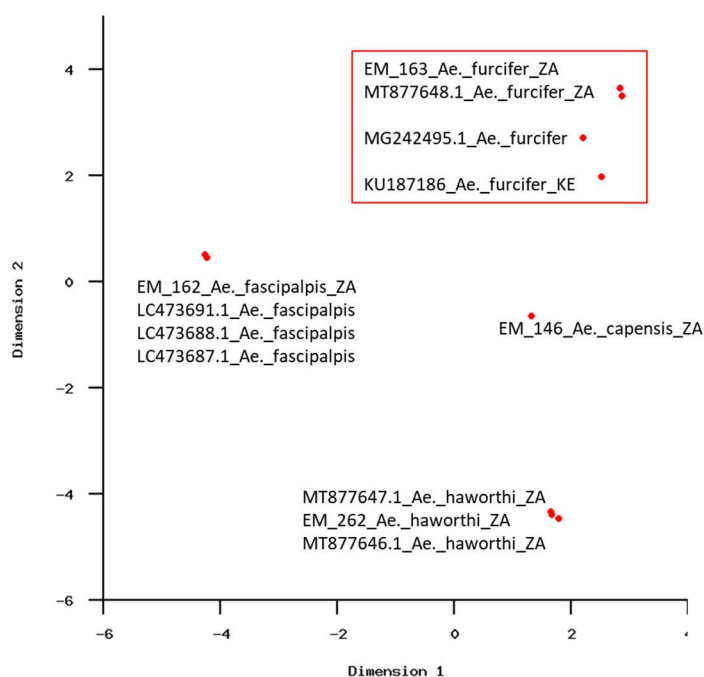
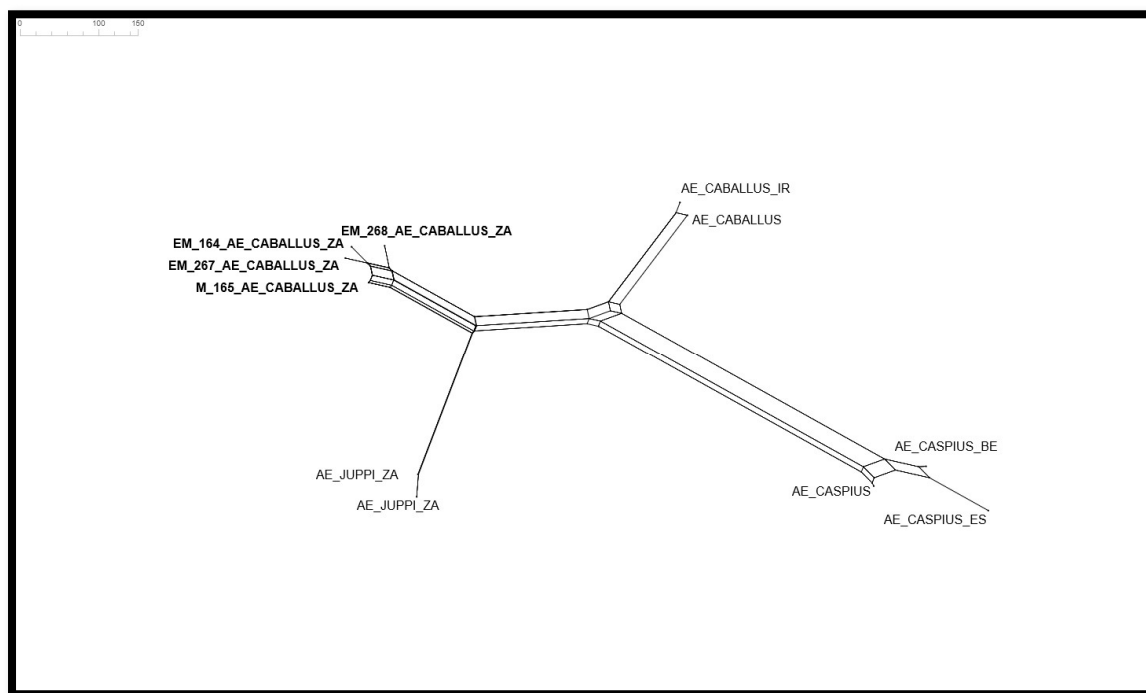


Figure S-IV 7. Networks a) and PCOORD b), analysis of 12 partial *coxI* nucleotide sequences from mosquitoes of the genus *Aedes*, presenting subgenera *Diceromyia* and *Albuginosus*. Sequences obtained in this work are indicated with the “EM”code, and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Kenya (KE)] are also indicated.

Table S-IV 6. Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Ae. caballus* from South Africa (ZA), Iran (IR) and *Ae. juppi* from ZA. The indicated values have been calculated using the K2P model.

	N° of sequences	Intraspecific variation (%)	Interspecific variation (%)	
			1	2
1 <i>Ae. caballus</i> ZA	4	0.5 (± 0.2)		
2 <i>Ae. juppi</i> ZA	2	0.2 (± 0.2)	2.8 (± 0.7)	
3 <i>Ae. caballus</i> IR	2	0.2 (± 0.2)	3.6 (± 0.8)	4.0 (± 0.9)

a)



b)

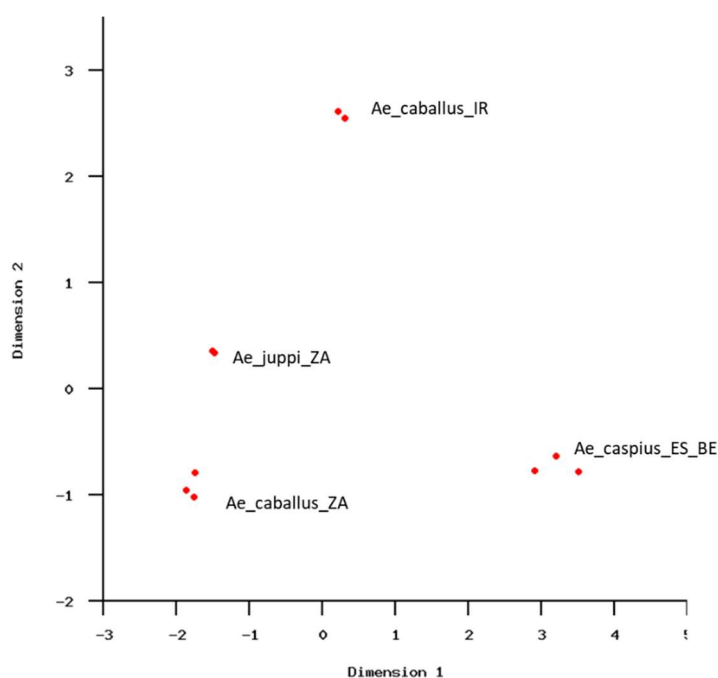
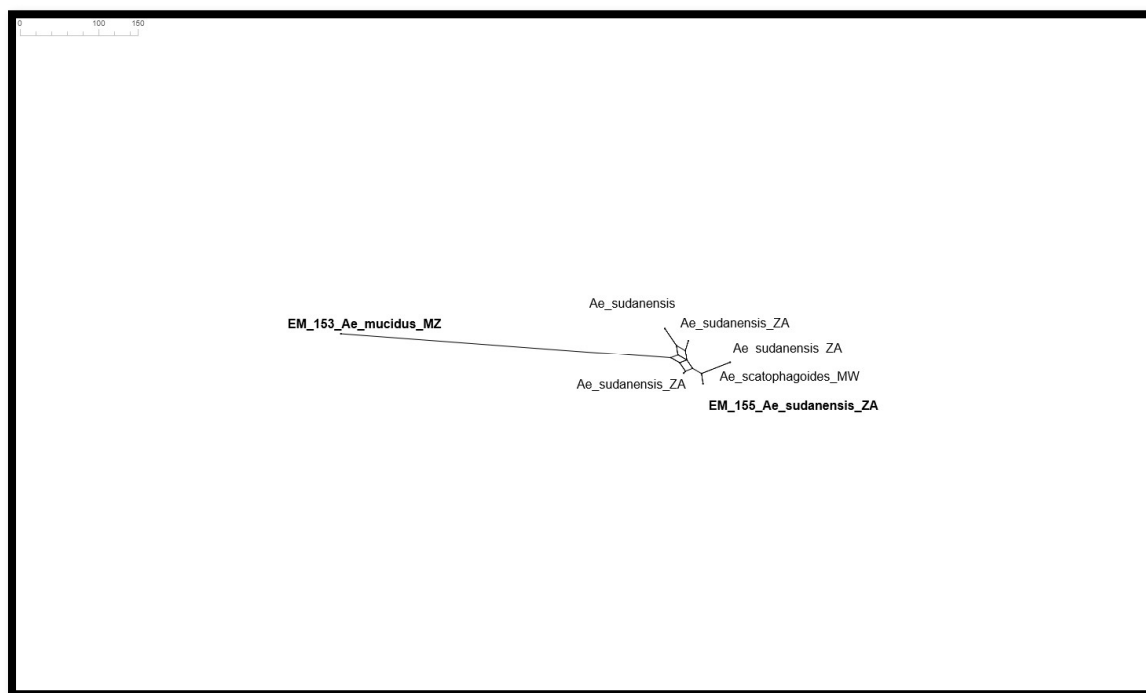


Figure S-IV 8. Networks **a)** and PCOORD **b)**, analysis of 11 partial *coxI* nucleotide sequences from mosquitoes of the genus *Aedes*, subgenus *Ochlerotatus*. The sequences obtained in this work are indicated with the “EM” code, and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA) , Belgium (BE), Spain (ES), Iran (IR)] are also indicated.

a)



b)

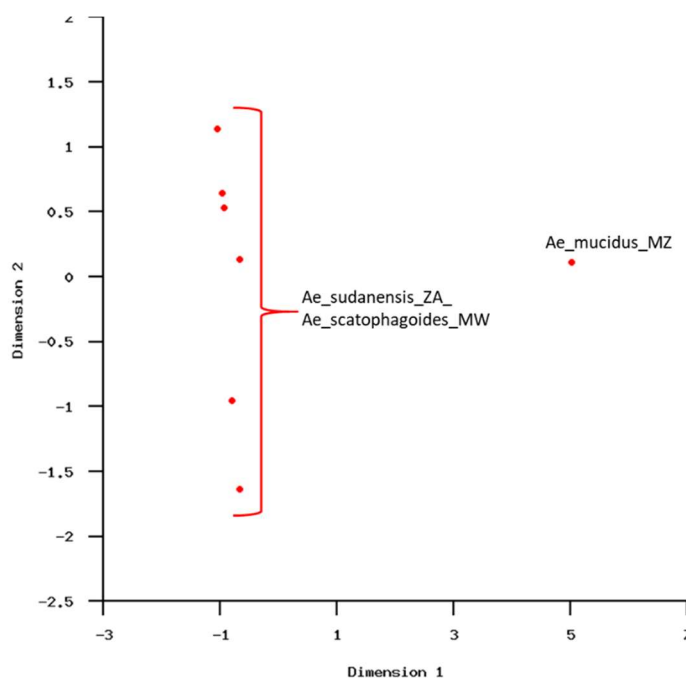


Figure S-IV 9. Networks a) and PCOORD b), analysis of 7 partial *coxI* nucleotide sequences from mosquitoes of the genus *Aedes*, subgenus *Mucidus*. The sequences obtained in this work are indicated with the “EM” code, and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Malawi (MW), Mozambique (MZ)] are also indicated.

Table S-IV 7. Intraspecific and interspecific variation of partial *coxI* sequences amplified from different *Eretmapodites* species. The indicated values have been calculated using the K2P model.

	N° of sequences	Intraspecific variation (%)	Interspecific variation (%)			
			1	2	3	4
1 <i>Er. quinquevittatus</i>	1	-	-			
2 <i>Er. silvestris</i>	1	-	-	0.0 (± 0.0)		
3 <i>Er. intermedius</i>	3	0.2 (± 0.2)	10.7 (± 1.6)	10.7 (± 1.6)		
4 <i>Er. subsimplicipes</i>	3	0.0 (± 0.0)	9.3 (± 1.5)	9.3 (± 1.5)	4.4 (± 1.0)	
5 <i>Er. chrysogaster</i>	2	0.0 (± 0.0)	10.6 (± 1.6)	10.6 (± 1.6)	9.7 (± 1.5)	9.1 (± 1.4)

Table S-IV 8. Intraspecific and inter-group variation based on the analysis of partial *coxI* sequences amplified from *Cx. perexiguus* from Europe (ER), Africa (AF), *Cx. neavei* and *Cx. univittatus*. The indicated values have been calculated using the K2P model.

	N° of sequences	Intraspecific variation (%)	Interspecific variation (%)		
			1	2	3
1 <i>Cx. perexiguus</i> MO-ER	4	0.5 (± 0.2)			
2 <i>Cx. perexiguus</i> AF	11	0.3 (± 0.1)	0.5 (± 0.2)		
3 <i>Cx. neavei</i>	6	1.8 (± 0.4)	5.0 (± 0.9)	5.0 (± 0.9)	
4 <i>Cx. univittatus</i>	14	1.4 (± 0.3)	5.0 (± 0.9)	5.1 (± 0.9)	4.9 (± 0.8)

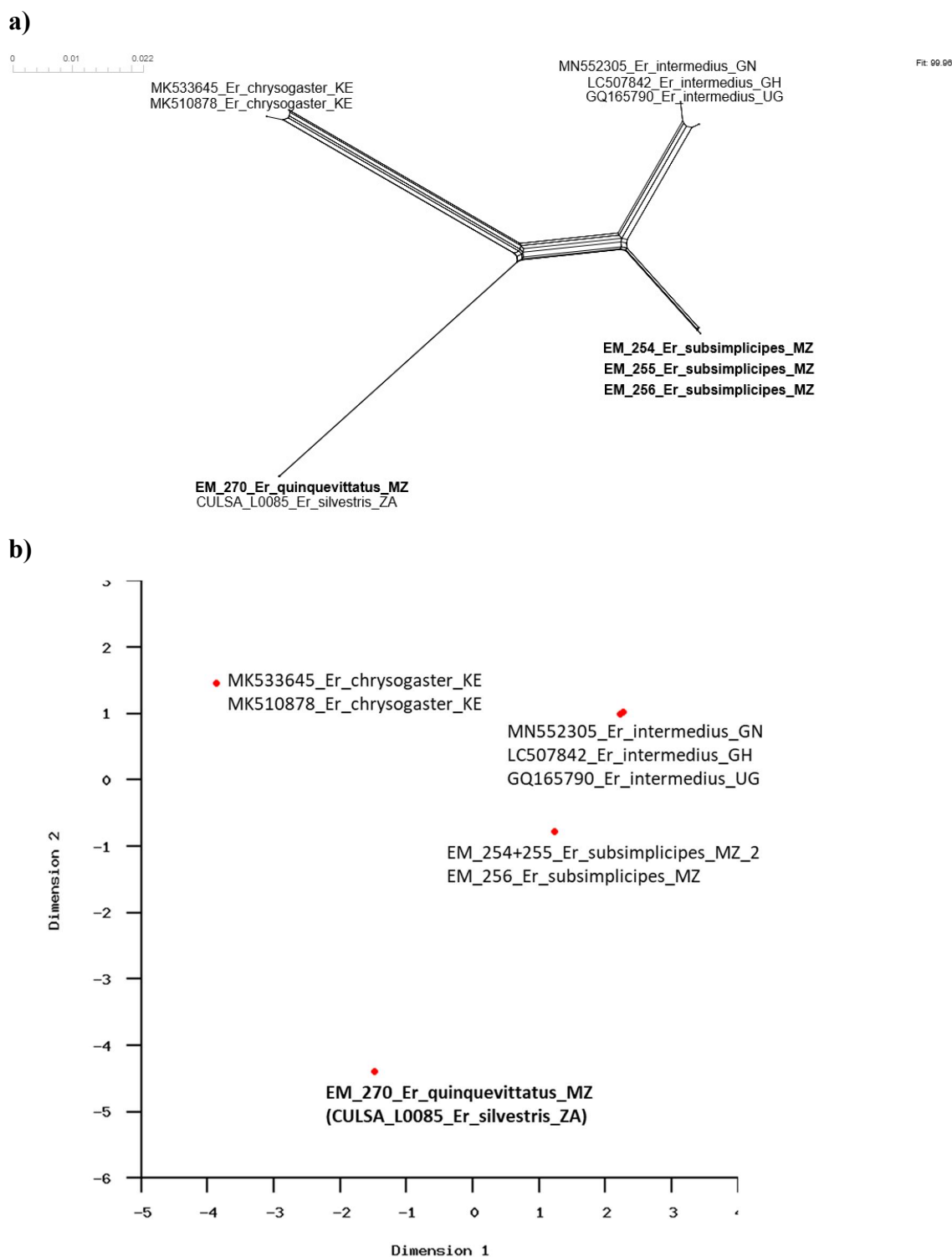
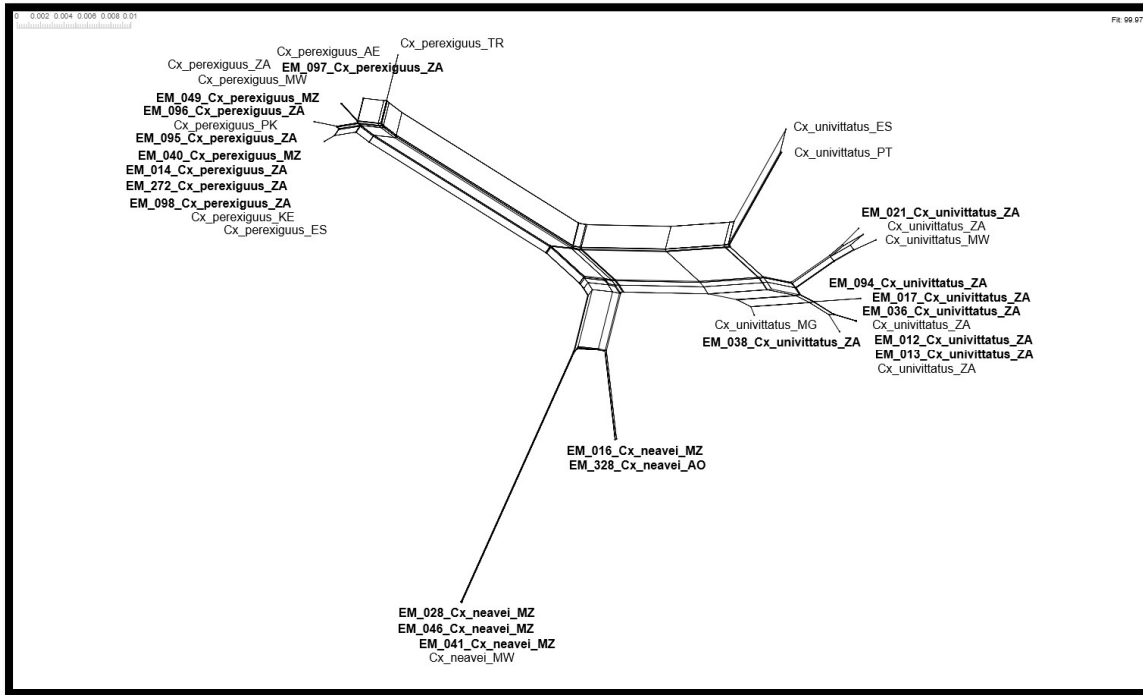


Figure S-IV 10. Networks **a)** and PCOORD **b)**, analysis of 10 partial *coxI* nucleotide sequences from mosquitoes of the genus *Eretmapodites*. The sequences obtained in this work are indicated with the “EM” code, and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Mozambique (MZ), Ghana (GH), Guinea (GN), Kenya (KE), Uganda (UG)] are also indicated.

a)



b)

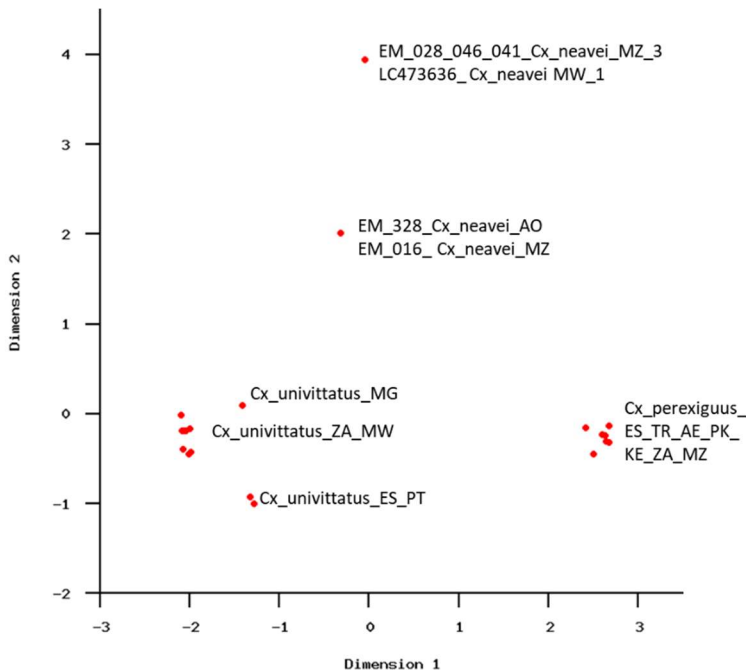
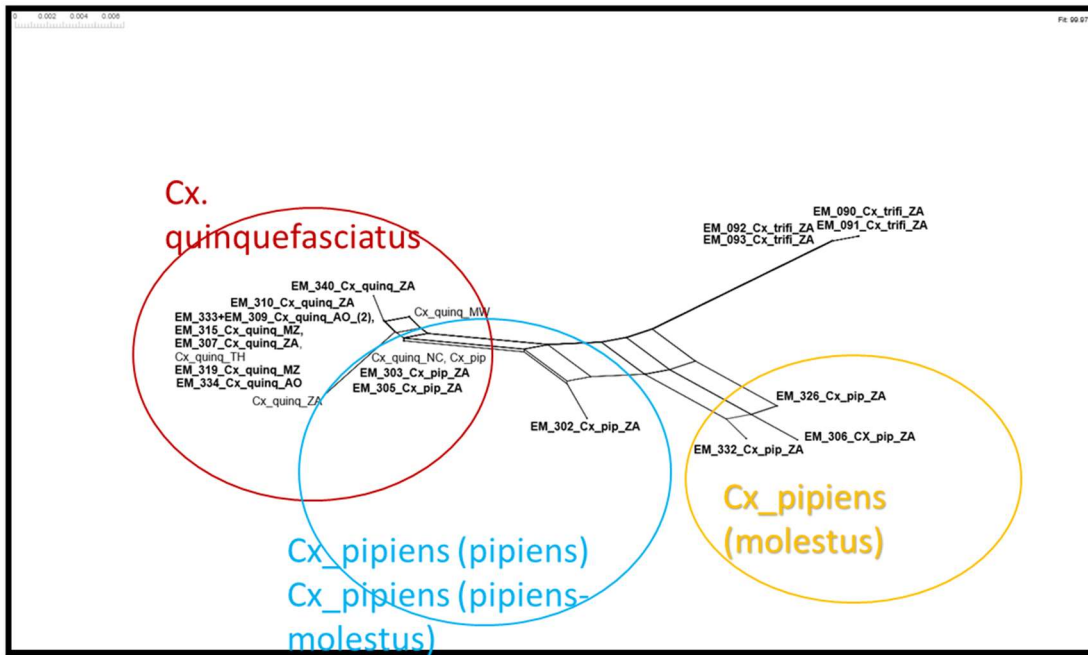


Figure S-IV 11. Networks a) and PCOORD b), analysis of 35 (21 unique, 14 duplicate) partial *coxI* nucleotide sequences from *Culex* mosquitoes, presenting the *Univittatus* subgroup of the *Culex* subgenus. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Angola (AO), United Arab Emirates (AE), Spain (ES), Madagascar (MG), Malawi (MW), Mozambique (MZ), Pakistan (PK), Portugal (PT), Kenya (KE), Turkey (TR)] are also indicated.

Table S-IV 9. Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Cx. pipiens* (pip-mol hybrid), *Cx. pipiens* (*pipiens* ecotype), *Cx. pipiens* (*molestus* ecotype), *Cx. quinquefasciatus* and *Cx. Trifilatus*. The indicated values have been calculated using the K2P model. “pip-mol hybrid” indicated hybrid from *pipiens* and *molestus* ecotypes.

	Nº of sequences	Intraspecific variation (%)	Interspecific variation (%)			
			1	2	3	4
1 <i>Cx. pipiens</i> (pip-mol hybrid)	2	1.3 (± 0.5)				
2 <i>Cx. Quinquefasciatus</i>	12	0.2 (± 0.1)	0.9 (± 0.3)			
3 <i>Cx. pipiens</i> (<i>pipiens</i> ecotype)	1	-	0.6 (± 0.2)	0.2 (± 0.1)		
4 <i>Cx. pipiens</i> (<i>molestus</i> ecotype)	3	0.5 (± 0.2)	2.2 (± 0.5)	2.8 (± 0.6)	2.6 (± 0.6)	

a)



b)

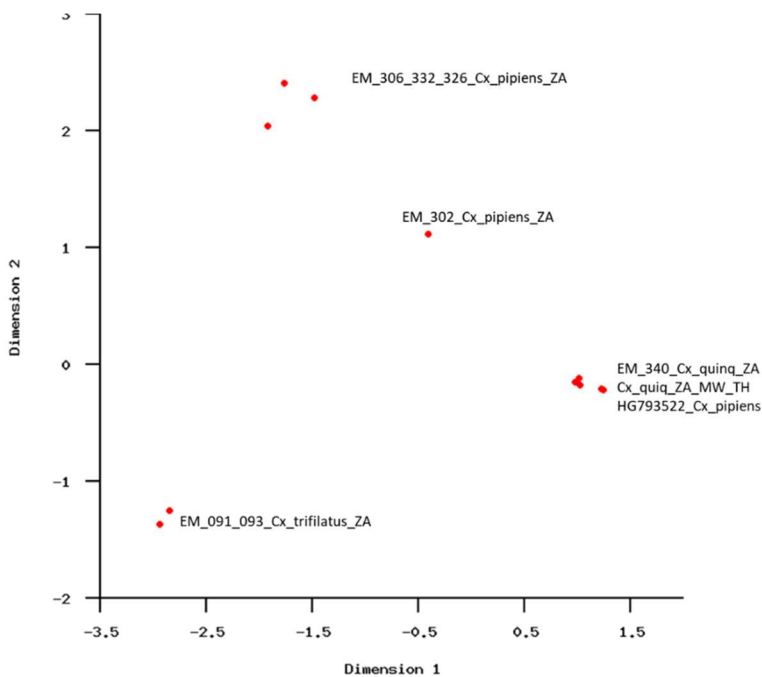
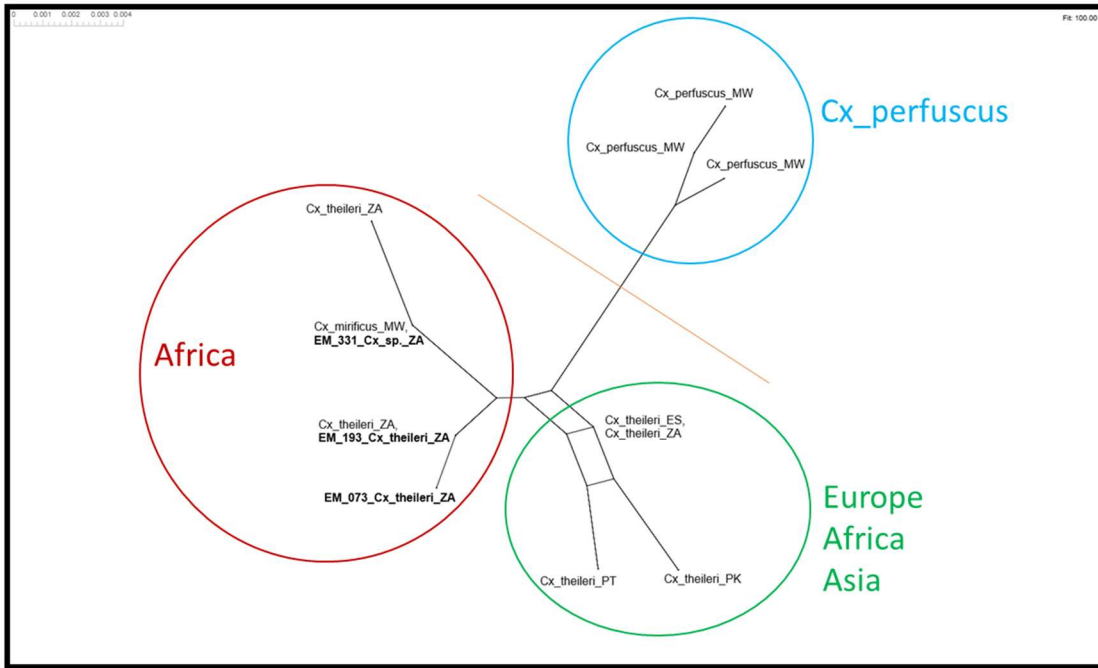


Figure S-IV 12. Networks a) and PCOORD b), analysis of 23 partial *coxI* nucleotide sequences from *Culex* mosquitoes, presenting the Pipiens subgroup of the *Culex* subgenus. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA) , Angola (AO), Malawi (MW), Mozambique (MZ), Thailand (TH)] are also indicated. In addition to the ecotypes of *Cx. pipiens*.

a)



b)

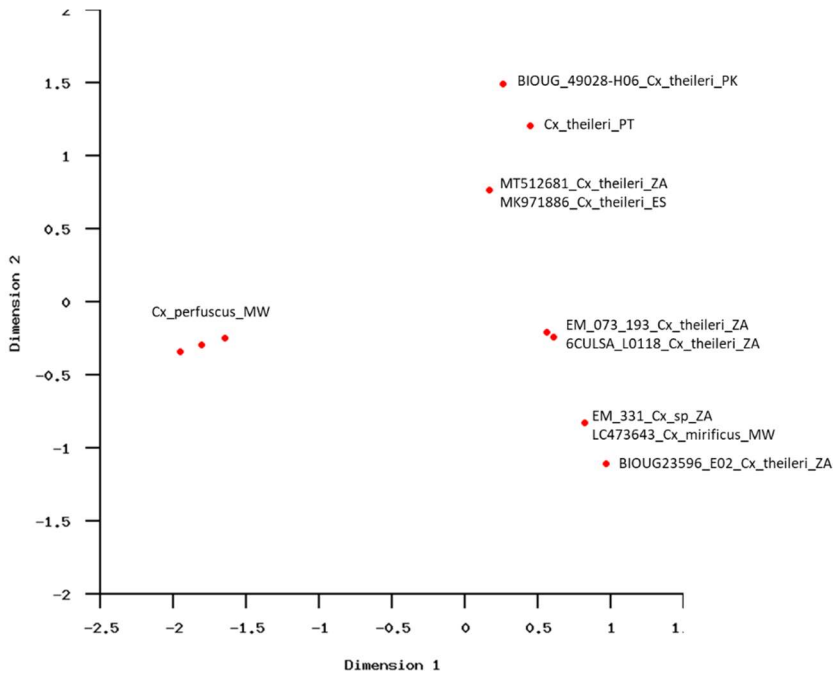
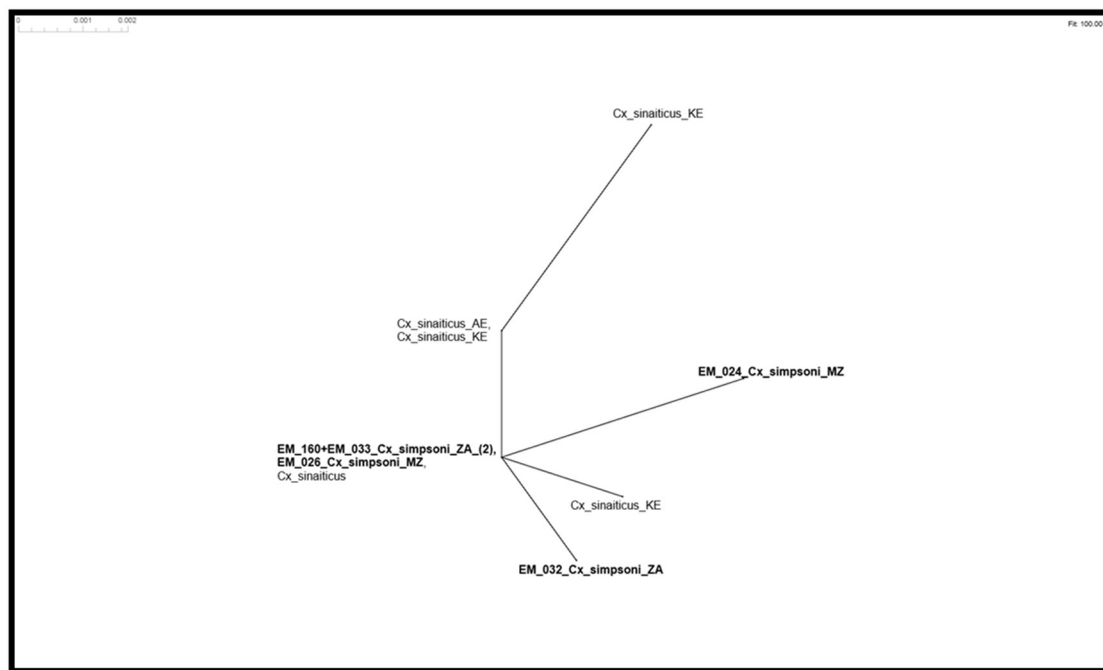


Figure S-IV 13. Networks a) and PCOORD b), analysis of 13 partial *coxI* nucleotide sequences from *Culex* mosquitoes, presenting the Theileri subgroup of the *Culex* subgenus. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Spain (ES), Malawi (MW), Pakistan (PK), Portugal (PT)] are also indicated.

a)



b)

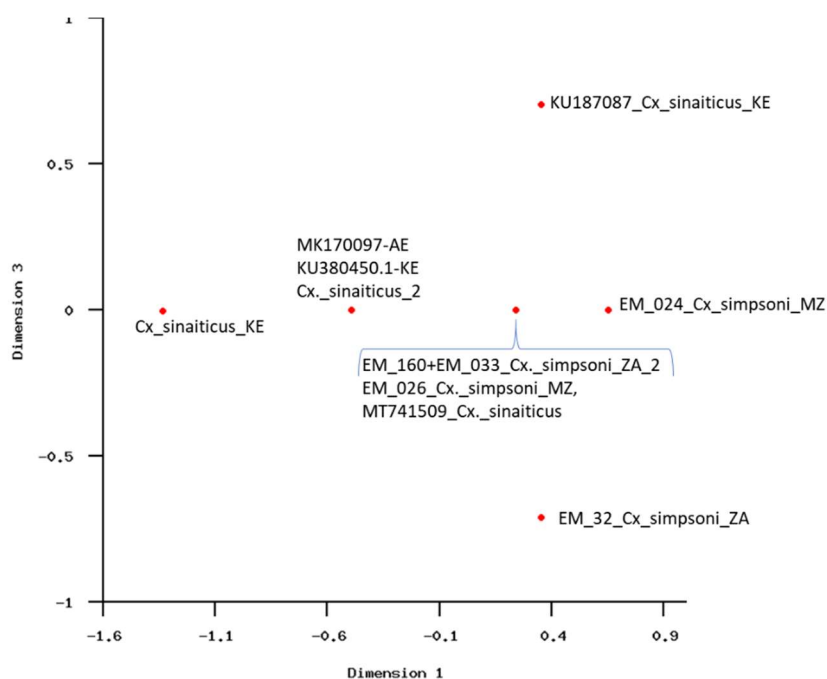


Figure S-IV 14. Networks a) and PCOORD b), analysis of 10 partial *coxI* nucleotide sequences from *Culex* mosquitoes, presenting the Simpsoni subgroup of the *Culex* subgenus. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), United Arab Emirates (AE), Mozambique (MZ), Kenya (KE)] are also indicated.

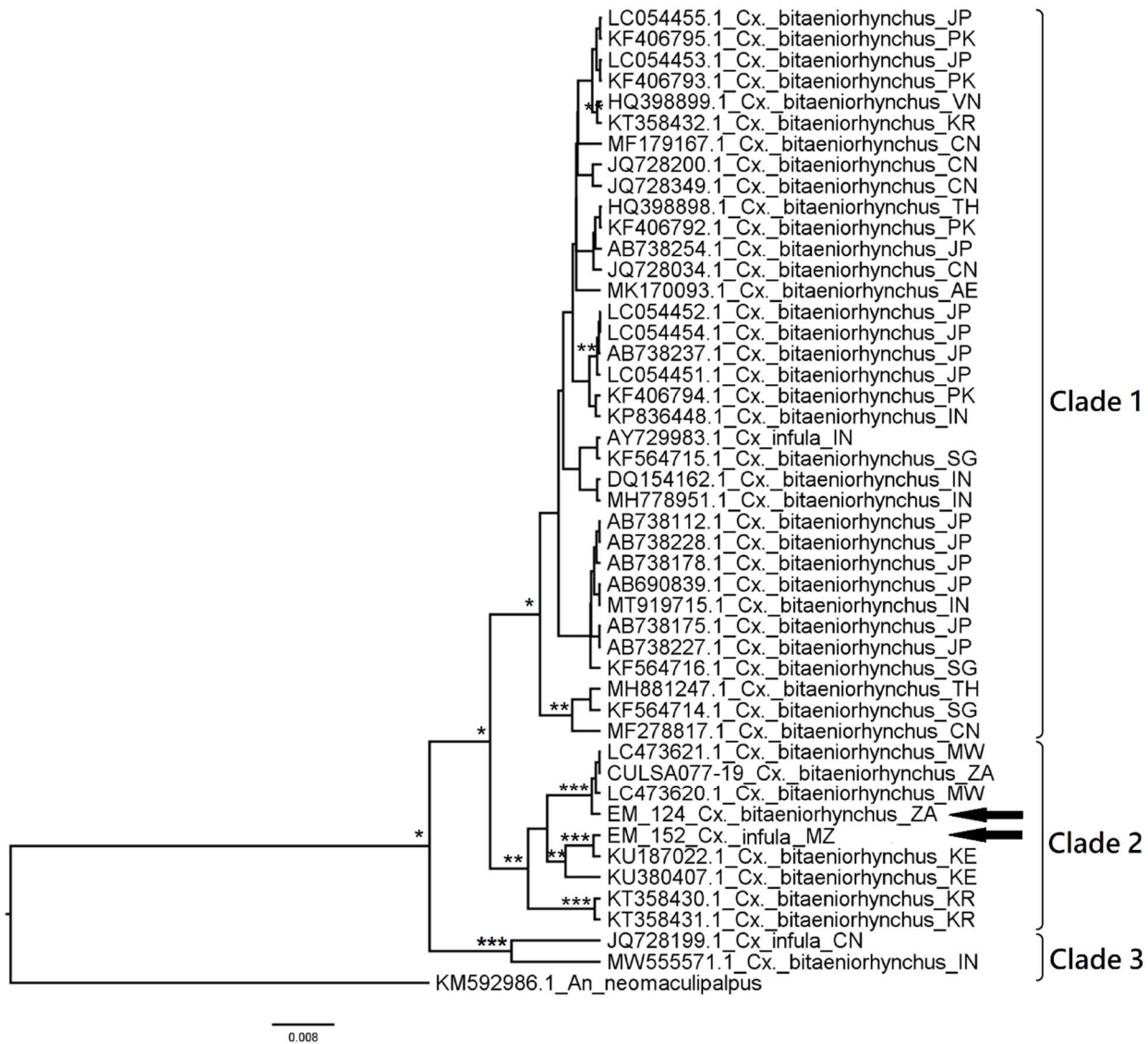


Figure S-IV 15. Phylogenetic analysis of 47 partial *coxI* nucleotide sequences from mosquitoes of the species *Cx. bitaeniorhynchus* and *Cx. infula*. . At specific branches, the number of * indicates the tree topological support revealed by the different phylogenetic reconstruction methods, assuming as relevant bootstrap and aLRT values above 75% and posterior probability values above 0.80. The sequences obtained in this work are indicated with the “EM” code, and those with an associated genitalia photo are indicated by the horizontal arrows. The sequences downloaded from GenBank and BOLDsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), China (CN), United Arab Emirates (AE), India (IN), Japan (JP), South Korea (KR), Malawi (MW), Mozambique (MZ), Pakistan (PK), Kenya (KE), Singapore (SG), Thailand (TH), Vietnam (VN)] are also indicated.

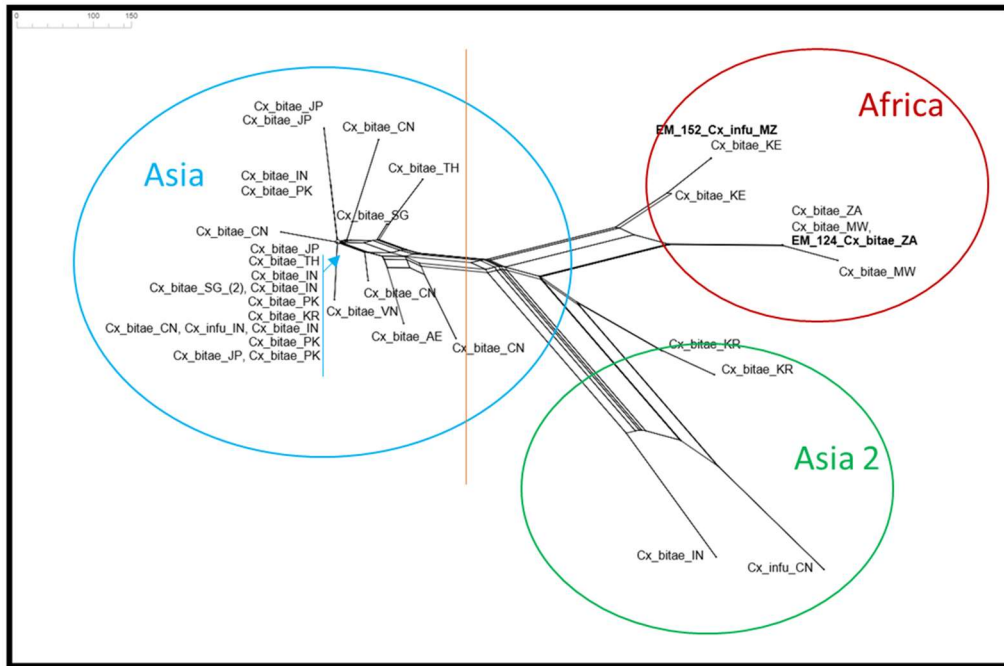
Table S-IV 10. a – Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Cx. bitaeniorhynchus* mosquitoes from South Africa (ZA), India (IN), Japan (JP), Pakistan (PK), Thailand (TH), Korean (KR), Vienna (VN), China (CN), Arab Emirates (AE), Malawi (MW), Kenya (KE), Singapore (SG) and *Cx. infula* from ZA, IN, CN. The indicated values have been calculated using the K2P model.

	N° of sequences	Intraspecific variation (%)	Interspecific variation (%)													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Cx. bitaeniorhynchus</i> IN	5	1.1 (± 0.3)														
2 <i>Cx. bitaeniorhynchus</i> JP	13	0.3 (± 0.2)	0.7 (± 0.2)													
3 <i>Cx. bitaeniorhynchus</i> PK	4	0.1 (± 0.1)	0.6 (± 0.2)	0.2 (± 0.1)												
4 <i>Cx. bitaeniorhynchus</i> TH	2	0.4 (± 0.3)	0.8 (± 0.2)	0.4 (± 0.2)	0.3 (± 0.2)											
5 <i>Cx. bitaeniorhynchus</i> KR	3	1.4 (± 0.4)	1.6 (± 0.4)	1.4 (± 0.4)	1.3 (± 0.4)	1.3 (± 0.4)										
6 <i>Cx. bitaeniorhynchus</i> VN	1	-	1.0 (± 0.3)	0.6 (± 0.3)	0.5 (± 0.3)	0.7 (± 0.4)	1.5 (± 0.5)									
7 <i>Cx. bitaeniorhynchus</i> CN	5	0.8 (± 0.3)	0.9 (± 0.2)	0.6 (± 0.2)	0.5 (± 0.1)	0.6 (± 0.2)	1.5 (± 0.4)	0.9 (± 0.3)								
8 <i>Cx. bitaeniorhynchus</i> AE	1	-	1.0 (± 0.3)	0.6 (± 0.3)	0.5 (± 0.3)	0.7 (± 0.4)	1.7 (± 0.5)	0.9 (± 0.5)	0.8 (± 0.3)							
9 <i>Cx. bitaeniorhynchus</i> MW	2	0.2 (± 0.2)	2.5 (± 0.7)	2.3 (± 0.7)	2.2 (± 0.7)	2.2 (± 0.7)	2.0 (± 0.6)	2.6 (± 0.8)	2.3 (± 0.7)	2.2 (± 0.7)						
10 <i>Cx. bitaeniorhynchus</i> ZA	2	0.0 (± 0.0)	2.4 (± 0.7)	2.2 (± 0.7)	2.1 (± 0.7)	2.0 (± 0.7)	1.9 (± 0.6)	2.5 (± 0.8)	2.2 (± 0.7)	2.0 (± 0.7)	0.1 (± 0.1)					
11 <i>Cx. bitaeniorhynchus</i> KE	2	0.7 (± 0.4)	2.1 (± 0.6)	1.8 (± 0.6)	1.8 (± 0.6)	1.9 (± 0.6)	2.0 (± 0.6)	2.0 (± 0.7)	1.9 (± 0.6)	1.7 (± 0.6)	1.5 (± 0.5)	1.4 (± 0.5)				
12 <i>Cx. infula</i> MZ	1	-	2.0 (± 0.6)	1.7 (± 0.6)	1.6 (± 0.6)	1.8 (± 0.6)	2.0 (± 0.6)	2.0 (± 0.7)	1.8 (± 0.6)	1.6 (± 0.6)	1.5 (± 0.6)	1.4 (± 0.6)	0.3 (± 0.2)			
13 <i>Cx. infula</i> IN	1	-	0.6 (± 0.2)	0.2 (± 0.1)	0.1 (± 0.1)	0.2 (± 0.2)	1.2 (± 0.4)	0.4 (± 0.3)	0.4 (± 0.1)	0.4 (± 0.3)	2.2 (± 0.7)	2.0 (± 0.7)	1.7 (± 0.6)	1.6 (± 0.6)		
14 <i>Cx. infula</i> CN	1	-	2.6 (± 0.7)	2.6 (± 0.7)	2.6 (± 0.7)	2.5 (± 0.7)	2.3 (± 0.6)	3.0 (± 0.8)	2.6 (± 0.7)	2.5 (± 0.8)	2.8 (± 0.8)	2.7 (± 0.8)	2.8 (± 0.8)	2.7 (± 0.8)	2.5 (± 0.7)	
15 <i>Cx. bitaeniorhynchus</i> SG	3	0.1 (± 0.1)	0.7 (± 0.2)	0.2 (± 0.1)	0.1 (± 0.1)	0.2 (± 0.2)	1.2 (± 0.4)	0.5 (± 0.3)	0.4 (± 0.2)	0.5 (± 0.3)	2.1 (± 0.7)	2.0 (± 0.7)	1.8 (± 0.6)	1.7 (± 0.6)	0.1 (± 0.1)	2.4 (± 0.7)

Table S-IV 10. b – Intraclade and interclade variation, referring to Fig. 15, S-IV; Clade 1: *Cx. bitaeniorhynchus* and *Cx. infula* - Asia; Clade 2: *Cx. bitaeniorhynchus* and *Cx. infula* - Africa and Korea; Clade 3: *Cx. bitaeniorhynchus* - IN and *Cx. infula* – CN.

	N° of sequences	Intraclade variation (%)	Interclade variation (%)	
			Clade 1	Clade 2
Clade 1	35	0,39 (± 0,10)		
Clade 2	9	1,29 (± 0,35)	2,00 (± 0,55)	
Clade 3	2	1,58 (± 0,57)	2,41 (± 0,64)	2,72 (± 0,67)

a)



b)

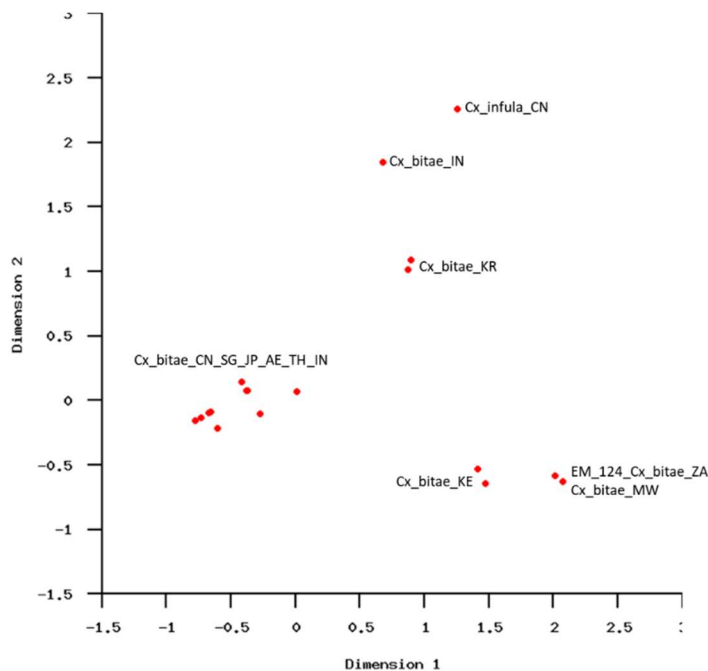
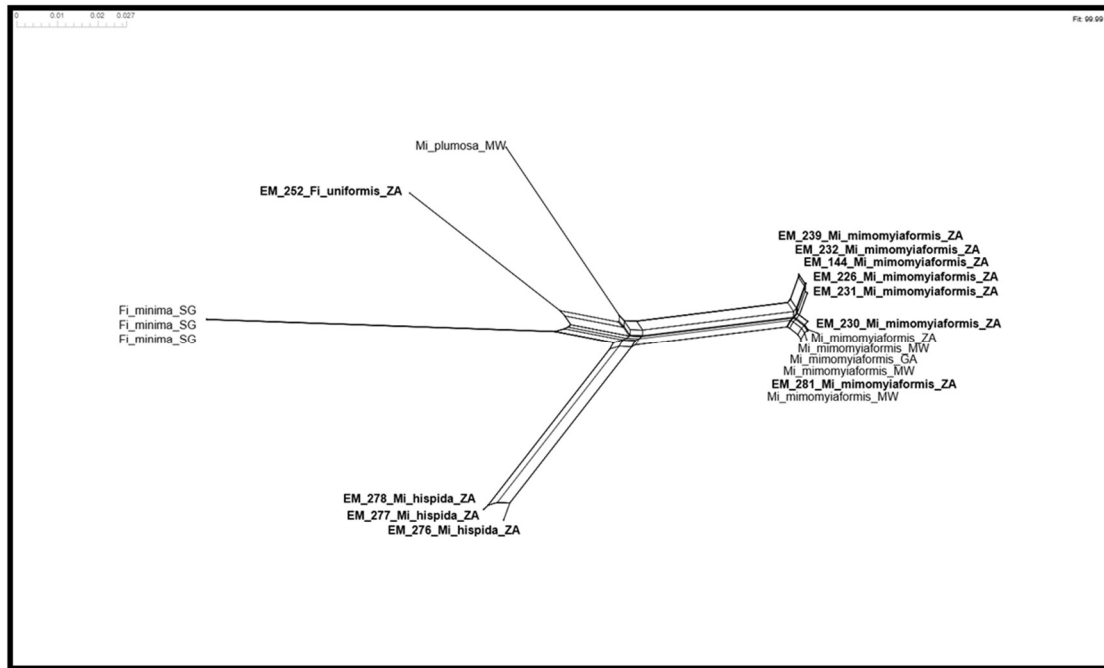


Figure S-IV 16. Networks analysis of 36 partial *coxI* nucleotide sequences from mosquitoes of the species *Cx. bitaeniorhynchus* and *Cx. infula*. The sequences obtained in this work are indicated with the “EM” code, and are boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), China (CN), United Arab Emirates (AE), India (IN), Japan (JP), South Korea (KR), Malawi (MW), Mozambique (MZ), Pakistan (PK), Kenya (KE), Singapore (SG), Thailand (TH), Vietnam (VN)] are also indicated.

Table S-IV 11. Intraspecific and interspecific variation based on the analysis of partial *coxI* sequences amplified from *Mimomyia* and *Ficalbia* mosquitoes. The indicated values have been calculated using the K2P model.

	N° of sequences	Intraspecific variation (%)	Interspecific variation (%)						
			1	2	3	4	5	6	7
1 <i>Mi. hispida</i>	3	0.7 (± 0.4)							
2 <i>Mi. mimomyiaformis</i>	12	0.9 (± 0.3)	10.9 (± 1.9)						
3 <i>Mi. plumosa</i>	1	-	12.0 (± 1.9)	11.1 (± 1.9)					
4 <i>Fi. uniformis</i>	1	-	13.3 (± 2.1)	12.1 (± 2.0)	12.5 (± 1.9)				
5 <i>Mi. splendens</i>	6	0.5 (± 0.3)	16.7 (± 2.4)	15.6 (± 2.2)	12.5 (± 2.0)	13.0 (± 2.0)			
6 <i>Mi. mediotineata</i>	4	2.0 (± 0.6)	14.5 (± 2.0)	14.9 (± 2.1)	13.9 (± 2.0)	16.7 (± 2.3)	13.7 (± 2.0)		
7 <i>Mi. luzonensis</i>	3	0.4 (± 0.3)	17.6 (± 2.4)	16.2 (± 2.4)	15.6 (± 2.3)	15.9 (± 2.3)	14.2 (± 2.1)	15.4 (± 2.2)	
8 <i>Fi. minima</i>	3	0.0 (± 0.0)	17.8 (± 2.4)	17.4 (± 2.5)	18.5 (± 2.5)	16.1 (± 2.4)	16.7 (± 2.3)	18.7 (± 2.4)	19.4 (± 2.6)

a)



b)

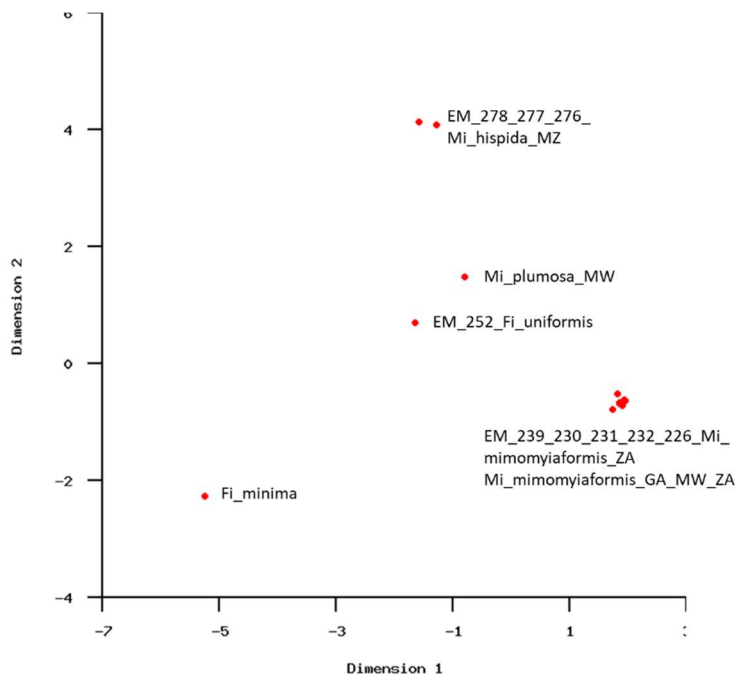
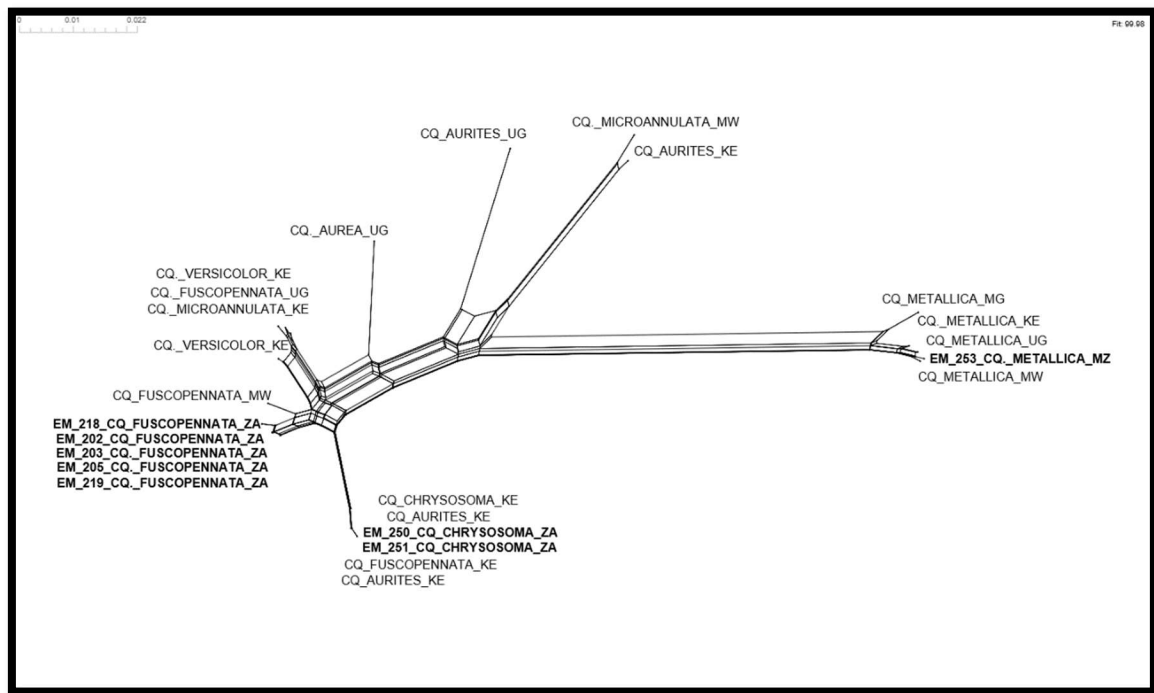


Figure S-IV 17. Networks and PCOORD, analysis of 20 partial *coxI* nucleotide sequences from mosquitoes of the *Ficalbia* and *Mimomyia* genera. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Gabon (GA), Malawi (MW), Mozambique (MZ), Kenya (KE)] are also indicated.

a)



b)

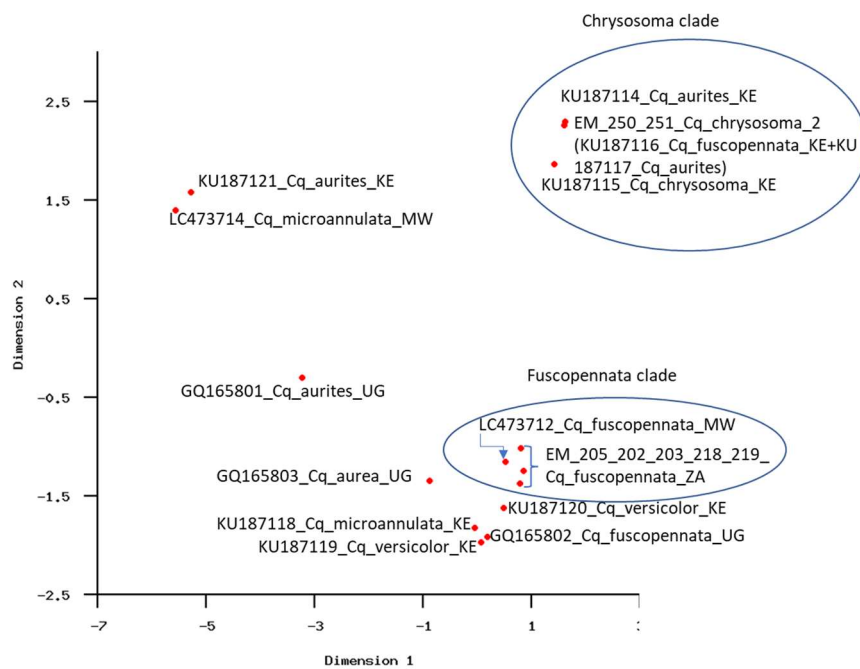
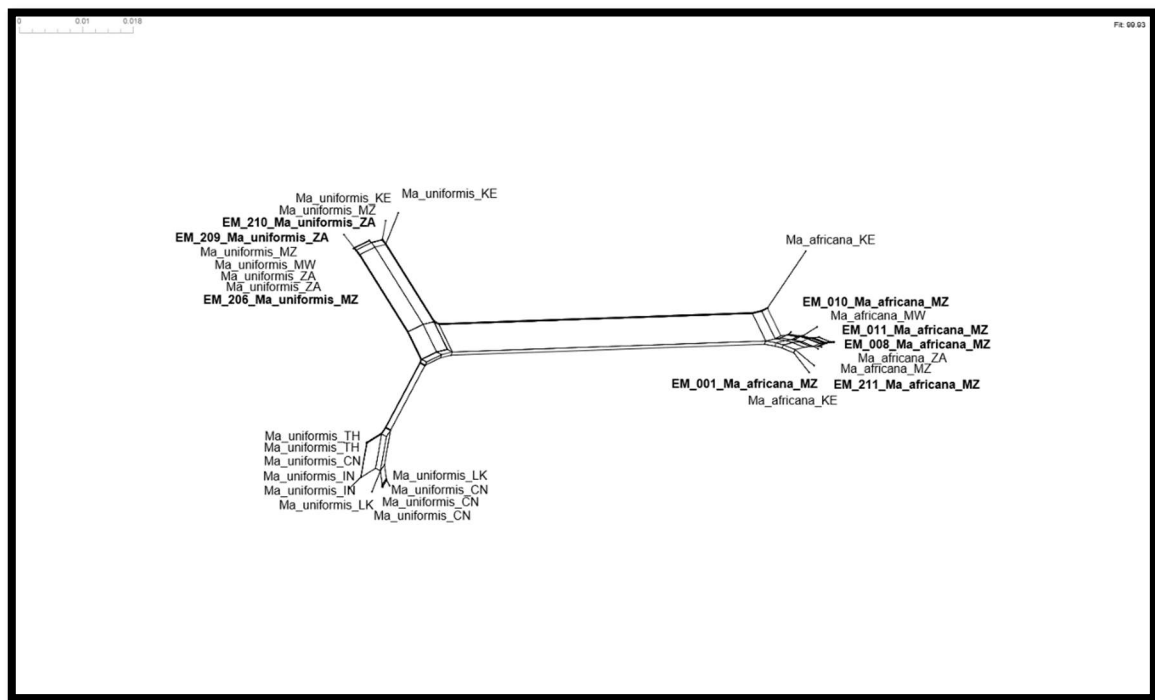


Figure S-IV 18. Networks and PCOORD, analysis of 25 and 20, respectively, partial *coxI* nucleotide sequences from mosquitoes of the genus *Coquillettidia*. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), Malawi (MW), Mozambique (MZ), Kenya (KE), Uganda (UG)] are also indicated.

a)



b)

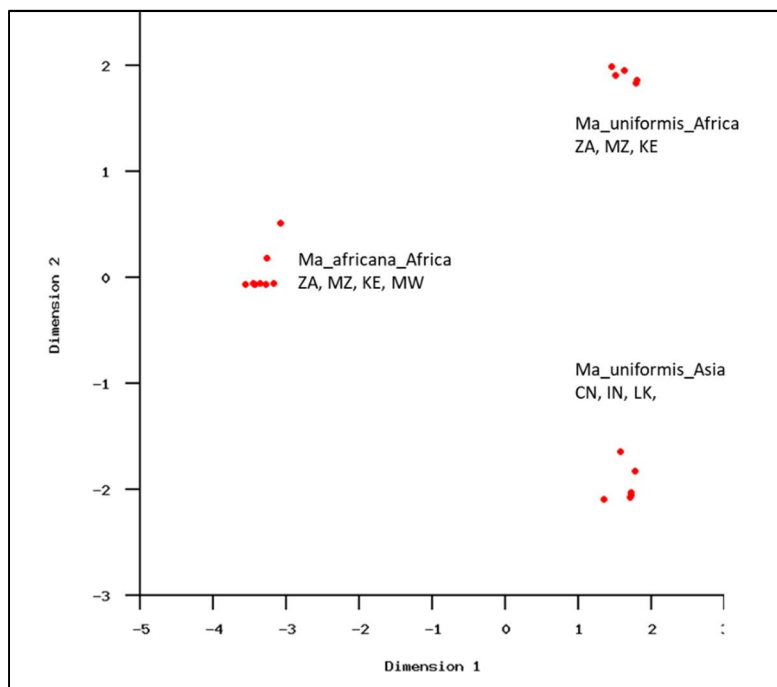


Figure S-IV 19. Networks **a)** and PCOORD **b)**, analysis of 30 partial *coxI* nucleotide sequences from mosquitoes of the genus *Mansonia*. The sequences obtained in this work are indicated with the “EM” code, and boldface. The sequences downloaded from GenBank and Boldsystems are indicated by their respective accession number and access code (respectively). Their country of origin [South Africa (ZA), China (CN), India (IN), Malawi (MW), Mozambique (MZ), Kenya (KE), Sri Lanka (LK), Thailand (TH)] are also indicated.