# Supplemental Data

# Figures

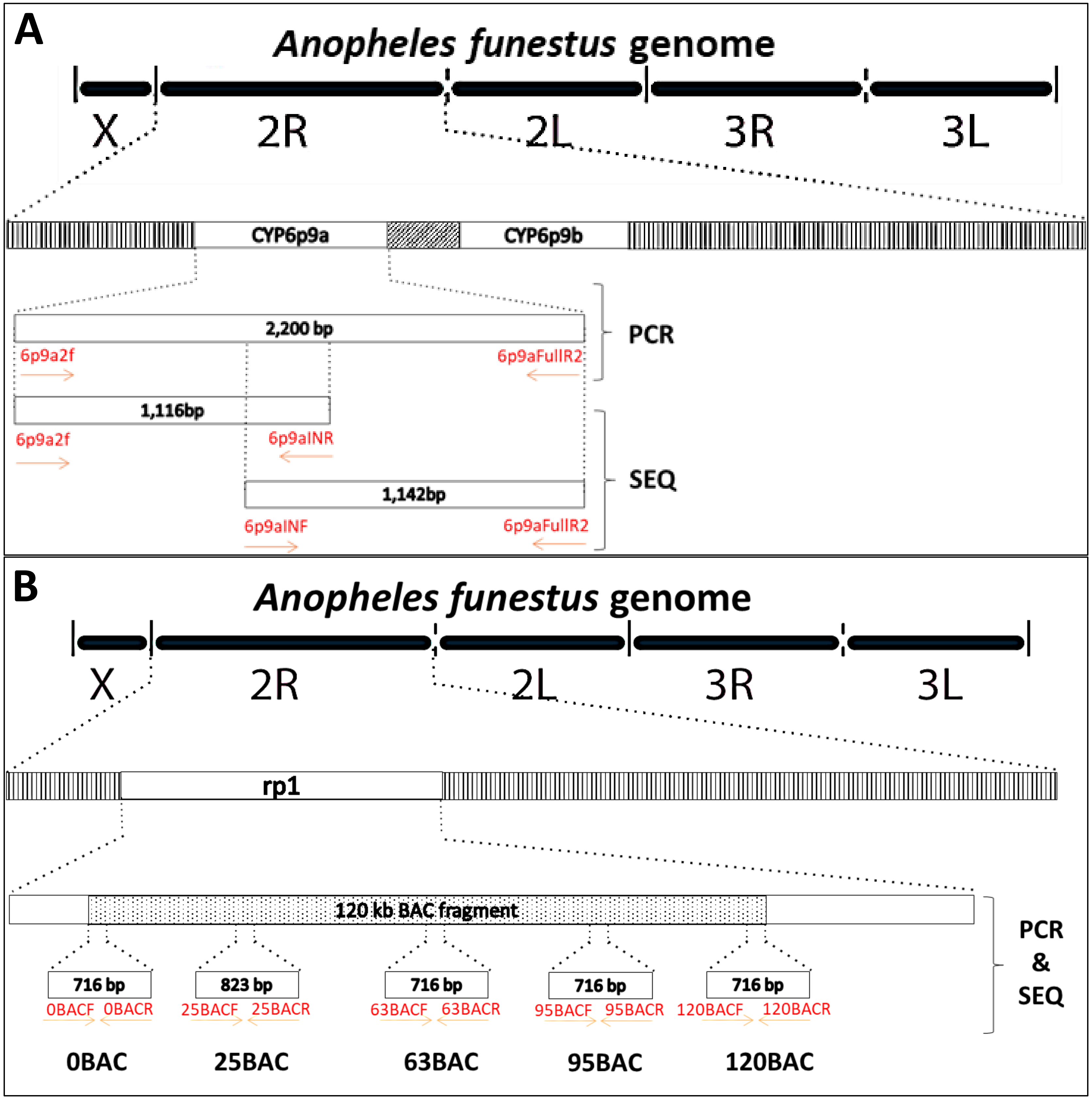


**Fig. S1:** amplification and sequencing scheme of tthe key insecticide resistance gene CYP6P9a (A) and the BAC clone (B)

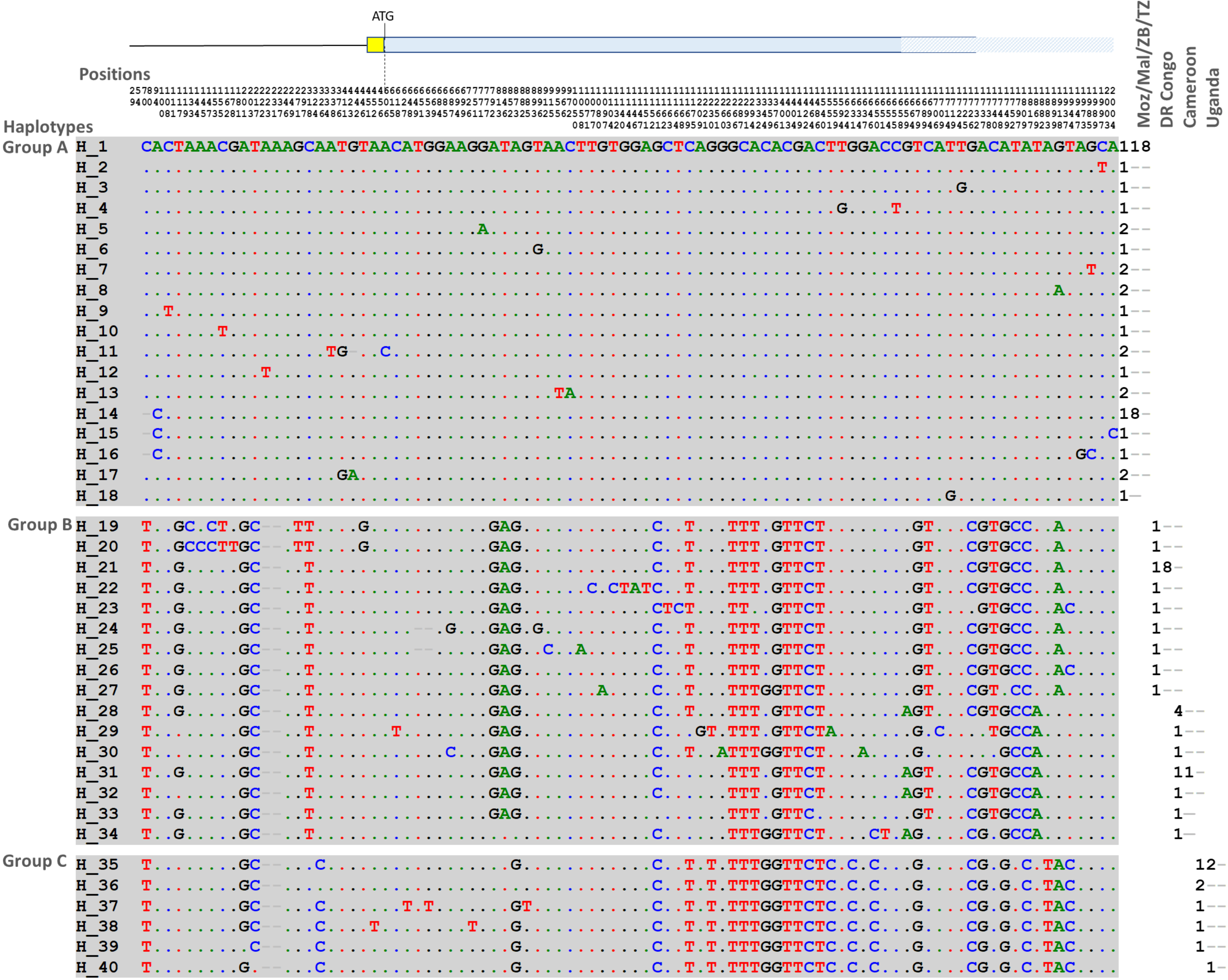


**Fig. S2: Schematic position of nucleotide polymorphisms at *CYP6P9a* and haplotypes across the gene.**

Nucleotide polymorphism was examined across the 1,139 bp of *CYP6P9a* gene including successively, 465 bp of the partial 5' upstream intergenic region (black line), 32 bp of 5'UTR (yellow box) and 642 bp of partial gene's first exon (blue box),. The number below the model indicate each nucleotide position on the sequence. In each haplotype, letters indicate alternative nucleotide at given position with reference to haplotype 1. The short line dash in haplotypes represents deletions. Numbers on the left-hand side of the figure indicate the number of sequences contained in each haplotype.

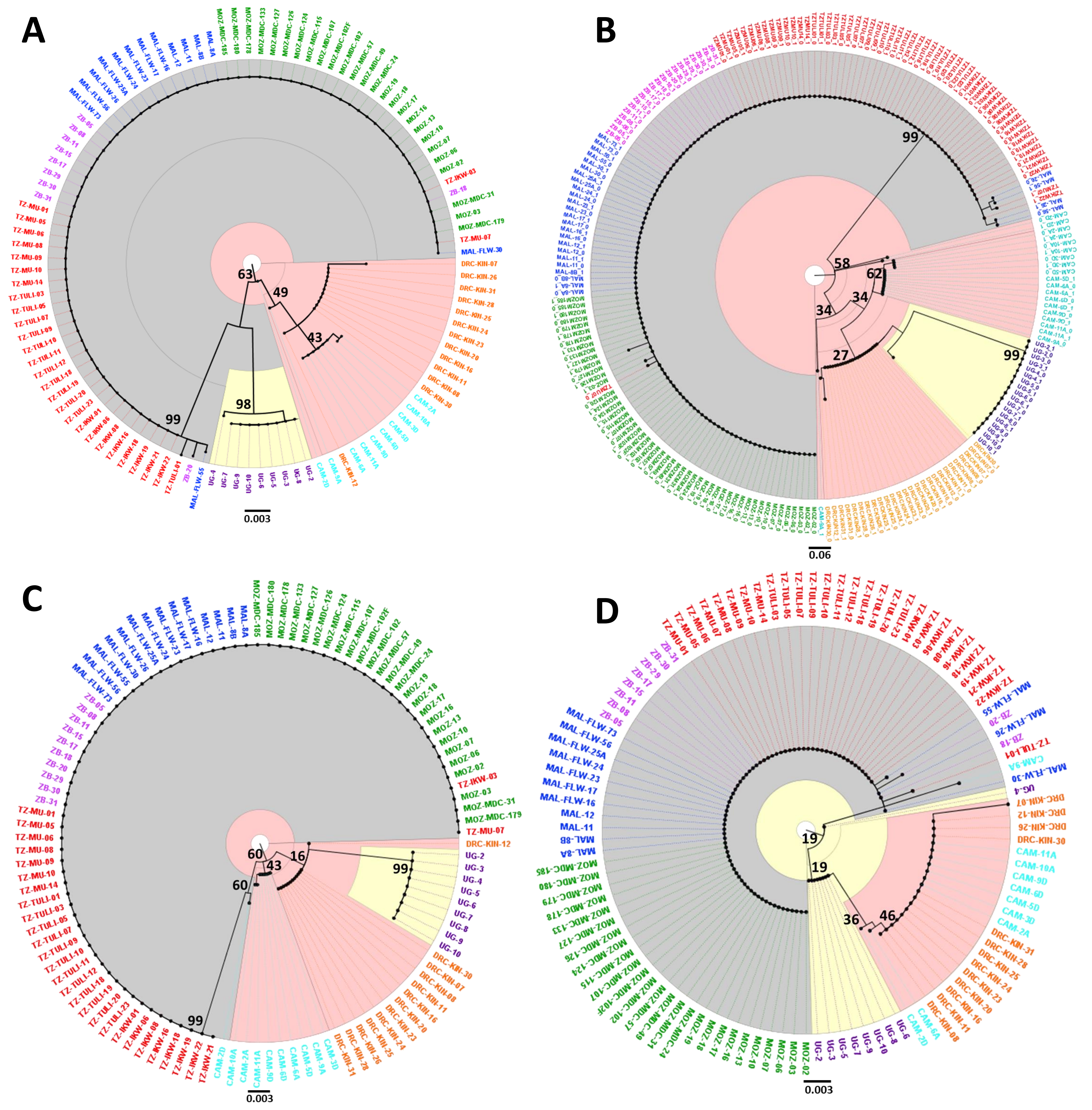


**Fig. S3 : Phylogenetic trees** generated by the Maximum likelyhood method based on the Hasegawa-Kishino-Yano model inferred on a 1,139-bp fragment of CYP6P9a gene (A), the 36 non-synonymous mutations found in the fragment (B), 498-bp fragment of CYP6P9a gene's coding region (C) and 461-bp of the gene's non-coding region (D). All but one trees include 111 *A. funestus* sequences from 7 sub-Saharan African countries generated in this work including 10 reference sequences from Cameroon and 9 from Uganda. Non synonymous mutations tree include 222 *A. funestus* unphased sequences. Trees with the highest log likelihood are shown. To test the robustness of each tree topology, 1,000 bootstrap replicates were performed. Trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

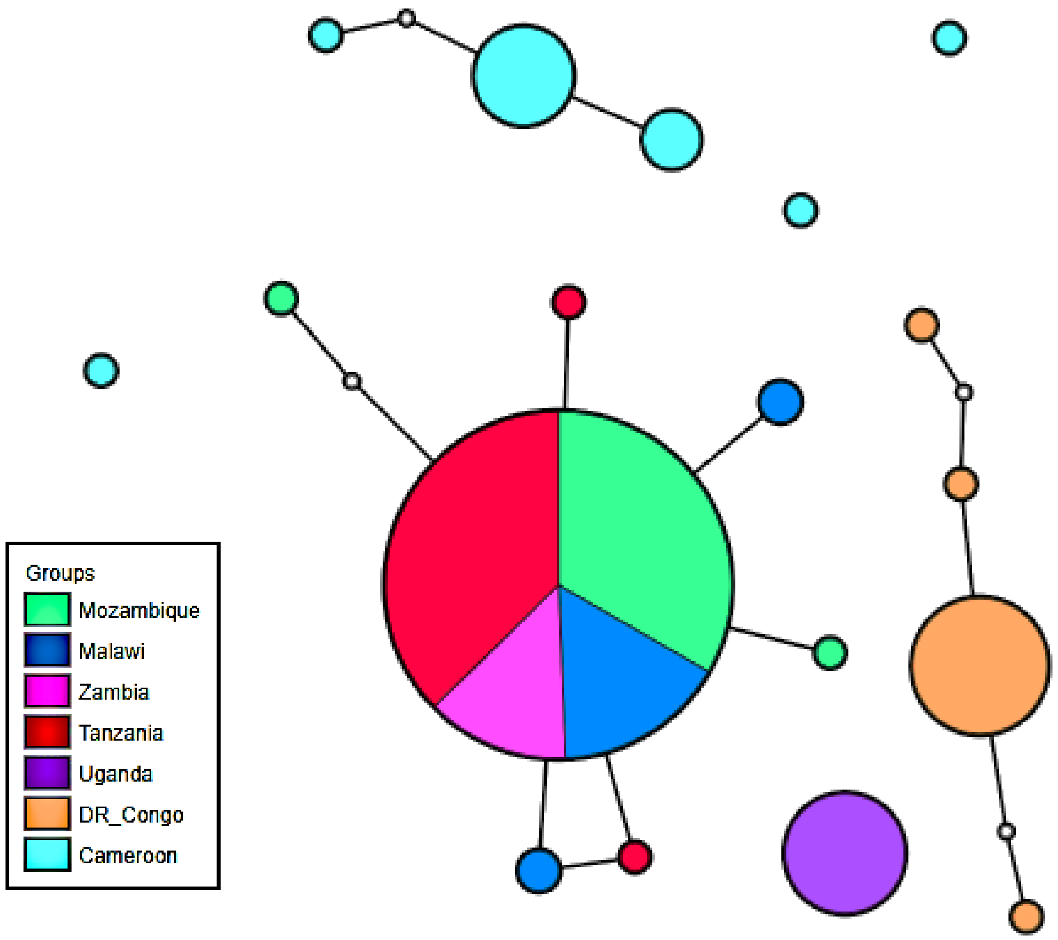


**Fig. S4 : Haplotype network for nucleotide Non-synonymous variants in *6p9a* resistance gene.** The green, Bleu, red and Pink parts of the circle constitute the major haplotype respectively shared between Mozambique (MOZ), Malawi (MAL), Zambia (ZB) and Tanzania (TZ). The Orange, Cyan and Purple circles shows three distinct haplotypes representing mosquitoes population from Democratic Republic of Congo (DRC), Cameroon (CAM) and Uganda (UG) respectively. The size of the shapes is proportional to the frequency of the haplotype and spots on each branch show the mutational steps separating haplotypes.

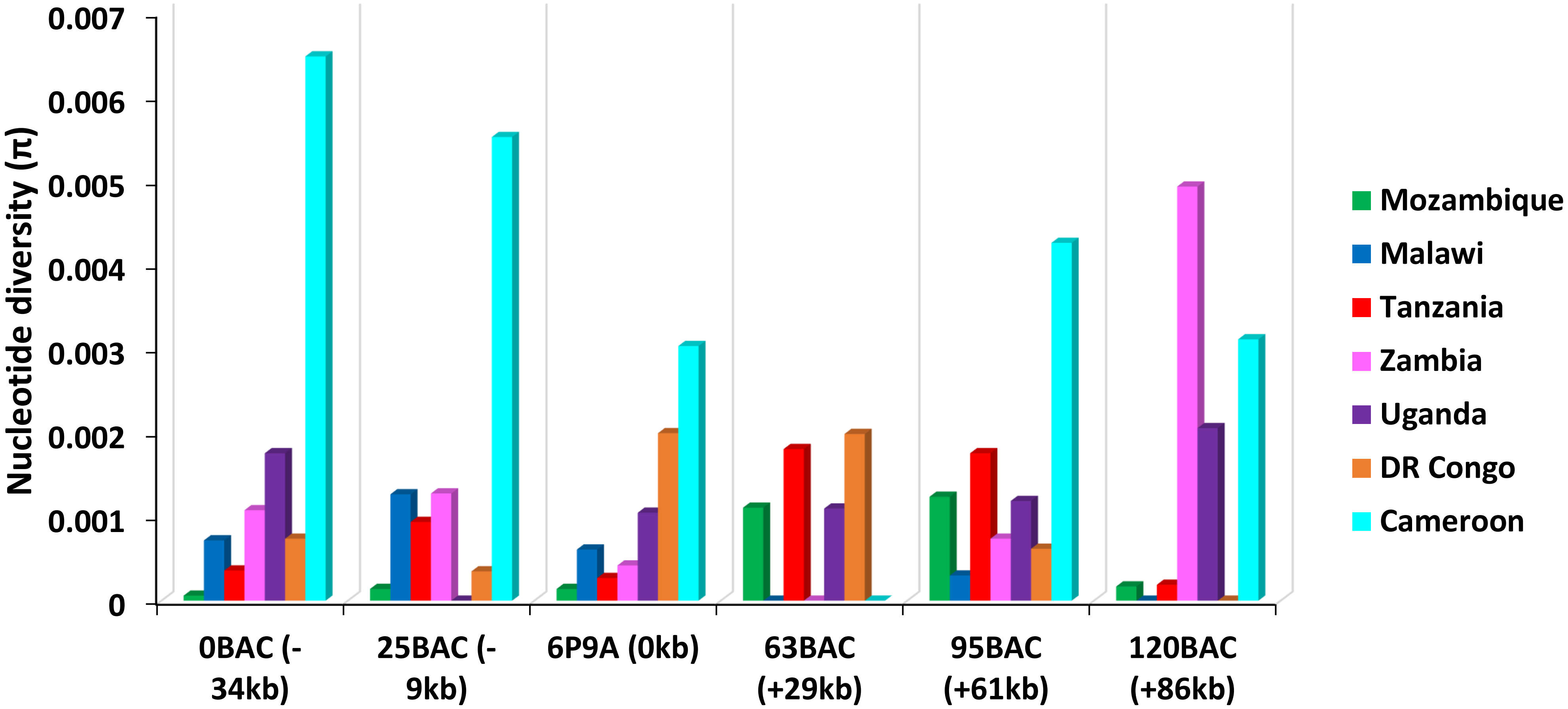


**Fig. S5: Levels of nucleotide diversity survey at *CYP6P9a* and five loci around the pyrethroid resistance gene.** For each locus, nucleotide diversity was investigated by sequencing *A. funestus* samples from seven sub-Saharan African countries. Positions (kb) of sequenced regions relative to CYP6P9a.

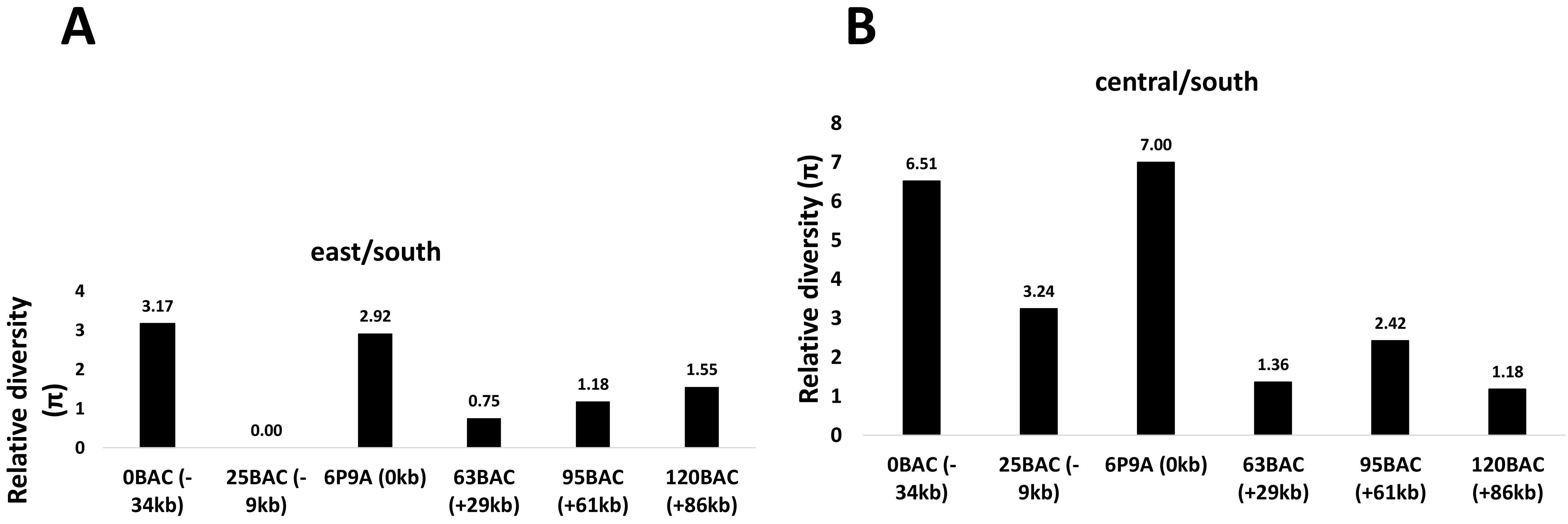
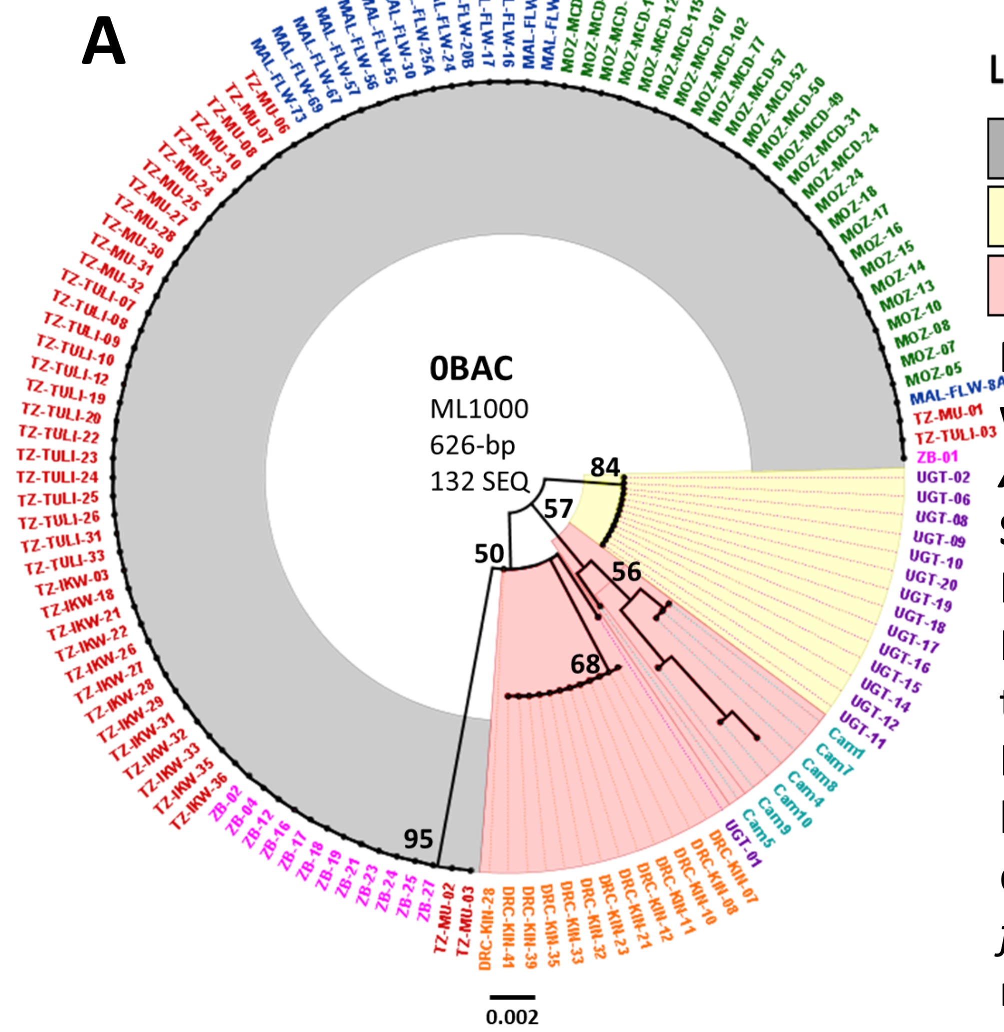


**Fig. S6: Ratio of nucleotide diversity at *CYP6P9a* and five loci around the pyrethroid resistance gene.** For each locus, nucleotide diversity was investigated by sequencing *A. funestus* samples from seven sub-Saharan African countries. Results were further grouped samples into Southern, Eastern and Central Africa respective to mosquitoes field population origin. Positions (kb) of sequenced regions relative to CYP6P9a. Ratio of relative nucleotide diversity at each loci was calculated between Eastern and Southern Africa **(A)**, and Central and Southern Africa **(B)**.



Legend

▨ Southern Africa : ● Mozambique ● Malawi ● Zambia

▨ Eastern Africa : ● Uganda ● Tanzania

▨ Central Africa : ● DR Congo ● Cameroon

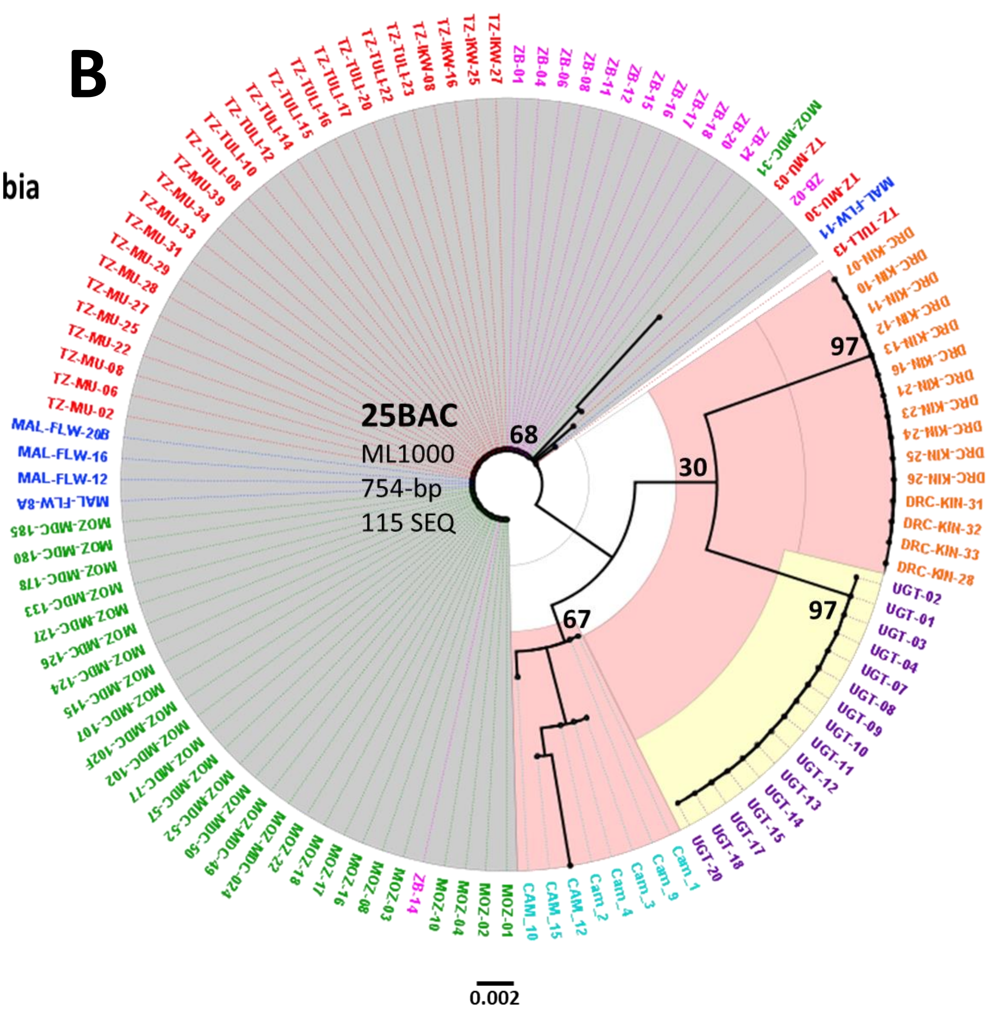
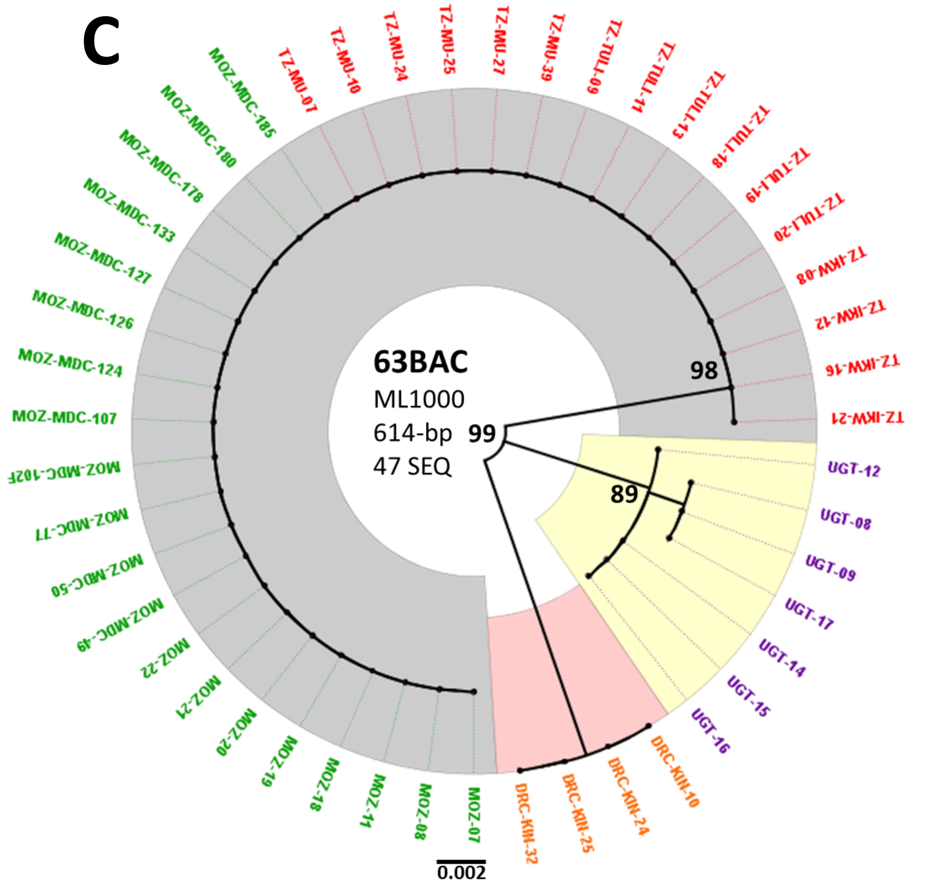
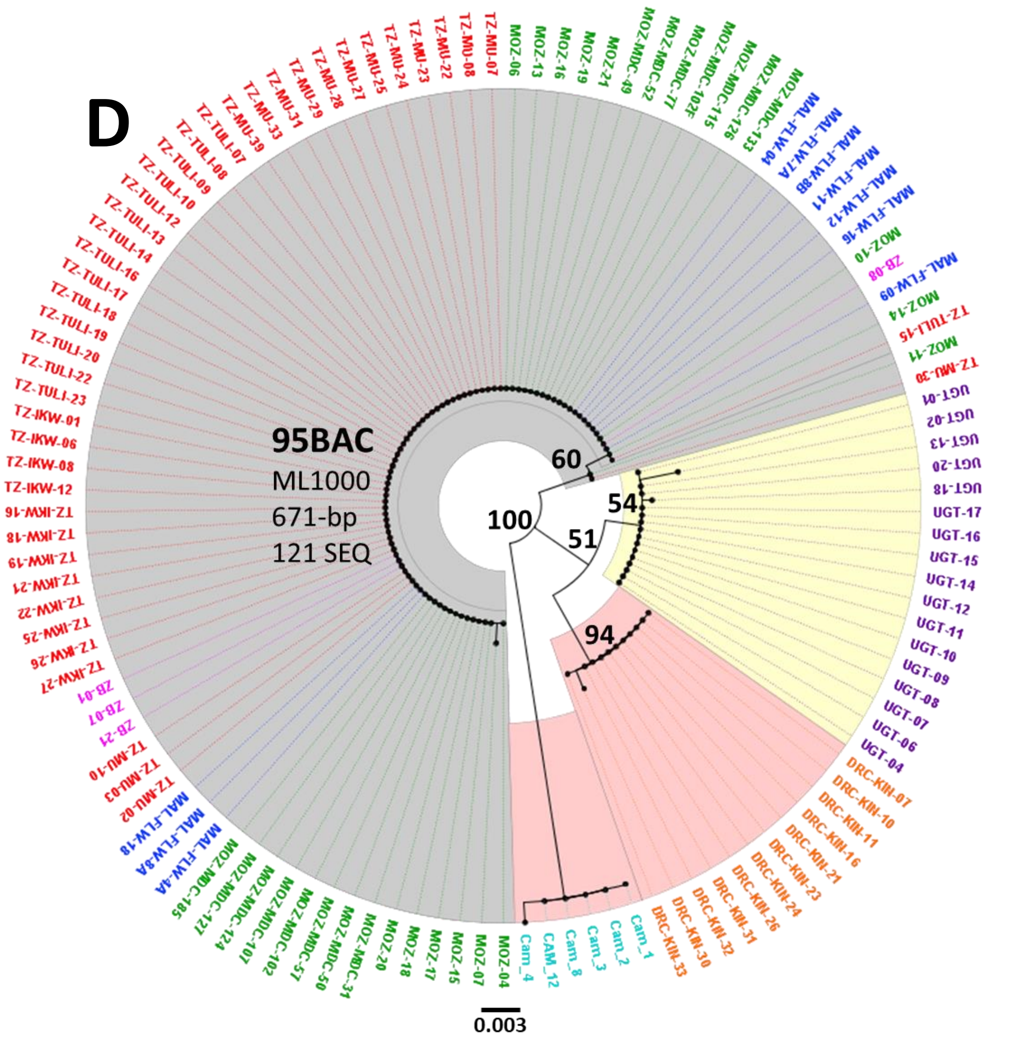


**Fig. S7: Phylogenetic trees generated with Maximum Likelihood method on *A. funestus* BAC clone from 7 sub-Saharan African countries.** Final alignments were submitted to MEGAX ModelTest program to select the best model according to the Akaike Information Criterion. The Hasegawa-kishino Yano Model plus a gamma distribution was inferred on 132 *A. funestus* 0BAC fragment sequences measuring 626-bp in length **(A)**, 115 *A. funestus* 25BAC fragment sequences measuring 754 bp **(B)** and 47 *A. funestus* 63BAC fragment sequences measuring 614bp **(C)**. The Kimura-2 model was inferred on 121 95BAC fragment sequences measuring 671bp **(D)**. To test the robustness of each tree topology, 1000 bootstrap replicates were calculated and the numbers at some nodes of the tree indicate frequencies of occurrence for 100 trees. Trees with the highest log likelihood are shown. The branch lengths are drawn to scale, and the scale bar indicates nucleotide replacements per site.





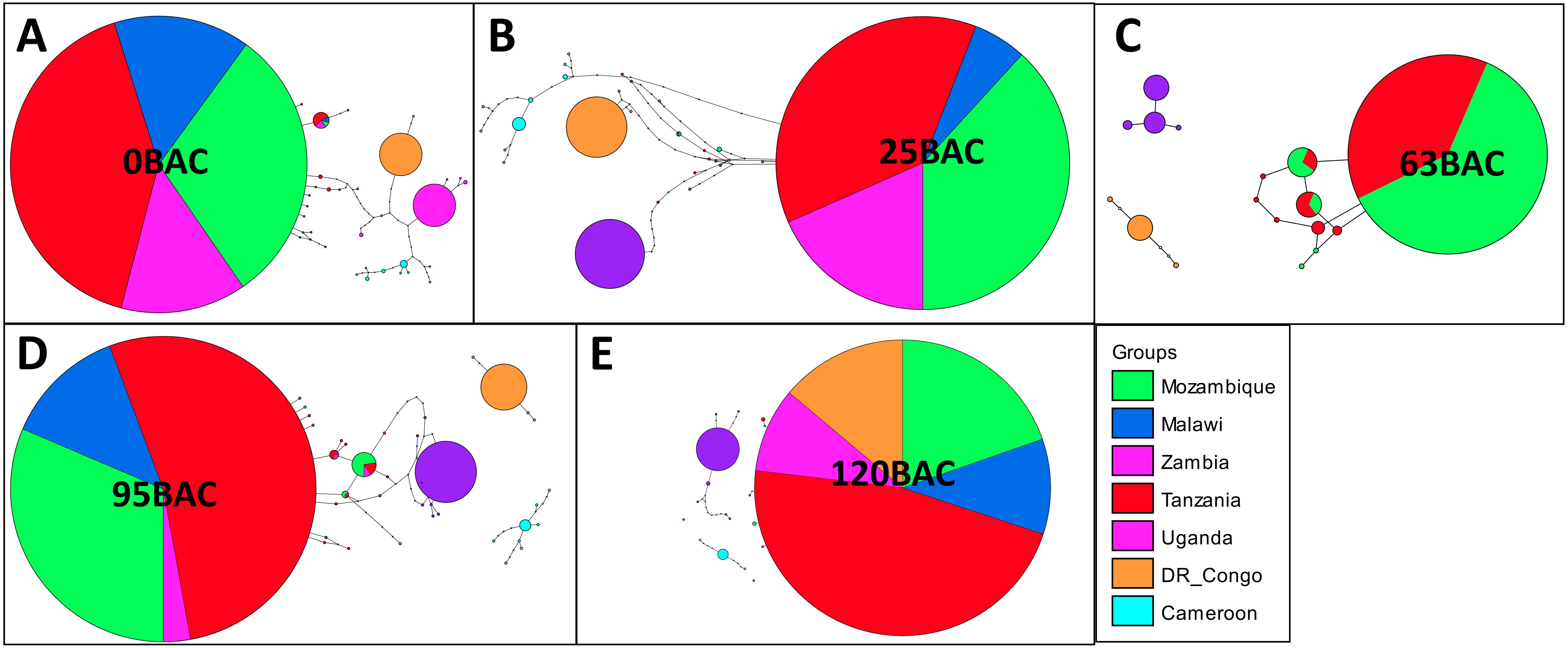


**Fig. S8 : Haplotype network for nucleotide variants in BAC fragments in 7 sub-Saharan African countries**

Africa-wide TCS network for the 0BAC **(A)**, 25BAC **(B)**, 63BAC**(C)**, 95BAC **(D)** and 120BAC **(E)** haplotypes showing predominant haplotypes in southern Africa and Tanzania later Joined by DR Congo. A nearly fixed haplotype is seen in Uganda (Purple) and DR Congo (Orange). Cameroon (Cyan) show a greater diversity. Lines connecting haplotypes and each node represent a single mutation event.

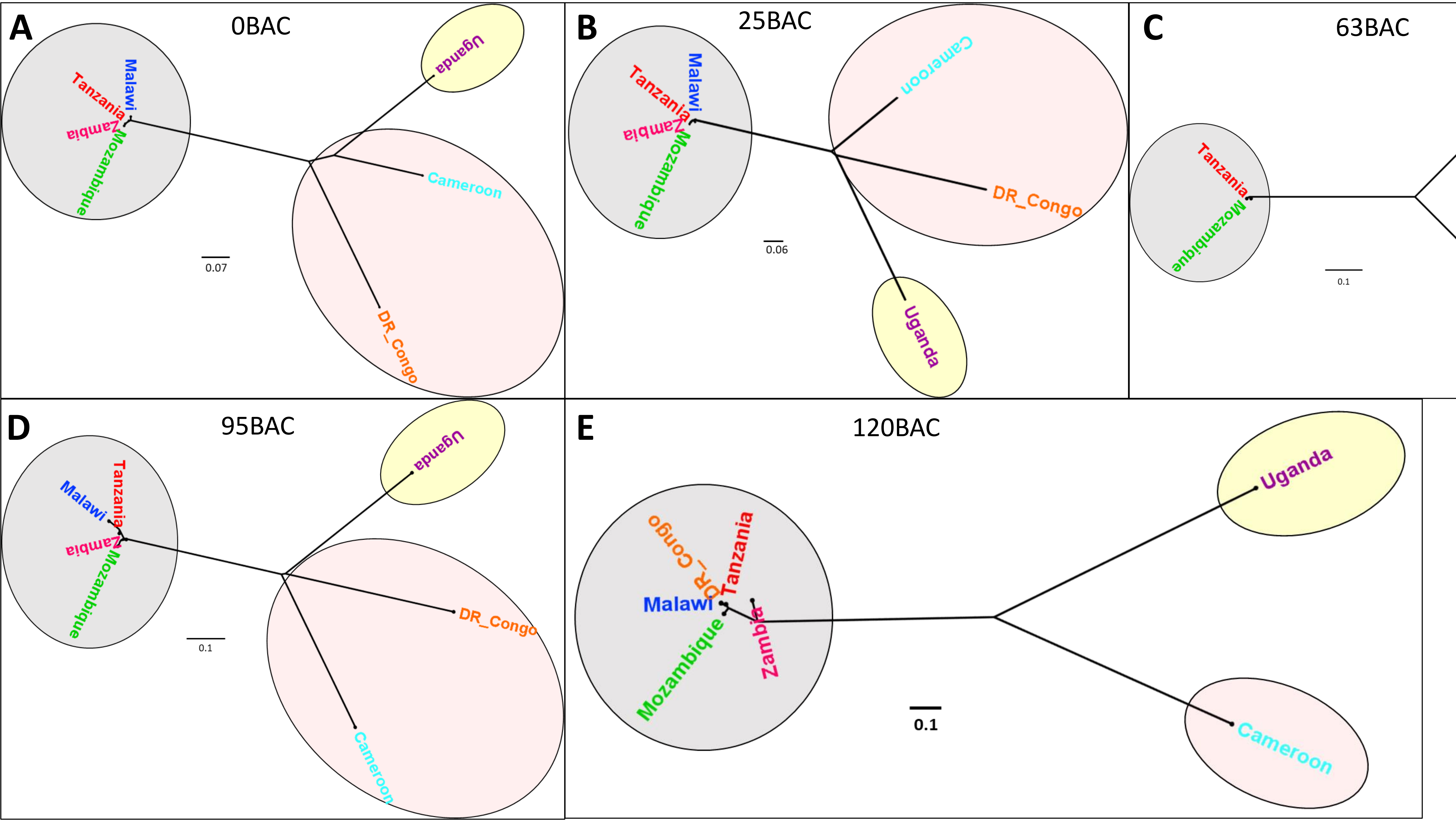


**Fig. S9: Phylogenetic tree generated by the Neighbor-Joining method based on pairwise *Fst* distance**
Trees based on $F_{st}$ distance data were computed from *A. funestus* 0BAC fragment sequences **(A)**, 25BAC fragment sequences **(B)**, 63BAC fragment sequences **(C)**, 95BAC fragment sequences **(D)** and 120BAC fragment sequences **(E)** from seven African countries, grouped, respective to each samples origin, into Southern Africa (Mozambique, Malawi, Zambia), Eastern Africa (Tanzania and Uganda) and Central Africa (DR Congo and Cameroon). Optimal trees with the sum of branch length is shown. Trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

# Tables

# Table S1: Country and sampling sites

| Countries | Sites | Coordinates | Collection date |
|---|---|---|---|
| Mozambique | Palmeira | 25° 15’ 19” S<br>32° 52’ 22” E | April 2017 |
|  | Magania da Costa | 17° 18' 40" S<br>37° 30' 53" E | March 2013 |
| Malawi | Fulirwa | 9° 55' 59.99" S<br>33° 55' 59.99" E | February 2017 |
| Zambia | Kaoma | 14° 48’00”S<br>24° 48’00”E | July 2013 |
| Tanzania | Muheza | 4° 54'18.00" S<br>38° 55'22.80" E | May 2018 |
|  | Tulizamoyo | 8.354497° S | May 2018 |
|  | Ikwambi | 7.98244° S<br>36.82167° E | May 2018 |
| Uganda | Tororo | 0° 45'N, 34° 5'E | March 2014 |
| DR-Congo | Kinshasa | 4° 19’39”S, 15° 18’48”E | May 2015 |
| Cameroon | Lagdo | 9° 05'N, 13° 40’E | June 2010 |

# Table S2: List of BAC clone and *CYP6P9a* primers used for PCRs and sequencing

| Primer | Size(bp) Range | Reverse | Forward | Tm (C) |
|---|---|---|---|---|
| 0 BAC | 716 | TCACACTAGCTGCCAAATCG | TGGTAGCTGCTTGAGGAGAAA | 57 |
| 25 BAC | 823 | TCATCTCCTTGAGTGCATCG | GTGACGGATCTGGACCTTGT | 57 |
| 63 BAC | 719 | ACCAACCGGTAAGTTTCGTG | ATTACGAACCGTCAGCCATC | 57 |
| 95 BAC | 755 | CTCCTCCGTAACGTGATCGT | CCTCTTTACTGGCCACCGTA | 57 |
| 120 BAC | 711 | AGTTCGAGCACCAGCTCAAG | TCTTCCGCCATTGTGTATCA | 57 |
| CYP6P9a | 815 | TCACAATTTTTCCACCTTCAAGTAATTACCCGC | ATCCCTAACTATTAAAAGGCAAT | 62 |
| CYP6P9aIN | - | ACTCAAACGCTTTGTTGCCG | GCTTGTTGCGTCAGAAGCTC | 67 |

# Table S3a: Gene flow and genetic differentiation patterns

| CYP6P9a | Fst | Gst | Nst | Hst | Kst |
|---|---|---|---|---|---|
| CYP6P9a full length 1,139bp | | | | | |
| Moz_P - Moz_M | 0.00 (-) | -0.01 (-32.5) | 0.00 (1*10E4) | -0.01 | 0.00 |
| TZ_MU - TZ_TULI | 0.02 (13.41) | -0.01 (-33.5) | 0.02 (13.35) | -0.01 | 0.01 |
| TZ_MU - TZ_IKW | 0.00 (-2*1E15) | -0.01 (-33.5) | 0.00 (2*10E4) | -0.01 | 0.00 |
| TZ_TULI - TZ_IKW | 0.03 (8.85) | 0.00 (191.2) | 0.03 (8.85) | -0.00 | 0.01 |
| Southern Africa | 0.02 (10.87) | 0.04 (5.86) | 0.02 (10.87) | 0.05** | 0.01*** |
| Central Africa | 0.53 (0.22) | 0.25 (0.76) | 0.53 (0.22) | 0.25*** | 0.37*** |
| South-East | 0.97 (0.01) | 0.28 (0.65) | 0.97 (0.01) | 0.28*** | 0.92*** |
| South-Central | 0.91 (0.02) | 0.30 (0.57) | 0.91 (0.02) | 0.31*** | 0.87*** |
| Central-East | 0.84 (0.05) | 0.15 (1.37) | 0.84 (0.05) | 0.15*** | 0.63*** |
| ALL | 0.93 (0.02) | 0.44 (0.32) | 0.93 (0.02) | 0.44*** | 0.93*** |
| CYP6P9a Coding Region 642 bp | | | | | |
| Moz_P - Moz_M | 0.00 (-) | -0.01 (-32.5) | 0.00 (6*10E3) | -0.01 | 0.00 |
| TZ_MU - TZ_TULI | 0.00 (-) | 0.02 (-11.5) | -0.00 (2*10E3) | 0.05 | 0.00 |
| TZ_MU - TZ_IKW | 0.00 (-2*1E15) | -0.01 (-31.6) | 0.00 (1*10E4) | -0.01 | 0.00 |
| TZ_TULI - TZ_IKW | 0.00 (-) | 0.00 (504.0) | 0.00 (-) | 0.02 | 0.00 |
| Southern Africa | 0.02 (14.52) | 0.01 (19.94) | 0.02 (14.53) | 0.01 | 0.01* |
| Central Africa | 0.68 (0.12) | 0.42 (0.35) | 0.68 (0.12) | 0.43*** | 0.54*** |
| South-East | 1.0 (0.00) | 0.61 (0.16) | 1.0 (0.00) | 0.61*** | 0.98*** |
| South-Central | 0.91 (0.02) | 0.46 (0.30) | 0.91 (0.02) | 0.46*** | 0.88*** |
| Central-East | 0.87 (0.04) | 0.35 (0.46) | 0.87 (0.04) | 0.34*** | 0.65*** |
| ALL | 0.96 (0.01) | 0.69 (0.11) | 0.96 (0.01) | 0.69*** | 0.95*** |
| CYP6P9a Non-Coding Region 497 bp | | | | | |
| Moz_P - Moz_M | - | - | - | - | - |
| TZ_MU - TZ_TULI | 0.03 (6.94) | 0.03 (9.74) | 0.03 (6.96) | 0.01 | 0.01 |
| TZ_MU - TZ_IKW | - | - | - | - | - |
| TZ_TULI - TZ_IKW | 0.03 (6.94) | 0.03 (9.30) | 0.03 (6.96) | 0.01 | 0.02 |
| Southern Africa | 0.03 (9.59) | 0.04 (6.33) | 0.03 (9.59) | 0.06** | 0.02** |
| Central Africa | 0.02 (10.5) | -0.01 (-28.2) | 0.02 (10.39) | -0.01 | 0.01 |
| South-East | 0.88 (0.03) | 0.40 (0.39) | 0.89 (0.03) | 0.40*** | 0.77*** |
| South-Central | 0.92 (0.02) | 0.53 (0.22) | 0.92 (0.02) | 0.53*** | 0.87*** |
| Central-East | 0.78 (0.07) | 0.29 (0.61) | 0.78 (0.07) | 0.29*** | 0.58*** |

# Table S3b: Gene flow and genetic differentiation patterns

| CYP6P9a | Fst | Gst | Nst | Hst | Kst |
|---|---|---|---|---|---|
| **CYP6P9a full length 1,139bp** | | | | | |
| Southern Africa | 0.02 (10.87) | 0.04 (5.86) | 0.02 (10.87) | 0.05** | 0.01*** |
| Central Africa | 0.53 (0.22) | 0.25 (0.76) | 0.53 (0.22) | 0.25*** | 0.37*** |
| South-East | 0.97 (0.01) | 0.28 (0.65) | 0.97 (0.01) | 0.28*** | 0.92*** |
| South-Central | 0.91 (0.02) | 0.30 (0.57) | 0.91 (0.02) | 0.31*** | 0.87*** |
| Central-East | 0.84 (0.05) | 0.15 (1.37) | 0.84 (0.05) | 0.15*** | 0.63*** |
| ALL | 0.93 (0.02) | 0.44 (0.32) | 0.93 (0.02) | 0.44*** | 0.93*** |
| **CYP6P9a Coding Region 642 bp** | | | | | |
| Southern Africa | 0.02 (14.52) | 0.01 (19.94) | 0.02 (14.53) | 0.01 | 0.01* |
| Central Africa | 0.68 (0.12) | 0.42 (0.35) | 0.68 (0.12) | 0.43*** | 0.54*** |
| South-East | 1.0 (0.00) | 0.61 (0.16) | 1.0 (0.00) | 0.61*** | 0.98*** |
| South-Central | 0.91 (0.02) | 0.46 (0.30) | 0.91 (0.02) | 0.46*** | 0.88*** |
| Central-East | 0.87 (0.04) | 0.35 (0.46) | 0.87 (0.04) | 0.34*** | 0.65*** |
| ALL | 0.96 (0.01) | 0.69 (0.11) | 0.96 (0.01) | 0.69*** | 0.95*** |
| **CYP6P9a Non-Coding Region 497 bp** | | | | | |
| Southern Africa | 0.03 (9.59) | 0.04 (6.33) | 0.03 (9.59) | 0.06** | 0.02** |
| Central Africa | 0.02 (10.5) | -0.01 (-28.2) | 0.02 (10.39) | -0.01 | 0.01 |
| South-East | 0.88 (0.03) | 0.40 (0.39) | 0.89 (0.03) | 0.40*** | 0.77*** |
| South-Central | 0.92 (0.02) | 0.53 (0.22) | 0.92 (0.02) | 0.53*** | 0.87*** |
| Central-East | 0.78 (0.07) | 0.29 (0.61) | 0.78 (0.07) | 0.29*** | 0.58*** |
| ALL | 0.87 (0.04) | 0.55 (0.20) | 0.87 (0.04) | 0.56*** | 0.87*** |

Table S4a: Pairwise Fst distance based on 6p9a sequences from different countries

| | MOZ_P | MOZ_M | MOZ | MAL | ZB | TZ-MU | TZ-TULI | TZ-IKW | TZ | DRC | CAM | UG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CYP6P9a_Full_1139 bp** | | | | | | | | | | | | |
| **MOZ_P** | - | | | | | | | | | | | |
| **MOZ_M** | **0.000** | - | | | | | | | | | | |
| **MOZ** | **0.000** | **0.000** | - | | | | | | | | | |
| **MAL** | **0.019** | **0.018** | **0.018** | - | | | | | | | | |
| **ZB** | **0.035** | **0.031** | **0.032** | **0.030** | - | | | | | | | |
| **TZ-MU** | **0.000** | **0.000** | **0.000** | **0.006** | **0.024** | - | | | | | | |
| **TZ-TULI** | **0.028** | **0.024** | **0.025** | **0.022** | **0.039** | **0.018** | - | | | | | |
| **TZ-IKW** | **0.000** | **0.000** | **0.000** | **0.019** | **0.034** | **0.000** | **0.027** | - | | | | |
| **TZ** | **0.006** | **0.005** | **0.005** | **0.013** | **0.029** | **0.000** | **0.000** | **0.000** | - | | | |
| **DRC** | **0.958** | **0.956** | **0.957** | **0.947** | **0.951** | **0.953** | **0.952** | **0.957** | **0.954** | - | | |
| **CAM** | **0.934** | **0.932** | **0.933** | **0.924** | **0.927** | **0.929** | **0.929** | **0.934** | **0.930** | **0.537** | - | |
| **UG** | **0.977** | **0.975** | **0.976** | **0.966** | **0.970** | **0.972** | **0.972** | **0.977** | **0.973** | **0.894** | **0.877** | - |
| **CYP6P9a_Coding_642 bp** | | | | | | | | | | | | |
| **MOZ_P** | - | | | | | | | | | | | |
| **MOZ_M** | **0.000** | - | | | | | | | | | | |
| **MOZ** | **0.000** | **0.000** | - | | | | | | | | | |
| **MAL** | **0.027** | **0.022** | **0.024** | - | | | | | | | | |
| **ZB** | **0.000** | **0.000** | **0.000** | **0.037** | - | | | | | | | |
| **TZ-MU** | **0.000** | **0.000** | **0.000** | **0.003** | **0.000** | - | | | | | | |
| **TZ-TULI** | **0.000** | **0.000** | **0.000** | **0.037** | **0.000** | **0.000** | - | | | | | |
| **TZ-IKW** | **0.000** | **0.000** | **0.000** | **0.027** | **0.000** | **0.000** | **0.000** | - | | | | |
| **TZ** | **0.000** | **0.000** | **0.000** | **0.019** | **0.000** | **0.000** | **0.000** | **0.000** | - | | | |
| **DRC** | **0.983** | **0.980** | **0.981** | **0.978** | **0.985** | **0.975** | **0.985** | **0.982** | **0.981** | - | | |
| **CAM** | **0.926** | **0.924** | **0.925** | **0.921** | **0.928** | **0.919** | **0.928** | **0.926** | **0.925** | **0.678** | - | |
| **UG** | **0.998** | **0.996** | **0.997** | **0.994** | **1.000** | **0.992** | **1.000** | **0.997** | **0.997** | **0.973** | **0.905** | - |
| **CYP6P9a_Non-Coding_497 bp** | | | | | | | | | | | | |
| **MOZ_P** | - | | | | | | | | | | | |
| **MOZ_M** | 0.000 | - | | | | | | | | | | |
| **MOZ** | 0.000 | 0.000 | - | | | | | | | | | |
| **MAL** | 0.012 | 0.012 | 0.012 | - | | | | | | | | |
| **ZB** | 0.042 | 0.042 | 0.042 | 0.028 | - | | | | | | | |
| **TZ-MU** | 0.000 | 0.000 | 0.000 | 0.012 | 0.042 | - | | | | | | |
| **TZ-TULI** | 0.035 | 0.035 | 0.035 | 0.016 | 0.039 | 0.035 | - | | | | | |
| **TZ-IKW** | 0.000 | 0.000 | 0.000 | 0.012 | 0.042 | 0.000 | 0.035 | - | | | | |
| **TZ** | 0.014 | 0.014 | 0.014 | 0.009 | 0.035 | 0.014 | 0.000 | 0.014 | - | | | |
| **DRC** | 0.912 | 0.912 | 0.912 | 0.893 | 0.891 | 0.912 | 0.894 | 0.912 | 0.904 | - | | |
| **CAM** | 0.950 | 0.950 | 0.950 | 0.928 | 0.925 | 0.950 | 0.929 | 0.950 | 0.941 | 0.023 | - | |
| **UG** | 0.899 | 0.899 | 0.899 | 0.869 | 0.865 | 0.899 | 0.870 | 0.899 | 0.887 | 0.766 | 0.806 | - |

# Table S4b: Pairwise Fst distance based on 6p9a sequences from different countries

| | Mozambique | Malawi | Zambia | Tanzania | Uganda | DR_Congo | Cameroon |
|---|---|---|---|---|---|---|---|
| **CYP6P9a_Full_1139 bp** | | | | | | | |
| **Mozambique** | - | | | | | | |
| **Malawi** | 0.018** | - | | | | | |
| **Zambia** | 0.032 | 0.030 | - | | | | |
| **Tanzania** | 0.005 | 0.013** | 0.029 | - | | | |
| **Uganda** | 0.957*** | 0.947*** | 0.951*** | 0.954*** | - | | |
| **DR_Congo** | 0.933*** | 0.924*** | 0.927*** | 0.930*** | 0.537*** | - | |
| **Cameroon** | 0.976*** | 0.966*** | 0.970*** | 0.973*** | 0.894*** | 0.877*** | - |
| **CYP6P9a_Coding_642 bp** | | | | | | | |
| **Mozambique** | - | | | | | | |
| **Malawi** | 0.024 | - | | | | | |
| **Zambia** | 0.000 | 0.037 | - | | | | |
| **Tanzania** | 0.000 | 0.019* | 0.000 | - | | | |
| **Uganda** | 0.981*** | 0.978*** | 0.985*** | 0.981*** | - | | |
| **DR_Congo** | 0.925*** | 0.921*** | 0.928*** | 0.925*** | 0.678*** | - | |
| **Cameroon** | 0.997*** | 0.994*** | 1.000*** | 0.997*** | 0.973*** | 0.905*** | - |
| **CYP6P9a_Non-Coding_497 bp** | | | | | | | |
| **Mozambique** | - | | | | | | |
| **Malawi** | 0.012*** | - | | | | | |
| **Zambia** | 0.042* | 0.028 | - | | | | |
| **Tanzania** | 0.014 | 0.009* | 0.035 | - | | | |
| **Uganda** | 0.912*** | 0.893*** | 0.891*** | 0.904*** | - | | |
| **DR_Congo** | 0.950*** | 0.928*** | 0.925*** | 0.941*** | 0.023 | - | |
| **Cameroon** | 0.899*** | 0.869*** | 0.865*** | 0.887*** | 0.766*** | 0.806*** | - |

# Table S5: Ratio of nucleotide diversity at *CYP6P9a* and five loci around the pyrethroid resistance gene.

| | 0BAC (-34kb) | 25BAC (-9kb) | 6P9A (0kb) | 63BAC (+29kb) | 95BAC (+61kb) | 120BAC (+86kb) |
|---|---|---|---|---|---|---|
| **Eastern / southern Africa** | **3.17117** | **0.00000** | **2.91667** | **0.75342** | **1.17822** | **1.55472** |
| **Central / southern Africa** | **6.51351** | **3.23967** | **7.00000** | **1.36301** | **2.42079** | **1.17736** |
| **East-Central / Southern Africa** | **4.84234** | **1.61983** | **1.40607** | **1.05822** | **1.79950** | **1.36604** |

**Table S6: Location of different BAC fragments regarding *An. funestus* genome annotation features from v**

| Chromosome | Start Position (Kb) | End Position (Kb) | Strand | Gene attribute |
|---|---|---|---|---|
| CM012071.1 | 8449150 | 8579233 | + | AFUN020405 (solute carrier family 8; sodium/calcium exchanger) |
| **0BAC (-34kb)** | **8510505** | **8511221** | **+** | **within AFUN020405 locus** |
| CM012071.1 | 8531151 | 8533251 | - | AFUN015786 (CYP6PAA1; cytochrome P450) |
| CM012071.1 | 8533523 | 8535535 | - | AFUN015785 (CYP6PAA2; cytochrome P450) |
| **25BAC (-9kb)** | **8535505** | **8536328** | **-** | **within CYP6PAA2 and AFUN015785 loci** |
| CM012071.1 | 8535644 | 8537599 | - | AFUN015787 |
| CM012071.1 | 8539121 | 8540985 | - | AFUN015793 (Carboxylic ester hydrolase) |
| CM012071.1 | 8541428 | 8543101 | - | AFUN008357 (Cytochrome P450) |
| **CM012071.1** | **8544505** | **8546181** | **-** | **AFUN015792 (CYP6P9A; cytochrome P450)** |
| CM012071.1 | 8554228 | 8555956 | - | AFUN015889 (CYP6P9b; cytochrome P450) |
| CM012071.1 | 8556748 | 8558550 | - | AFUN015888 (CYP6P5; cytochrome P450) |
| CM012071.1 | 8559923 | 8562096 | - | AFUN020895 (cytochrome P450) |
| CM012071.1 | 8562645 | 8564463 | - | AFUN019365 (cytochrome P450) |
| CM012071.1 | 8565228 | 8566912 | - | AFUN015802 (CYP6P1; cytochrome P450) |
| CM012071.1 | 8567086 | 8569039 | - | AFUN015801 (CYP6P2; cytochrome P450) |
| CM012071.1 | 8569231 | 8571137 | - | AFUN015714 (CYP6AD1; cytochrome P450) |
| **63BAC (+29kb)** | **8573505** | **8574224** | **-** | **Close to AFUN008362 locus** |
| CM012071.1 | 8582748 | 8584347 | - | AFUN008362 |
| CM012071.1 | 8586822 | 8589035 | + | AFUN008363 |
| CM012071.1 | 8597859 | 8599357 | - | AFUN021910 (lipase) |
| **95BAC (+61kb)** | **8605505** | **8606260** | **-** | **Within AFUN021912 locus** |
| CM012071.1 | 8602367 | 8608947 | - | AFUN021912 |
| CM012071.1 | 8609970 | 8613744 | - | AFUN021911 |
| **120BAC (+86kb)** | **8630505** | **8631216** | **+** | **within AFUN008365 locus** |

**Table S7: Gene flow and genetic differentiation patterns**

| | Fst (Nm) | Gst (Nm) | Nst (Nm) | Hst | Kst |
|---|---|---|---|---|---|
| **0BAC** | | | | | |
| **Southern Africa** | 0.02 (15.85) | 0.02 (15.10) | 0.02 (15.87) | 0.01 | 0.01 |
| **Central Africa** | 0.76 (0.08) | 0.34 (0.49) | 0.76 (0.08) | 0.36*** | 0.65*** |
| **South-Central** | 0.77 (0.07) | 0.34 (0.49) | 0.77 (0.07) | 0.34*** | 0.68*** |
| **South-East** | 0.93 (0.02) | 0.38 (0.41) | 0.93 (0.02) | 0.38*** | 0.83*** |
| **East-Central** | 0.51 (0.24) | 0.32 (0.53) | 0.51 (0.24) | 0.32*** | 0.32*** |
| **ALL** | 0.87 (0.04) | 0.51 (0.24) | 0.87 (0.04) | 0.51*** | 0.86*** |
| **25BAC** | | | | | |
| **Southern Africa** | 0.004 (56.20) | 0.01 (26.32) | 0.004 (55.94) | 0.02 | 0.002 |
| **Central Africa** | 0.80 (0.06) | 0.37 (0.42) | 0.80 (0.06) | 0.40*** | 0.72*** |
| **South-Central** | 0.67 (0.12) | 0.45 (0.31) | 0.67 (0.12) | 0.45*** | 0.57*** |
| **South-East** | 0.98 (0.01) | 0.66 (0.13) | 0.98 (0.01) | 0.66*** | 0.88*** |
| **East-Central** | 0.75 (0.08) | 0.48 (0.27) | 0.75 (0.08) | 0.48*** | 0.55*** |
| **ALL** | 0.87 (0.04) | 0.69 (0.11) | 0.87 (0.04) | 0.70*** | 0.88*** |
| **63BAC** | | | | | |
| **Southern Africa** | 0.00 (00) | 0.006 (40.0) | 0.00 (00) | 0.007 | 0.00 |
| **South-Central** | 0.93 (0.02) | 0.17 (1.26) | 0.93 (0.02) | 0.15*** | 0.73*** |
| **South-East** | 0.92 (0.02) | 0.19 (1.08) | 0.92 (0.02) | 0.18*** | 0.75*** |
| **East-Central** | 0.92 (0.02) | 0.28 (0.64) | 0.92 (0.02) | 0.28*** | 0.85*** |
| **ALL** | 0.91 (0.03) | 0.26 (0.72) | 0.91 (0.03) | 0.26*** | 0.82*** |
| **95BAC** | | | | | |
| **Southern Africa** | 0.02 (11.82) | 0.03 (9.62) | 0.02 (11.82) | 0.03* | 0.02* |
| **Central Africa** | 0.92 (0.02) | 0.32 (0.54) | 0.92 (0.02) | 0.40*** | 0.88*** |
| **South-Central** | 0.62 (0.15) | 0.27 (0.66) | 0.62 (0.15) | 0.28*** | 0.53*** |
| **South-East** | 0.93 (0.02) | 0.36 (0.45) | 0.92 (0.02) | 0.36*** | 0.77*** |
| **East-Central** | 0.50 (0.25) | 0.34 (0.48) | 0.50 (0.25) | 0.34*** | 0.33*** |
| **ALL** | 0.91 (0.02) | 0.46 (0.29) | 0.91 (0.02) | 0.47*** | 0.87*** |
| **120BAC** | | | | | |
| **Southern Africa** | 0.03 (8.54) | 0.02 (10.64) | 0.03 (8.57) | 0.03* | 0.02** |
| **Central Africa** | 0.91 (0.02) | 0.71 (0.10) | 0.91 (0.02) | 0.76*** | 0.89*** |
| **South-Central** | 0.19 (1.08) | 0.05 (4.37) | 0.19 (1.08) | 0.06*** | 0.12*** |
| **South-East** | 0.94 (0.01) | 0.57 (0.19) | 0.94 (0.01) | 0.58*** | 0.87*** |
| **East-Central** | 0.84 (0.05) | 0.42 (0.34) | 0.84 (0.05) | 0.42*** | 0.73*** |
| **ALL** | 0.88 (0.03) | 0.60 (0.16) | 0.88 (0.03) | 0.61*** | 0.88*** |

*, $0.01<P<0.05$; **, $0.001<P<0.01$; ***, $P<0.001$

# Table S8: Pairwise Fst distance between different countries

**0BAC**

| | Mozambique | Malawi | Tanzania | Zambia | Uganda | DR_Congo | Cameroon |
|---|---|---|---|---|---|---|---|
| Mozambique | - | | | | | | |
| Malawi | 0.031* | - | | | | | |
| Tanzania | 0.019* | 0.017 | - | | | | |
| Zambia | 0.001 | 0.021 | 0.002 | - | | | |
| Uganda | 0.949*** | 0.932*** | 0.920*** | 0.941*** | - | | |
| DR_Congo | 0.977*** | 0.959*** | 0.947*** | 0.968*** | 0.874*** | - | |
| Cameroon | 0.849*** | 0.836*** | 0.825*** | 0.843*** | 0.601*** | 0.759*** | - |

**25BAC**

| | Mozambique | Malawi | Tanzania | Zambia | Uganda | DR_Congo | Cameroon |
|---|---|---|---|---|---|---|---|
| Mozambique | - | | | | | | |
| Malawi | 0.018 | - | | | | | |
| Tanzania | 0.008 | 0.000 | - | | | | |
| Zambia | 0.004 | 0.025 | 0.000 | - | | | |
| Uganda | 0.995*** | 0.967*** | 0.965*** | 0.972*** | - | | |
| DR_Congo | 0.979*** | 0.943*** | 0.943*** | 0.951*** | 0.989*** | - | |
| Cameroon | 0.740*** | 0.706*** | 0.704*** | 0.717*** | 0.780*** | 0.780*** | - |

**63BAC**

| | Mozambique | Tanzania | Uganda | DR_Congo |
|---|---|---|---|---|
| Mozambique | - | | | |
| Tanzania | 0.000 | - | | |
| Uganda | 0.932*** | 0.913*** | - | |
| DR_Congo | 0.927*** | 0.913*** | 0.916*** | - |

**95BAC**

| | Mozambique | Malawi | Tanzania | Zambia | Uganda | DR_Congo | Cameroon |
|---|---|---|---|---|---|---|---|
| Mozambique | - | | | | | | |
| Malawi | 0.115 | - | | | | | |
| Tanzania | 0.036* | 0.024 | - | | | | |
| Zambia | 0.000 | 0.049* | 0.000* | - | | | |
| Uganda | 0.896*** | 0.946*** | 0.924*** | 0.873*** | - | | |
| DR_Congo | 0.937*** | 0.970*** | 0.956*** | 0.921*** | 0.906*** | - | |
| Cameroon | 0.920*** | 0.935*** | 0.928*** | 0.913** | 0.923*** | 0.925*** | - |

**120BAC**

| | Mozambique | Malawi | Tanzania | Zambia | Uganda | DR_Congo | Cameroon |
|---|---|---|---|---|---|---|---|
| Mozambique | - | | | | | | |
| Malawi | 0.029 | - | | | | | |
| Tanzania | 0.019 | 0.010 | - | | | | |
| Zambia | 0.030 | 0.03* | 0.027** | - | | | |
| Uganda | 0.956*** | 0.959*** | 0.955*** | 0.860*** | - | | |
| DR_Congo | 0.029 | 0.000 | 0.010 | 0.030** | 0.959*** | - | |
| Cameroon | 0.906*** | 0.911*** | 0.906*** | 0.797*** | 0.936*** | 0.911*** | - |