The Anopheles coluzzii range extends into Kenya: Detection, insecticide resistance profiles and population genetic structure in relation to conspecific populations in West and Central Africa

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Research Article

Abstract

Background

Anopheles coluzzii is a primary vector of malaria found in West and Central Africa, but its presence has hitherto never been documented in Kenya. A thorough understanding of vector bionomics is important as it enables the implementation of targeted and effective vector control interventions. Malaria vector surveillance efforts in the country have tended to focus on historically known primary vectors. In the current study, we sought to determine the taxonomic status of samples collected from five different malaria epidemiological zones in Kenya as well asdescribe the population genetic structure and insecticide resistance profiles in relation to other *An. coluzzi* populations.

Methods

Mosquitoes were sampled as larvae from Busia, Kwale, Turkana, Kirinyaga and Kiambu counties, representing the range of malaria endemicities in Kenya, in 2019 and 2021 and emergent adults analysed using Whole Genome Sequencing data processed in accordance with the *Anopheles gambiae* 1000 Genomes Project phase 3. Where available, historical samples from the same sites were included for WGS.

Results

This study reports the detection of *Anopheles coluzzii* for the first time in Kenya. The species was detected in Turkana County across all three time points sampled and its presence confirmed through taxonomic analysis. Additionally, we found a lack of strong population genetic differentiation between *An. coluzzii* from Kenya and those from the more northerly regions of West and Central Africa, suggesting they represent a connected extension to the known species range. Mutations associated with target-site resistance to DDT and pyrethroids and metabolic resistance to DDT were found at high frequencies of ~60%. The profile and frequencies of the variants observed were similar to *An. coluzzii* from West and Central Africa but the ace-1 mutation linked to organophosphate and carbamate resistance present in *An. coluzzii* from coastal West Africa was absent in Kenya.

Conclusions

These findings emphasise the need for the incorporation of genomics in comprehensive and routine vector surveillance to inform on the range of malaria vector species, and their insecticide resistance status to inform the choice of effective vector control approaches.

Introduction

Malaria is transmitted through the infectious bite of the *Anopheles* female mosquito and is a major cause of morbidity and mortality in Kenya and sub-Saharan Africa in general. In 2022, there were an estimated 249 million cases of malaria worldwide with 233 million of these occurring in the WHO African region and accounting for about 94% of all cases (1). In Kenya, approximately 70% of the population is at risk of malaria with the disease accounting for an estimated 13 - 15% of outpatient consultations (2). Despite concerted global efforts to control malaria, elimination remains a challenge in both low and high burden settings. Historically, *Anopheles gambiae* and *Anopheles funestus* species complexes have been known to transmit malaria in Kenya. Within the

Anopheles gambiae complex, *An. gambiae* sensu stricto (s.s.) and *An. arabiensis* were considered the major vectors, with *An. merus* contributing to transmission in coastal Kenya. Invasive *An. stephensi* has also recently been detected in the country but it's contribution to malaria control is yet to be evaluated (3).

Anopheles coluzzii is another member of the *An. gambiae s.l.* species complex which is morphologically indistinguishable from at least ten sibling species of malaria in sub-Saharan Africa (SSA). It is responsible for a significant proportion of the malaria transmission across SSA along with *An. gambiae* and *An. arabiensis*, although *An. funestus* is of increasing concern in East and Southern Africa (4). *Anopheles coluzzii* was formally named in 2013 following accumulating evidence of subdivision between the previously described M (Mopti) and S (Savana) molecular forms of *An. gambiae,* evidence whichincluded the presence of pre-mating barriers and genome-wide divergence and independent evolutionary trajectories; consequently, the M form was assigned the name *An. coluzzii* and the S form *An. gambiae* (5). *Anopheles coluzzii* is widely distributed in West and Central Africa and found in sympatry with other members of the species complex and has also been documented in Somalia (6). To date however, no record of its presence in Kenya is available. Similar to other vector species within the *Anopheles gambiae* s.l. species complex, the distribution and role of *An. coluzzii* in malaria transmission as well as the development of insecticide resistance varies greatly in different settings (7–9).

The heterogeneities with respect to ecological characteristics and trophic habits of members of the *An. gambiae* s.l. species complex have allowed the expansion of its range and contributed to its success in malaria transmission (10,11). Compared to *An. gambiae* which prefers to breed in unpolluted environments typical of rural areas*, An. coluzzii* possess a greater capacity to survive in ecologically complex environments characterised by the presence of a variety of stressors. *An. coluzzii* is likely to have a greater resistance to desiccation because it predominates in arid regions (12,13). Studies have shown *An. coluzzii* to have greater tolerance to salinity as well as xenobiotics and ammonia pollutants in larval habitat compared to *An. gambiae,* enabling extension of its range beyond traditional rural settings to densely urbanised settings (14–16). With the trends in urban migration seen in many African countries expected to continue and unplanned human settlements in urban settings presenting a potential risk factor of increased malaria transmission (17), comprehensive vector surveillance is crucial. Such surveillance will improve the understanding of vector bionomics thereby enabling the implementation of targeted and effective vector control interventions (18–20). These objectives are in line with the WHO Global Vector Control Response 2017–2030 strategy's recommendation of strengthening national surveillance systems and integration with health information systems to guide vector control and effectiveness (21).

Most entomological studies and vector surveillance efforts, including those in Kenya tend to focus on the historically known primary vectors of malaria to the exclusion of unanticipated or novel vector species. Recently however, the potential role of secondary vectors in malaria transmission has been highlighted aided by the use of molecular identification tools (22,23). The current study used whole genome sequence (WGS) data to investigate the taxonomic relationship of samples collected across five counties in Kenya with different epidemiological parameters. This investigation describes the first report on *An. coluzzii* in Kenya while seeking to characterise their population structure and insecticide resistance profiles.

Materials and Methods

Mosquito sampling, identification and rearing

The study utilised archived mosquitoes collected from previous studies in addition to samples collected between December 2019 and February 2021 from five study sites (Fig. 1). These were i) Teso in Busia County ii) Kwale in Kwale County iii) Kakuma in Turkana County iv) Mwea in Kirinyaga County and v) Thika in Kiambu County (Table). These five locations represent different ecological and malaria epidemiological zones within Kenya (24). Teso on the western border of Kenya with Uganda is within the lake endemic zone. Kwale in the southeast is within the coastal endemic zone. Turkana in the northwest is relatively arid and within the seasonal transmission zone. Mwea and Thika are within the central highlands with Mwea being in the seasonal transmission zone and Thika in the low-risk zone.

The figure shows the sampling locations in Kenya in relation to *Plasmodium falciparum* prevalence rate in 2015 standardised to the age group 2 to 10 years using data obtained from the malariaAtlas R package (25).

For both sets of mosquitoes, *Anopheles gambiae* s.l. larvae identified based on morphology (26) were collected, using standard larval 350 ml dippers, from multiple breeding sites to minimize chances of sampling siblings. The larvae were transported to the laboratory for rearing to adults (except for Turkana where rearing was carried out in the field due to the long distance to the laboratory). Larvae were reared in water collected from the larval sampling site or dechlorinated tap water at temperatures between 28°C – 31°C and humidity between 80% – 85% and fed on finely ground *Sera Vipan staple diet™* (Sera, Germany) fish food. The resultant adult mosquitoes were analysed using WGS.

Sequencing and SNP calling

Sequencing and single nucleotide polymorphisms (SNP) calling was performed following the Ag1000G phase 3 project protocol. Briefly, paired-end multiplex libraries were prepared using Illumina's DNA preparation protocol with fragmentation using Covaris Adaptive Focused Acoustics. Multiplexes of 12 tagged individual mosquitoes were sequenced in three replicates using Illumina HiSeq 2000 and the Illumina HiSeq X technologies. Reads were aligned to the AgamP4 reference genome using BWA version 0.7.15 and indel realignment and SNP calling performed using GATK version 3.7.0. Quality control filters applied included the exclusion of individuals with median coverage < 10X, with no coverage across > 50% of the reference genome, or samples identified as cross-contaminated by a percentage of ≥ 4.5% using the protocols set out by the AG1000G project. Only technical replicates with the best sequencing coverage were retained. Additionally, site filters defined by the Ag1000G project were applied to exclude sites where SNP calling and genotyping was less reliable because the observed genotypes were not consistent with Mendelian inheritance in laboratory crosses.

Taxonomic assignment

To investigate taxonomic status, individual mosquitoes were assessed against two sets of ancestry-informative markers (AIMs) used to distinguish *An. gambiae* from its sister taxa *An. coluzzii* and *An. gambiae/ An. coluzzii* from *An. arabiensis* using publicly available data from the *Anopheles* 16 genomes project (27–29). The dataset, described by the *Anopheles gambiae* 1000 Genomes project (30), includes a set of AIMs SNPs informative in distinguishing taxa because they are exclusive to each taxonomic group discounting multiallelic sites and those with missing data. In total, 2612 and 700 AIMs were used to differentiate *An. gambiae/ An. coluzzii* from *An. arabiensis* and *An. gambiae* from *An. coluzzii*, respectively. Individuals assessed against AIMs for distinguishing *An. gambiae* from *An. coluzzii* were called as *An. gambiae* when the fraction of coluzzii-like calls was < 0.12 and *An. coluzzii* where this fraction was > 0.9. Individuals assessed against AIMs distinguishing *An. gambiae/ An.*

coluzzii from *An. arabiensis* were called as the latter when the fraction of arabiensis-like alleles was > 0.6. Individuals in-between these fractions represent other taxa.

Population Structure

To compare the genomic composition of *An. coluzzii* in Kenya with other *An. coluzzii* cohorts, we performed a Principal Component Analysis (PCA) dimensionality reduction on the allele counts of 100,000 biallelic SNPs equally distributed across chromosome three. Chosen SNPs had a minor allele frequency greater than 0.2% and no missing data. Using the same criteria for SNP selection, we then compared the evolutionary relationships of African *An. coluzzii* by constructing an unrooted Neighbour-Joining tree with a cityblock distance metric. To determine whether *An. coluzzii* across Africa are connected to *An. coluzzii* in Kenya, we computed genomic differentiation between populations using Hudson's pairwise FST (31). To further investigate whether *An. coluzzii* in Kenya have a similar demography to other *An. coluzzii*, we calculated informative summary statistics including Nucleotide diversity (θ_π), Watterson's theta (θ_W) and Tajima's D. All analyses were performed using the open source and freely available malariagen_data python package.

Insecticide Resistance

To investigate whether *An. coluzzii* in Kenya have target site mutations associated with insecticide resistance similar to other African *An. coluzzii*, we used the malariagen_python package to calculate amino acid substitution frequencies based on the occurrence of non-synonymous SNPs at genomic sites of interest. These included the gene targeted by pyrethroid insecticides, the voltage-gated sodium channel (Vgsc; AGAP004707), the glutathione S-transferase gene conferring resistance to DDT (*Gste2*; AGAP009194), the Resistance to dieldrin gene (*Rdl*; AGAP006028) and the organophosphate target gene, acetylcholinesterase (*Ace1*; AGAP001356). To account for sequencing error and remove substitutions unlikely to be under selection, only amino acid substitutions present at a frequency greater than 5% in at least one population were retained.

Results

Population sampling and sequencing

A total of 1,130 individual mosquitoes were collected during this study from five locations in Kenya (Fig. 1; Table). All locations included relatively recent sampling (2019–2021) and samples from earlier collections (2006–2014) were also available for Mwea, Teso and Turkana. A total of 744 mosquitoes with good quality extracted DNA were submitted for WGS, of which 564 passed all data quality control filters, with an average median coverage of 36 X and minimum of 10 X. After alignment to the AGAMP4 reference genome, we discovered a total of 83,052,633 SNPs segregating within the samples from this study, of which 43,701,680 passed all site quality filters previously established by the *Anopheles gambiae*1000 Genomes Project phase 3 (30)

Table Sampling locations in Kenya and the number of samples successfully sequenced, grouped by taxon

Location	Latitude	Longitude	Year	Month	Taxon			
					An. arabiensis	An. coluzzii	An. gambiae	An. quadriannulatus
Kwale	-4.572	39.257	2019	7	7	0	0	2
Mwea	-0.717	37.382	2007	6	18	0	0	0
			2014	9	15	0	0	0
			2020	2	46	0	0	0
			2020	12	26	0	0	0
			2021	1	29	0	0	1
			2021	2	16	0	0	0
Teso	0.626	34.236	2013	8	24	0	4	0
			2019	7	17	0	14	0
			2019	12	43	0	17	0
Thika	-1.061	37.181	2019	7	45	0	1	0
			2019	8	19	0	0	0
Turkana	3.717	34.857	2006	2	19	5	1	0
			2019	1	51	13	0	0
			2019	9	123	8	0	0

Taxon assignment

Taxon assignment within the *An. gambiae* complex is challenging because taxa are morphologically indistinguishable, and conventional genetic markers are based on a single locus that does not always reflect the ancestry of the rest of the genome (28, 29). All samples in this study were morphologically identified as *Anopheles gambiae s.l.*, then the genomic data used to investigate the species. Using a set of ancestry-informative markers (AIMs) previously ascertained from samples with known species status (28,29) we identified 498 samples as *Anopheles arabiensis* (arabiensis AIM fraction > 0.85; Supplementary Figure 1). Of the remaining samples, 37 were identified as *Anopheles gambiae* (coluzzii AIM fraction < 0.1) and 26 were identified as *Anopheles coluzzii* (coluzzii AIM fraction > 0.9). To provide additional confirmation of taxonomic status, we performed a principal components analysis (PCA) and constructed a neighbour-joining tree (NJT) using genomic data from this study together with samples from other African countries from the Anopheles gambiae 1000 Genomes Project and the study of Fontaine et al. (29)(Supplementary Figure 2). These analyses showed a clear grouping by species, with the position of Kenyan *An. gambiae*, *An. arabiensis* and *An. coluzzii* samples entirely consistent with the AIM results. On comparison of data originating from multiple taxa (29), a further 3 individuals that could not be identified via AIMs were confirmed to be *An. quadriannulatus* based on the PCA and NJT.

Anopheles coluzzii populations in West Africa are commonly found to have experienced adaptive introgression of genetic material from *An. gambiae* within a genomic region towards the centromere of chromosome arm 2L,

driven by selection for pyrethroid target-site resistance alleles (34–36). AIM profiles revealed the majority of *An. coluzzii* from Kenya are either homozygous (38%) or heterozygous (46%) for introgression from *An. gambiae* towards the centromere of 2L (Supplementary Fig. 1).

Anopheles coluzzii has not previously been reported in Kenya but is a highly competent malaria vector in West and Central Africa. The species were detected across three different sampling time points at frequencies of 20.0% (5 out of 25) in month 2 of 2009, 20.3% (13 out of 64) in month 1 of 2019 and 6.1% (8 out of 131) in month 9 of 2019. Given the importance of this finding for malaria vector surveillance and control, we focus the remainder of this report on a full characterisation of the Kenyan *An. coluzzii*. Analysis of genomic data from the other *Anopheles* taxa sequenced in this study will be reported separately.

Geographical population structure and genetic diversity

To explore the genetic relationship between the Kenyan *An. coluzzii* and conspecific populations from other countries, we combined data from this study with previous sequence data of *An. coluzzii* populations from inland West Africa (Burkina Faso, Mali), coastal West Africa (Côte d'Ivoire, Ghana), and Central Africa (Cameroon, the Central African Republic, Angola) (37). We used SNPs from Chromosome 3, which is free from polymorphic inversions, to perform a principal components analysis (PCA), compute a neighbour-joining tree (NJT) and quantify the degree of allele frequency differentiation (FST) between cohorts from different locations. The PCA and NJT analyses grouped the Kenyan *An. coluzzii* most closely with *An. coluzzii* from inland West Africa (Mali, Burkina Faso) and northern Cameroon (Fig. 2). The FST results were consistent with these analyses, finding the lowest FST between Kenya, Mali and Burkina Faso (FST 0.006–0.007 for both comparisons; Supplementary Figure S3). These results show a lack of strong population structure between *An. coluzzii* from Kenya and more northerly regions of West and Central Africa.

The figure shows the analysis of geographical population structure within *An. coluzzii*, comparing samples from Turkana, Kenya collected in this study with reference samples from the Ag1000G project and Fontaine et al. (29). Kenyan *An. coluzzii* are most closely related to *An. coluzzii* from inland West Africa (Mali, Burkina Faso) and Northern Cameroon. a, PCA. b, NJT.

To further explore whether Kenyan *An. coluzzii* share a genomic profile similar to northern West and Central Africa, we investigated whether they have the same 2La and 2Rb inversion karyotype by performing a PCA targeting these regions of the genome. *An. coluzzii* from Kenya have the same 2La/2Lb karyotype found in *An. coluzzii* from Burkina Faso and Mali only, supporting the finding that they are genetically most similar to these populations (Supplementary Figure S4). To investigate whether the Kenyan *An. coluzzii* have a similar demographic history to *An. coluzzii* populations from other countries in Africa, we computed genetic diversity summary statistics for mosquito cohorts grouped by geographical region and year of sampling. Nucleotide diversity, the density of segregating sites (Watterson's theta) and allele frequency spectra (Tajima's D) in Kenyan *An. coluzzii* were similar to *An. coluzzii* from West Africa (Nucleotide diversity, 0.026; Watterson's theta, 0.033; Tajima's D, -0.961; Supplementary Figure S5), suggesting lack of genetic isolation.

Insecticide resistance

To investigate whether Kenyan *An. coluzzii* have mutations associated with target-site resistance to insecticides, we computed amino acid allele frequencies across four genes encoding insecticide binding targets: *Vgsc* (AGAP004707), *Gste2* (AGAP009194), *Rdl* (AGAP006028) and *Ace1* (AGAP001356). We found that *An. coluzzii* in

Kenya have the *Vgsc-L995F* substitution associated with resistance to DDT and pyrethroids, at 62% frequency (Fig. 3a)(38). This allele is also found at high frequency in *An. coluzzii* populations throughout West and Central Africa. In addition, Kenyan *An. coluzzii* displayed the double mutant Vgsc-V402L + I1527T substitution at 38% frequency. This double mutant is also present in other *An. coluzzii* populations. Additionally, Kenyan *An. coluzzii* have the Gste2-I114T mutation at high frequency (67%), which confers metabolic resistance to DDT (39) (Fig. 3b) and also observed at high frequency in *An. coluzzii* populations from West and Central Africa.

The figure shows amino acid substitution frequencies in genes associated with target-site resistance to DDT and pyrethroids. a, Vgsc (AGAP004707). b, Gste2 (AGAP009194).

Two haplotypes have been previously associated with resistance to dieldrin in *An. coluzzii*, Rdl-296G/345M widely distributed across Africa and Rdl-296S/345S previously only reported from Burkina Faso (40). We observed the 296/345 substitution pair in Burkina Faso and also in Mali and Kenya, but the frequency was very low, ~ 5%, in *An. coluzzii* from Kenya (Supplementary Fig. 6). A mutation in the Acetylcholinesterase gene, Ace1-G280S, was previously linked to organophosphate and carbamate resistance (41). Although the mutation is present in *An. coluzzii* from coastal West Africa (i.e., Côte d'Ivoire and Ghana), it was not present in *An. coluzzii* from Kenya (Supplementary Fig. 7).

Discussion

A proper understanding of vector bionomics within the context of the malaria transmission system is important for the choice and successful implementation of vector control interventions. The current study sought to improve this understanding by analysing the distribution of *An. gambiae s.l.* sibling species from five sites in Kenya, one each in the different malaria epidemiological zones. Based on analysis of whole-genome data we report, for the first time in Kenya, the presence of *Anopheles coluzzii*.

Anopheles coluzzii is a highly competent malaria vector in West and Central Africa. All the *An. coluzzii* samples found in this study were collected from Turkana in Northwest Kenya. This included mosquitoes collected across multiple years and months, confirming the presence of *An. coluzzii* in the region since at least 2006 (Table). Past failure to detect *An. coluzzii* in Kenya is likely associated with the fact that malaria vector surveillance and identification has tended to focus on historically known primary vectors using species-specific markers. For example, in Turkana, a past study reported all mosquitoes identified as *An. arabiensis* but only 85% of samples reacted in PCR diagnostic tests (42). Failure to identify specimens in such molecular assays is usually explained away as failure in the assay itself due to factors such as poor-quality DNA or other associated factors. Our analysis exploited WGS allowing the use of a large number of markers distributed across the entire genome to detect cryptic and/or unknown species demonstrating how whole genomic sequencing can be configured to support routine surveillance to accurately identify present and shifting vector distributions, adaptive evolutionary changes and populations structuring, integral to understanding of malaria transmission dynamics (42).

When the Kenya *An. coluzzii* were compared with populations from the northerly regions of West and Central Africa, they showed a lack of strong population genetic structure evidenced by clustering on the PCA and NJT and low FST, suggesting relatively unrestricted gene flow across a northerly belt spanning continental Africa. In support of this notion, Kenyan *An. coluzzii* had a similar genetic diversity to other West and Central African populations. Exploration of the 2La/2Lb inversion karyotypes, which has previously been linked to aridity and

increases in frequency with this cline (43, 44) revealed that Kenyan *An. coluzzii* have the same inversion karyotype found in Burkina Faso and Mali at both loci, supporting the finding of genetic similarity to these populations. Our finding that the 2La/2Rb karyotype is the same as that found in arid West and Central Africa is consistent with its environmental association, since Turkana in Kenya is arid to semi-arid, experiencing high temperatures and highly seasonal rainfall. The presence of *An. coluzzii* has also been documented in Somalia, which shares a similar arid to semi-arid ecosystem (6). Our findings suggest Kenyan *An. coluzzii* are not an isolated population, nor have they experienced any recent bottlenecks or other distinct demographic events.

Investigation of the insecticide resistance profiles of Kenyan *An. coluzzii* revealed the presence of the *Vgsc-L995F* substitution associated with resistance to DDT and pyrethroids (38) at 62% frequency and the Gste2-I114T mutation which confers metabolic resistance to DDT (39) at a frequency of 67%. These alleles are also found at high frequency in *An. coluzzii* populations from West and Central Africa (45–47). In addition, Kenyan *An. coluzzii* displayed the double mutant Vgsc-V402L + I1527T substitution at 38% frequency also present in other *An. coluzzii* populations and recently observed to be increasing in frequency in *An. coluzzii* in Burkina Faso (48). The V402L substitution has been functionally validated to confer insecticide resistance with reduced fitness cost to the mosquito when compared to L995F (49) and the fact that V402L is almost exclusively found in combination with I1527T suggests a strongly synergistic effect that may further increase fitness in the presence of insecticides.

Previous studies have documented resistance to pyrethroids in *An. gambiae s.l.* in Kakuma (Turkana County) with enzymatic resistance being implicated (50). Both ITNs and IRS have historically been used to prevent malaria in the area. Our finding of mutations associated with resistance to insecticides is therefore not surprising and fits with the picture of widespread and increasing insecticide resistance in the country (51). The presence of the V402L + I1527T substitution in Kenya is concerning, because variants conferring stronger pyrethroid resistance could compromise the efficacy of new pyrethoid + PBO LLINs, currently considered among the most effective defense against resistant populations (52).

Increased urbanisation promotes retention of surface water and is associated with water distribution and drainage systems that provide suitable habitat for Anopheles mosquitoes. The refugee camp from which the samples were collected is a highly populated region that experiences low level local transmission and has recently suffered recurrent epidemics (42). Historically, the area has received little attention regarding malaria intervention, because the climate was considered unsuitable for known vector species, except *An. arabiensis*, considered a less competent vector.

Conclusion

The observation that *An. coluzzii*, an efficient vector of malaria which can tolerate more arid conditions and thrive in both rural and urban settings occurs in Kenya but has not been reported to date means that this vector is potentially contributing to malaria transmission in Turkana County and malaria control interventions currently in place may be ineffective against it. This finding alongside the recent finding of *An. stephensi* in Turkana and other regions of northern Kenya emphasizes the need for re-evaluation of the distribution, bionomics and epidemiological significance of the local vector populations in the country. This is the only way the country will be able to ensure vector control approaches are sufficiently targeted at the myriad of *Anopheles* vectors responsible for transmission in the different settings in Kenya.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The sequences for the specimens identified in this study were submitted to The European Nucleotide Archive (*ENA*) (accession nos. ERR11811571-ERR11811786, ERR11812004-ERR11812101 and ERR11840239-ERR12031958).

Competing interests

The authors declare that they have no competing interests.

No chatbot or artificial intelligence tool were used in any of this work.

Authors' contributions

LK, EO, JH, DMM, DM, JM, EJ, CM conceptualized and designed the study, data interpretation and assisted in drafting the manuscript.

LK, EO, SA, SO, JM, designed the study, conducted sample collection and processing, data collection.

LK, KLB, EO, DO, SB, AM conducted data analysis and interpretation and assisted in drafting the manuscript.

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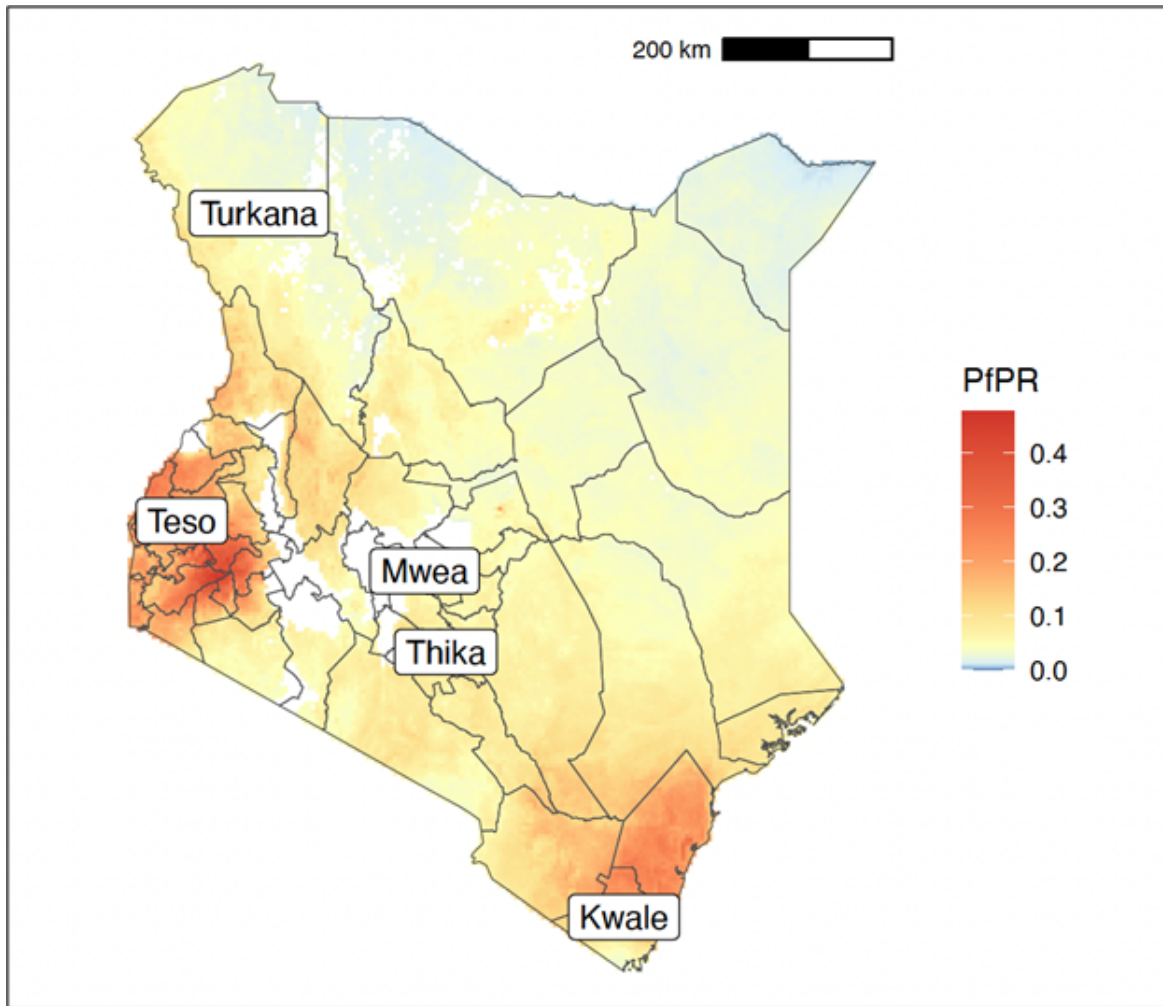
Figures

Figure 1

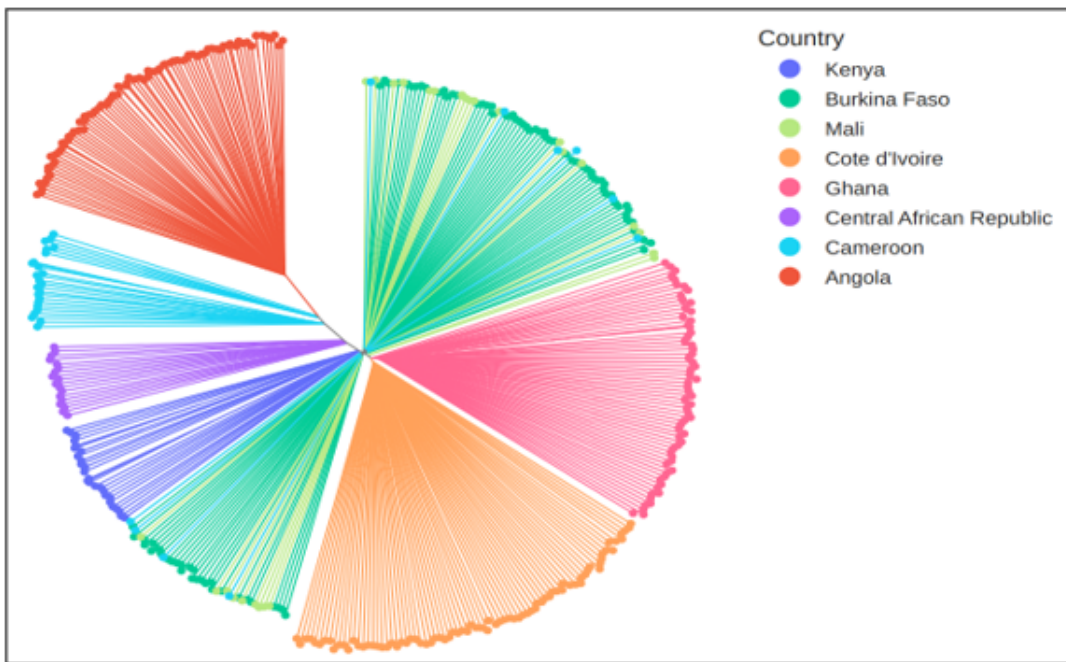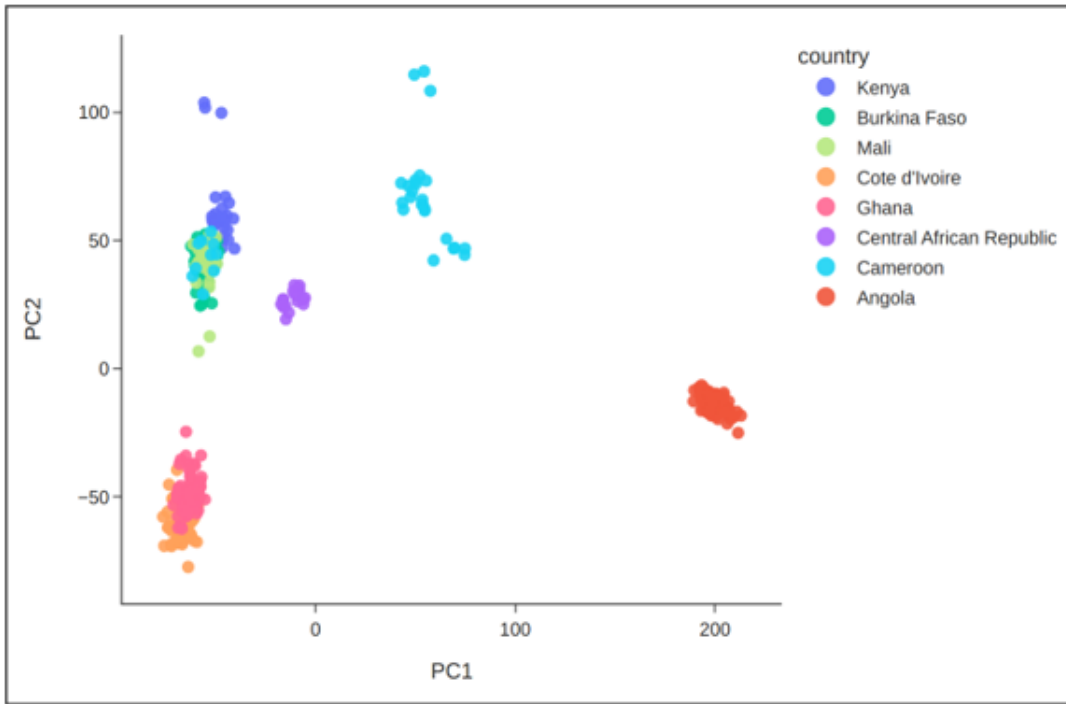Map of Kenya showing the study sites

Figure 2

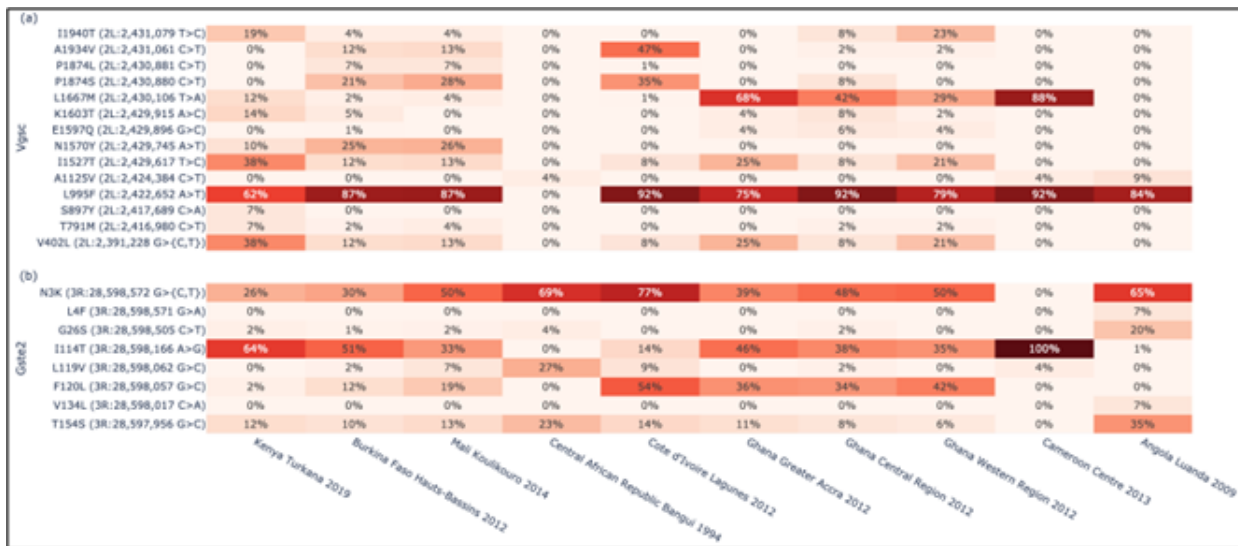Population genetic structure of Kenyan *An. coluzzii*

Figure 3

Insecticide resistance profiles of Kenyan *An. coluzzii*

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