Molecular surveillance leads to the first detection of *Anopheles stephensi* in Kenya

Eric O. Ochomo (ericochomo@yahoo.com)
Centre for Global Health Research, Kenya Medical Research Institute

Sylvia Milanoi
Centre for Global Health Research, Kenya Medical Research Institute

Bernard Abong'o
Centre for Global Health Research, Kenya Medical Research Institute

Brenda Onyango
Centre for Global Health Research, Kenya Medical Research Institute

Margaret Muchoki
Centre for Global Health Research, Kenya Medical Research Institute

Diana Omoke
Centre for Global Health Research, Kenya Medical Research Institute

Evelyne Olanga
PMI Kinga Malaria Project, Abt Associates

Laban Njoroge
National Museums of Kenya

Elijah Juma
Pan African Mosquito Control Association

James Dan Otieno
World Health Organization, Kenya Country Office

Damaris Matoke
Centre for Biotechnology Research and Development, Kenya Medical Research Institute

Luna Kamau
Centre for Biotechnology Research and Development, Kenya Medical Research Institute

Cristina Rafferty
Centers for Disease Control and Prevention

John E. Gimnig
Centers for Disease Control and Prevention

Mildred Shieshia
President's Malaria Initiative (PMI)

Daniel Wacira
President's Malaria Initiative (PMI)

Joseph Mwangangi

Centre for Geographical Medicine Research, Kenya Medical Research Institute
Marta Maia
Centre for Geographical Medicine Research, Kenya Medical Research Institute
Charles Chege
Division for National Malaria Program, Ministry of Health
Ahmeddin Omar
Division for National Malaria Program, Ministry of Health
Charles Mbogo
Centre for Geographical Medicine Research, Kenya Medical Research Institute
Lenson Kariuki
Division for National Malaria Program, Ministry of Health

Article

Keywords:

Posted Date: January 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2498485/v1

Abstract

Anopheles stephensi is an invasive malaria vector that is endemic to south Asia and the Arabian Peninsula. It was recently reported in the Horn of Africa countries including Djibouti (2012), Ethiopia, Sudan (2019), Somalia (2019) and most recently Nigeria (2020). This mosquito is a competent vector for both *Plasmodium falciparum* and *P. vivax.* It is characterized by a high degree of behavioral plasticity and the ability to reproduce in various types of breeding sites including containers and therefore has the potential to propagate malaria transmission in rapidly urbanizing settings with poor drainage and disposal of waste containers. The World Health Organization (WHO) has called on all countries to scale up surveillance efforts to detect and report invasion by this vector and institute appropriate and effective control mechanisms. In Kenya, the Division for National Malaria Program (DNMP) and its partners have been conducting entomological surveillance in all coastal and northern counties that are suspected to be at risk of *An. stephensi* invasion as well as in all counties at risk of malaria. These efforts were supported by molecular surveillance of all unidentified *Anopheles* mosquitoes from other studies conducted by the Kenya Medical Research Institute (KEMRI) to try and identify *An. stephensi*. In this article, we report the first detection of *An. stephensi* in two sub counties of Marsabit County, Kenya in December 2022. We used Polymerase Chain Reaction (PCR) as the primary method of identification and confirmed results using morphological keys and sequencing of the ITS2 region. With the detection of this vector in Kenya, there is an urgent need for intensified surveillance to determine its occurrence and distribution and develop tailored approaches towards control to prevent further spread.

Background

Anopheles stephensi is a major vector of malaria in south Asia, Middle East and southern China where it is endemic and is known to transmit both *Plasmodium falciparum* and as *P. vivax*. This vector differs from other malaria vectors due to its ability to develop in man-made containers, preferably in clean water, but also in contaminated water. These traits have enabled *An. stephensi* to colonize urban settings where it can sustain transmission of malaria. Additionally, it is an efficient vector in rural areas. *Anopheles stephensi* was first reported in the Horn of Africa in Djibouti in 2012 [1]. Since then, it has now been reported in multiple urban and rural settings in Ethiopia, Sudan and Somalia [2–4] and could be responsible for sustaining malaria transmission in Ethiopia with the potential to increase *P. falciparum* incidence by 50% according to recent mathematical modelling of the potential impact of *An. stephensi* [5, 6] as has been observed in Djibouti [7].

There is a risk of *An. stephensi* spreading further south and west from its original *foci* of detection in the Horn of Africa region, as has been observed in Nigeria (2020) [3]. Importantly, this vector has the potential to establish or increase transmission in urban settings where the malaria burden is generally lower compared to rural settings, particularly where there are poorly planned drainage and waste disposal systems, creating conducive larval habitats [8]. The adult mosquito behaviour is poorly understood, especially as it colonizes new areas in Africa. WHO recently called for heightened surveillance and the development of response strategies to limit the spread of the vector[3]. This document highlights five key

areas of focus; increased collaboration across sectors and borders, strengthening surveillance, improving information exchange, developing national guidelines, and prioritizing research to evaluate tools against this vector.

In Kenya, the Division for National Malaria Program (DNMP) at the Ministry of Health and its partners have been on high alert and instituted surveillance efforts following WHO's initiative[3]. Surveillance efforts have been focused along the Kenyan coast and the northern counties bordering Sudan and Ethiopia. The current surveillance efforts are aimed at the collection of both larval and adult mosquito samples. Samples collected are identified using morphological keys and further by PCR at the reference laboratories located at the Kenya Medical Research Institute (KEMRI). Here we report the process leading to the first detection and identification of *An. stephensi* in Kenya.

Methods

Surveillance sites

Mosquitoes were collected in 14 Counties in December 2022 as part of a routine surveillance exercise conducted by the DNMP and its partners. The following counties where DNMP supported vector surveillance in December 2022 are categorized as malaria endemic (Kilifi, Taita Taveta), highland epidemic prone (Elgeyo Marakwet, West Pokot, Kisii, Nandi), low risk (Garissa, Makueni, Kajiado, Kirinyaga and Laikipia), or seasonal (Marsabit, Baringo and Turkana) (Fig. 1). For the purpose of this work, we shall only describe and present results for Marsabit County, where samples were collected, identified and confirmed to be *An. stephensi*. Marsabit County is in the northern part of Kenya and borders Ethiopia to the North, Turkana County to the west, Samburu County to the South and Wajir and Isiolo Counties to the East. The county lies at between 300m and 900m above sea level. The major economic activities are livestock rearing and cross-border trade. Sampling was conducted in Laisamis and Saku sub-counties of Marsabit County and focused on urban and rural settings along the northern transport corridor connecting Kenya and Ethiopia (Table 1). The main economic activities of the rural population is nomadic pastoralism due to the mostly semi-arid terrain while urban trade centers are set up the northern transport corridor and was the focus of the sampling efforts.

Table 1
Characteristics of the habitats from which larvae were collected

County	Subcounty	Locality Village	Habitat Type	Larvae presence	Latitude	Longitude	Elevation
Marsabit	Saku	Mountain	Tank	Yes	2.329833	37.996728	1319.14
Marsabit	Saku	Nagayo	Tyre	Yes	2.337666	37.990045	1353.7
Marsabit	Saku	Larare	Stream bed	No	2.227091	37.938076	1057.33
Marsabit	Laisamis	Malgis	River bed	Yes	1.832333	37.86083	453.76
Marsabit	Laisamis	Laisamis	Animal drinking points	Yes	1.587676	37.80819	541.84

Sampling: Mosquito sampling was conducted for both adult and larval samples. Adults were collected using CDC light traps while larvae were collected by dipping. CDC light traps were set overnight from 1800 to 1900hrs and collected the next morning between 0700-0800hrs. Additionally, the teams dipped for larvae in animal watering pens, containers, tyres, and other water bodies in the area (Fig. 2). *Anopheles* larvae were collected and placed in whirlpacks for transportation to the entomology laboratory at KEMRI in Kisumu for additional assays. The mosquitoes were reared in the infection room, with a triple door and curtains at the entrance and sealed windows to prevent any escapees. Surviving larvae were reared to adults for morphological identification using standard conditions (25 ± 2°C; 80% ± 4% relative humidity with a 12 h: 12 h light/dark cycle). Larvae were fed on Tetramin baby fish food and brewer's yeast daily and adults maintained on 10% sugar solution.

Molecular characterization

DNA from 55 either whole or legs and wings of the mosquito samples was isolated using the ethanol precipitation method [9]. Amplification was carried out using an endpoint PCR assay previously described in Singh and others alongside a positive control using 0.15ul of the DNA template [10]. Thereafter, 15 µl of each of the PCR products was run on 2% agarose gel alongside 3 µl of a 100bp DNA ladder for size comparison. The products were visualized in the gel documentation system for an expected amplicon size of approximately 438bp. This was used as the primary method of identification given the relative inexperience of the laboratory teams in morphological identification of *An. stephensi.*

Morphological identification

Emerging adults were taxonomically identified using the keys described by Coetzee [11] to detect the distinct banding on the maxillary palps, pale scales on the scutum, and the 3 dark spots on wing vein 1A.

Sequencing

A sample of four adult specimens identified as *An. stephensi* by morphology were randomly selected and shipped to the US Centers for Disease Control and Prevention (CDC), Atlanta, USA where DNA from a single mosquito leg was extracted using the Extracta DNA Prep for PCR ®kit (Quantabio Beverly, MA) and Sanger sequencing was conducted using ITS2 primers[12]. Thereafter, Basic Local Alignment Search Tool (BLAST) homology searches of sequences was done using the default parameters on the sequences to confirm what species they matched to. Additionally, the phylogeny of ITS2 sequences from *An. stephensi* isolates from Kenya and other countries was performed using the construct/Test Maximum likelihood Tree approach on MEGA software. We used the default phylogeny reconstruction parameters apart from the number of bootstrap replications which was set to 100.

Results

Anopheles larvae were collected from the sources illustrated in Table 1 in 4 of the 5 villages the team visited. A total of 59 larvae were collected. Eleven died in transit and were immediately prepared for PCR identification using the *An. stephensi* protocol, 7 of which were confirmed as *An. stephensi* (Table 2). The remaining larvae were pooled by sub-county for rearing.

Table 2
Numbers of mosquitoes sampled as larvae and either used for PCR or reared to adults then used for PCR. Unamplified samples are those that failed to amplify with the An. stephensi, An. gambiae and An. funestus PCR protocols

		Larvae used for PCR			**Adults used for PCR**		
Sub-County	Total larvae collected	Sample size	*An. stephensi*	Unamplified	Sample size	*An. stephensi*	Unamplified
Saku	42	9	6	3	33	8	25
Laisamis	17	2	1	1	15	8	7
Total	59	11	7	4	48*	16	32
* Four adults were shipped to CDC for sequencing.							

The 48 remaining larvae were used to rear adult samples. Of the first 11 samples that emerged, 9 adults were identified by morphology (Fig. 3). The team correctly identified 7 of the 9 samples as *An. stephensi* which were later confirmed by PCR (Table 2), the other two were identified as *An. gambiae* by morphology but confirmed to be *An. stephensi by PCR.* Four of these samples were shipped to the US Centers for Disease Control and Prevention (CDC) for sequencing. Thirty-six samples did not amplify using *An. stephensi*, *An. gambiae* or *An. funestus* PCR protocols and are the subject of further investigation. Morphological identification was not conducted on these samples prior to DNA extraction; therefore, they will be sequenced to determine species at a later date. Light traps did not collect any adult mosquitoes.

Of the 59 mosquito samples tested by PCR, 23 were confirmed to be *An. stephensi*. The experiment was repeated with different concentrations of DNA but 0.15ul gave the clearest distinction.

Sequencing

The sequence for 3 of the 4 samples matched and were confirmed as *An. stephensi.* One sample failed to amplify possibly due to DNA degradation (Table 3).

Table 3
Results for sequencing analysis of the 4 samples from Kenya

Origin Sample ID	CDC Sample ID	Morphology ID	Confirmed Species	Accession Number of closest match	%Identity match
KE83GY	2023KEN0001	Suspected *An. stephensi*	*An. stephensi*	FJ526599.1	99.40%
KE83QF	2023KEN0002	Suspected *An. stephensi*	*An. stephensi*	MW732931.1	100%
KE83FZ	2023KEN0003	Suspected *An. stephensi*	*An. stephensi*	MW732931.1	100%
KE831Q	2023KEN0004	Suspected *An. stephensi*	*Did not amplify*		

BLAST searches using default parameters for isolates 2 and 3 matched to *An. stephensi* sequences with 100% identity to hap 10 5.8S ribosomal RNA gene, ITS2 while isolate 1 was at 99.4% identity to the same gene but 100% identity to *An. stephensi* isolate 141 steph 5.8S ribosomal RNA gene, ITS2.

Phylogenetic analysis of sequenced isolates with 31 other isolates of ITS2 for *An. stephensi* available on GenBank showed that our isolates closely matched the Iraq, India, Yemen and Nigerian isolates (Fig. 4).

Discussion

In this article, we report the first collection and detection of *An. stephensi* from Marsabit County in northern Kenya. From the samples collected, multiple methods were used to conduct the identification, including morphological keys, standard PCR, and Sanger sequencing. Molecular methods were instrumental in confirming the presence of *An. stephensi* in Marsabit county, Kenya. Additional surveys conducted at the same time in Turkana County in the northwestern parts of the country, bordering Marsabit County did not yield a similar finding; there have been no recent surveys on the northeastern part of the country. The *An. stephensi* mosquitoes were collected as larvae but no adults were found in the light traps set at the same locations indicating the need for studies to characterize vector bionomics and behavior to understand its contribution/potential contribution to malaria transmission and to design appropriate tools for conducting surveillance for adult *An. stephensi*.

From our surveillance efforts, we collected 59 larvae but did not trap any adults in the same area using CDC light traps. Reports from other sites have documented the difficulty in trapping adult mosquitoes [5]. The bionomics and behaviour of this vector in its recent invasive geographic foci are poorly understood, with the only detailed descriptions available to this point being from Asia [3, 13, 14]. However, reports have indicated that crepuscular biting and resting outside houses could translate to reduced efficacy of core vector control interventions, Insecticide treated nets (ITNs) and indoor residual spray (IRS) indicating the importance of accurate parameterization [6]. Additionally, the potential for any insecticide-based control methods to be effective will depend on the insecticide resistance status of the *An. stephensi* mosquitoes and therefore necessitating insecticide resistance surveys [6, 15]. Based on the phylogenetic analysis, the Kenyan *An. stephensi* isolates matched closely with isolates from India, Iraq, Yemen, Iran and Nigeria but more distant to the isolates from Ethiopia which could indicate multiple clades of *An. stephensi* invading different parts of Africa. There is a need for additional population genetics studies using whole genome sequencing approaches to describe these clades, coupled with intensive surveillance to describe their bionomics and behaviour. Our findings also suggest potential introduction routes, the *An. stephensi* were found along the main highway that connects Kenya to Ethiopia, highlighting the need for increased surveillance along major transportation routes and targeting areas such as truck stops and resting sites, weighbridges, and borders. That said, it is important to note that phylogenetic analyses of the ITS2 isolates from Kenya did not match those from Ethiopia which may mean multiple introductions of different clades simultaneously in different parts of Africa. Further, it will be important to track parasites causing malaria cases around the areas where *An. stephensi* has been introduced given that it is an efficient vector of both *P. falciparum* and *P. vivax*.

With rapid, often unplanned urbanization in Africa, many of the urban centers have poor refuse disposal and drainage systems which are potential larval habitats of *An. stephensi* [1, 8]. Coupled with climate change, which creates suitable climatic conditions for mosquito breeding, there is great potential for the spread and establishment of *An. stephensi* in African cities. At the point of introduction of *An. stephensi* into Djibouti [1], the country was at pre-elimination, with under 3000 malaria cases reported in 2013, one year after this mosquito was first reported. Six years later in 2019, Djibouti reported 49,402 malaria cases [16]. Modelling of the potential impact of *An. stephensi* establishment in Ethiopia predicts a surge in *P. falciparum* cases by 50% overall if no additional interventions are put in place with areas of lowest transmission (~ 0.1%) being impacted the most [6]. There is a need for similar models to be conducted in all the areas that are newly invaded such as Kenya, to predict the spread and potential impact of the vector and to understand the potential impact of additional interventions.

Anopheles stephensi breeding habitats are similar to *Aedes aegypti* habitats, with a poorer understanding of adult resting and biting behaviour [17, 18]. Evidence of outdoor, crepuscular feeding by this species suggests it may be less affected by ITNs or IRS. Furthermore, *An. stephensi* in Ethiopia was reported to be highly resistant to pyrethroids, carbamates and organophosphates [13]. These traits indicate that alternative vector control and non-vector control measures may be needed to address the threat of this invasive mosquito. As the national malaria control program develops a vector control strategy, integrated vector management (IVM) approaches offer advantages due to of the potential benefit of targeting

additional vectors based on WHO guidance [19, 20] but more importantly, because of how little of this vector's behaviour is understood, especially as it colonizes new areas. Deploying an integrated approach provides opportunities to target the two vectors for surveillance and control operations using similar interventions thus optimizing resource allocation and use in resource limited settings where this vector is currently being reported. Other potential vector control tools, including those currently under evaluation, include spatial repellents [21], attractive targeted sugar baits [22], endectocides [23], and genetically modified mosquitoes [24]. Given the outdoor, early evening biting behaviors, resistance to multiple insecticides, and the threat to malaria control efforts, these alternative vector control approaches may be necessary to sustain the gains made against malaria over the last two decades.

Conclusions

We confirm the presence of *An. stephensi* in northern Kenya. This finding is critical for the DNMP as it points to the urgent need to re-examine and expand the vector surveillance and control efforts to include *An stephensi* which is likely to sustain and possibly increase transmission in northern Kenya and spread further southwards to highly populated urban areas and existing malaria-endemic counties further compounding the problem of malaria control in the country. This report emphasizes the need for heightened and tailored surveillance to understand the scope of the spread of this invasive vector and to advise on targeted control using all the existing interventions, including those currently under trial given the emergency.

References

1. Faulde MK, Rueda LM, Khaireh BA: **First record of the Asian malaria vector Anopheles stephensi and its possible role in the resurgence of malaria in Djibouti, Horn of Africa**. Acta Trop 2014, **139**:39–43.
2. Ahmed A, Khogali R, Elnour MB, Nakao R, Salim B: **Emergence of the invasive malaria vector Anopheles stephensi in Khartoum State, Central Sudan**. Parasit Vectors 2021, **14**:511.
3. WHO: **WHO initiative to stop the spread of Anopheles stephensi in Africa**. Geneva, Switzerland: World Health Organization; 2022.
4. Carter TE, Yared S, Gebresilassie A, Bonnell V, Damodaran L, Lopez K, Ibrahim M, Mohammed S, Janies D: **First detection of Anopheles stephensi Liston, 1901 (Diptera: culicidae) in Ethiopia using molecular and morphological approaches**. Acta Trop 2018, **188**:180–186.
5. Tadesse FG, Ashine T, Teka H, Esayas E, Messenger LA, Chali W, Meerstein-Kessel L, Walker T, Wolde Behaksra S, Lanke K, et al: **Anopheles stephensi Mosquitoes as Vectors of Plasmodium vivax and falciparum, Horn of Africa, 2019**. Emerg Infect Dis 2021, **27**:603–607.
6. Hamlet A, Dengela D, Tongren JE, Tadesse FG, Bousema T, Sinka M, Seyoum A, Irish SR, Armistead JS, Churcher T: **The potential impact of Anopheles stephensi establishment on the transmission of Plasmodium falciparum in Ethiopia and prospective control measures**. BMC Med 2022, **20**:135.

7. de Santi VP, Khaireh BA, Chiniard T, Pradines B, Taudon N, Larreche S, Mohamed AB, de Laval F, Berger F, Gala F, et al: **Role of Anopheles stephensi Mosquitoes in Malaria Outbreak, Djibouti, 2019**. Emerg Infect Dis 2021, **27**:1697–1700.
8. Sinka ME, Pironon S, Massey NC, Longbottom J, Hemingway J, Moyes CL, Willis KJ: **A new malaria vector in Africa: Predicting the expansion range of Anopheles stephensi and identifying the urban populations at risk**. Proc Natl Acad Sci U S A 2020, **117**:24900–24908.
9. Collins FH, Mendez MA, Rasmussen MO, Mehaffey PC, Besansky NJ, Finnerty V: **A ribosomal RNA gene probe differentiates member species of the Anopheles gambiae complex**. The American journal of tropical medicine and hygiene 1987, **37**:37–41.
10. Singh OP, Mishra S, Sharma G, Sindhania A, Kaur T, Sreehari U, Das MK, Kapoor N, Gupta B: **Evaluation of intron-1 of odorant-binding protein-1 of Anopheles stephensi as a marker for the identification of biological forms or putative sibling species**. PLoS One 2022, **17**:e0270760.
11. Coetzee M: **Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae)**. Malaria Journal 2020, **19**:70.
12. Beebe NW, Saul A: **Discrimination of all members of the Anopheles punctulatus complex by polymerase chain reaction–restriction fragment length polymorphism analysis**. Am J Trop Med Hyg 1995, **53**:478–481.
13. Balkew M, Mumba P, Dengela D, Yohannes G, Getachew D, Yared S, Chibsa S, Murphy M, George K, Lopez K, et al: **Geographical distribution of Anopheles stephensi in eastern Ethiopia**. Parasit Vectors 2020, **13**:35.
14. WHO: **Vector Alert: Anopheles stephensi invasion and spread: Horn of Africa, the Republic of the Sudan and surrounding geographical areas, and Sri Lanka: information note**. World Health Organization: Geneva, Switzerland; 2019.
15. Balkew M, Mumba P, Yohannes G, Abiy E, Getachew D, Yared S, Worku A, Gebresilassie A, Tadesse FG, Gadisa E, et al: **An update on the distribution, bionomics, and insecticide susceptibility of Anopheles stephensi in Ethiopia, 2018–2020**. Malar J 2021, **20**:263.
16. Seyfarth M, Khaireh BA, Abdi AA, Bouh SM, Faulde MK: **Five years following first detection of Anopheles stephensi (Diptera: Culicidae) in Djibouti, Horn of Africa: populations established-malaria emerging**. Parasitol Res 2019, **118**:725–732.
17. Takken W, Lindsay S: **Increased Threat of Urban Malaria from Anopheles stephensi Mosquitoes, Africa**. Emerg Infect Dis 2019, **25**:1431–1433.
18. Mnzava A, Monroe AC, Okumu F: **Anopheles stephensi in Africa requires a more integrated response**. Malar J 2022, **21**:156.
19. WHO: **Global vector control response 2017–2030**. Geneva: World Health Organization; 2017.
20. WHO: **Global framework for the response to malaria in urban areas**. Geneva: World Health Organization; 2022.
21. Achee NL, Perkins TA, Moore SM, Liu F, Sagara I, Van Hulle S, Ochomo EO, Gimnig JE, Tissera HA, Harvey SA, et al: **Spatial repellents: The current roadmap to global recommendation of spatial**

repellents for public health use. Curr Res Parasitol Vector Borne Dis 2023, **3**:100107.

22. Attractive Targeted Sugar Bait Phase IIITG: **Attractive targeted sugar bait phase III trials in Kenya, Mali, and Zambia**. Trials 2022, **23**:640.
23. The Ivermectin R, Billingsley P, Binka F, Chaccour C, Foy B, Gold S, Gonzalez-Silva M, Jacobson J, Jagoe G, Jones C, et al: **A Roadmap for the Development of Ivermectin as a Complementary Malaria Vector Control Tool**. Am J Trop Med Hyg 2020, **102**:3–24.
24. Schairer CE, Najera J, James AA, Akbari OS, Bloss CS: **Oxitec and MosquitoMate in the United States: lessons for the future of gene drive mosquito control**. Pathog Glob Health 2021, **115**:365–376.

Abbreviations

ITNs
Insecticide Treated Nets
IRS
Indoor Residual Spraying
An
Anopheles
KEMRI
Kenya Medical Research Institute
CDC
Centers for Disease Control and Prevention
DNMP
Division for National Malaria Program
IVM
Integrated Vector Management
WHO
World Health Organization
BLAST
Basic Local Alignment Search Tool
ITS2
Internal Transcribed Spacer 2

Declarations

Acknowledgement

We acknowledge the support of Paul Omondi Osodo, Stephen Okeyo, George Marube, James Opalla who conducted the survey leading to the discovery of *An. stephensi* in Marsabit. Ian Mabiria and Blantine Akinyi who supported the molecular identification process, and Richard Amito who supported the rearing

of the collected larvae. James Kiarie of DNMP and of KEMRI who helped with the data computation and development of the maps.

Authors' contribution

EOO, EO, LN, EJ, JDO, DM, LK, JEG, MS, DW, JM, Marta Maia, CC, AO, CM, Lenson Kariuki: Conceptualization of the study, data analysis, drafting of the manuscript

SM, BA, BO, Margaret Muchoki, DO, CR: Study design, sample collection, sample processing, data collection, data analysis, drafting the manuscript

All authors read and approved this manuscript

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by funding from NIH_NIAID through the Liverpool School of Tropical Medicine (LSTM) and Bill and Melinda Gates Foundation Grant #INV-024969 to KEMRI and GF NFM III to the DNMP.

Data Availability

The *An. stephensi* sequences have now been uploaded on GenBank with accession numbers: OQ275144 (https://www.ncbi.nlm.nih.gov/nuccore/OQ275144), OQ275145 (https://www.ncbi.nlm.nih.gov/nuccore/OQ275145) and OQ275146 (https://www.ncbi.nlm.nih.gov/nuccore/OQ275146).

Consent for publication

This manuscript is published with the permission of the Director-General of the Kenya Medical Research Institute.

Participation of human subjects

Humans were not involved in this study

Disclaimer

The findings and conclusions expressed herein are those of the author(s) and do not necessarily represent the official position of USAID, PMI, or the Centers for Disease Control and Prevention (CDC).

Figures

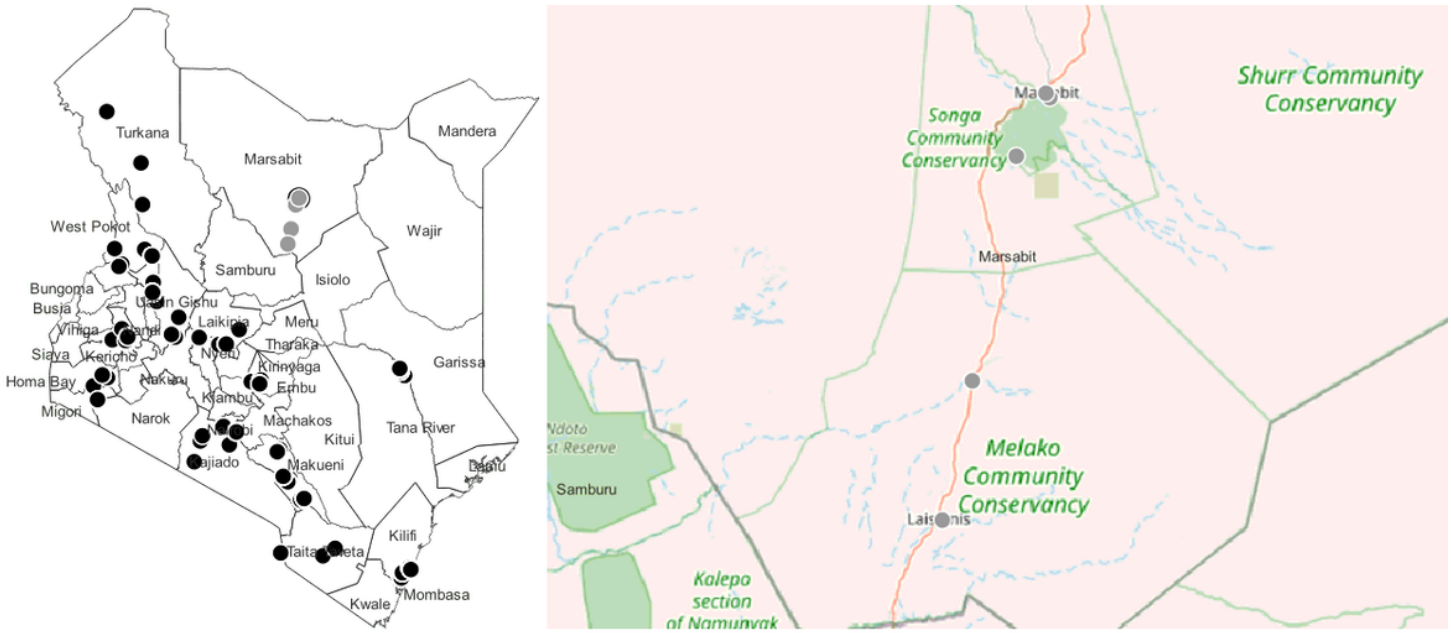

Figure 1

Map of Kenya showing the locations of mosquito collection during the surveillance conducted by DNMP and partners in December 2022 (left). Part of Marsabit showing the sampling points along the Northern transport corridor (Right). Grey dots indicate the sites where An. stephensi were present while the Black dots indicate where only other vectors (An. gambiae s.l. and An funestus s.l.) were collected.

Figure 2

Pictures of the range of habitats where An. stephensi was collected in Marsabit: (a) An animal watering pan, (b) disposed containers with water and (c) old tyre

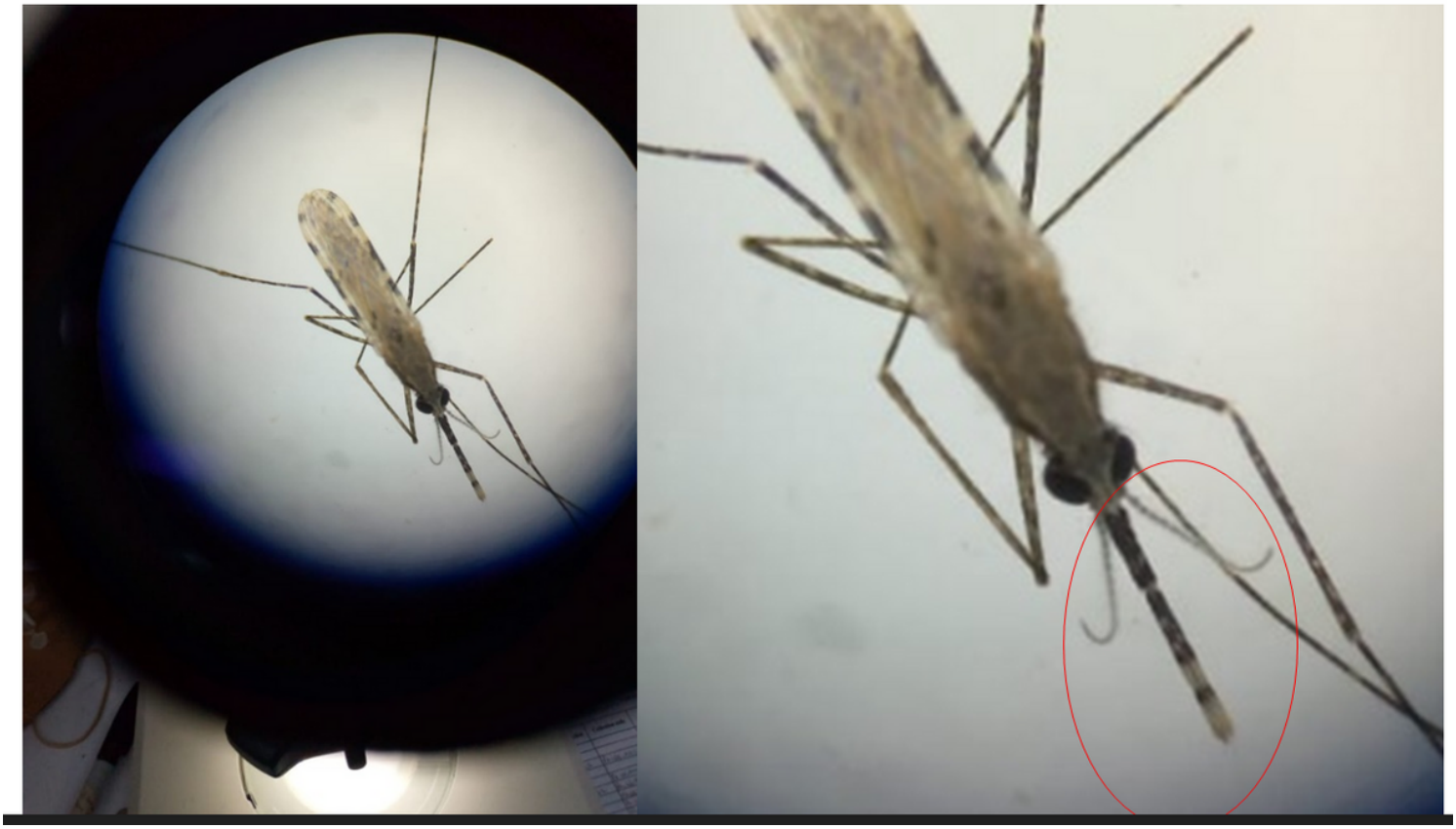

Figure 3

Picture of An stephensi as observed under a microscope with the second picture showing the dual banding on the palps characteristic of An stephensi. Other distinguishing features are not clear on this image.

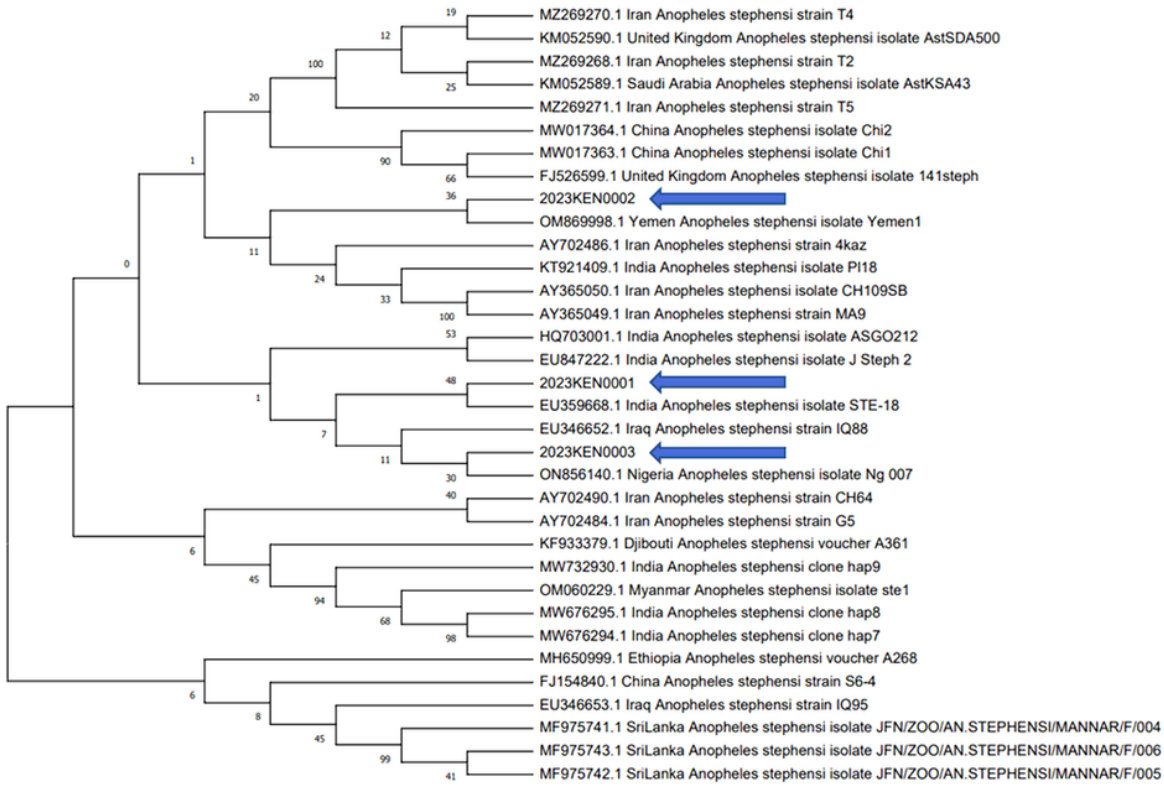

Figure 4

Phylogenetic analysis of isolates from Kenya (2023KEN002, 2023KEN003 and 2023KEN001) in comparison to *An. stephensi* isolates from other parts of the world.