REVIEW ARTICLE

ISSN (ONLINE): 3027-2998

CURRENT ISSUE ON THE EMERGENCE OF *ANOPHELES STEPHENSI* MOSQUITO IN AFRICA: A REVIEW

Azike Chidinma Anthonia[1]*, Sandra Ujunwa Ekesiobi[2], Chinonye Oluchi Ezenwaka[3], Jonathan Nyebuchi[1] and Brenda Anyakwe Nnokam[4]

[1] Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria

[2] Department of Medical Laboratory Science, Niger Delta University, Bayelsa State, Nigeria

[3] Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria

[4] Department of Family Medicine, College of Medicine, Rivers State University, Port Harcourt

Corresponding author's email address: chidimma.azike@ust.edu.ng

Received: 6th February, 2024
Accepted: 25st February, 2024
Published: 28th February, 2024
Available: https//www.nmlsj.org
Publisher: ScholarlyFeed

Cite as: Azike CA, Ekesiobi SU, Ezenwaka CO, Nyebuchi J, Nnokam BA. Current issue on the emergence of *Anopheles stephensi* mosquito in Africa: A review. Nexus Med Lab Sci J. 2024; 1(1):46-54

Abstract

Anopheles stephensi belongs to the same subgenus as Anopheles gambiae, which is the predominant mosquito vector of malaria in Africa and is the primary malaria vector in urban India. This research presents a comprehensive overview of the emergence and spread of *An. stephensi*, a significant malaria vector, focusing on its recent incursion into Africa. Originating from Southern and Western Asia, *An. stephensi* has expanded to seven African nations, posing a severe threat to malaria control efforts, particularly in urban areas. The mosquito's unique biology, diverse forms, and genetic markers are explored, emphasizing the importance of precise identification for effective disease management. The preferred habitats, life cycle, and feeding patterns of *An. stephensi* underscore its adaptability and resilience. Factors contributing to its spread, including climate change, urbanization, and vector resistance, are outlined. The current geographic distribution in the Horn of Africa raises concerns, demanding increased surveillance and research for a clearer understanding of its impact on malaria transmission. Finally, potential interventions, such as environmental management and genetic modification, are discussed as critical strategies to control *An. stephensi* and mitigate its implications for public health. This review serves as a valuable resource for fostering awareness, encouraging proactive measures, and informing targeted interventions and management techniques to curb the spread of this invasive malaria vector in Africa.

Keywords: *Biology, malaria, management, region, spread, transmission, urban, vector.*

Introduction

Background on *Anophelis stephensi*

An. stephensi belongs to the same subgenus as *Anopheles gambiae*, which is the predominant mosquito vector of malaria in Africa and is the primary malaria vector in urban India [1]. According to the World Health Organization (WHO), *An. stephensi* is a type of mosquito that can spread the malaria pathogens *Plasmodium falciparum* and *P. vivax* [2]. It is more likely to flourish in urban and artificial contexts than the other primary malaria mosquito vectors. *An. stephensi* was once only found in some regions of Southern and Western Asia where the species acts as a highly effective reservoir for malaria [3], as well as in Pakistan, and the Arabian Peninsula, but it has since been found in seven African nations [2]. Carter et al. [4] reported that *An. stephensi* mosquitoes were discovered for the first time in Ethiopia in 2016, and since then, the species has established itself there. This finding came after the species had been reported in nearby Djibouti previously [5, 6]. *An. stephensi* mosquitoes are present in both rural and urban regions in Africa, unlike other malaria vectors. They spawn in artificial water containers such garden reservoirs and home water storage containers [3]. According to another study by Gayan Dharmasiri et al. [7], the species was recently discovered for the first time in Sri Lanka, proving its capacity to spread over vast bodies of water and effectively establish itself in new geographical areas. A report by Nolan [8] of the New York Times revealed that 1,300 students were impacted by a malaria outbreak that occurred at Dire-Dawa University in Dire Dawa, the second-largest city in Ethiopia, in April of 2023. Because the outbreak happened in an urban setting and during the dry season, two circumstances that are unusual for frequent occurrences of malaria in this part of the world, it remained mysterious. Researchers finally concluded that *An. stephensi*, a parasite that causes malaria and flourishes in urban areas during dry seasons and is resistant to insecticides, was responsible for this after blood tests revealed the presence of the ring-shaped malaria parasite.

A WHO Vector Alert from 2019 highlighted the risk of *An. stephensi* emergence and spread outside of its natural geographic range and recommended action [9]. *An. stephensi* has now been documented in Yemen (2021), Kenya (2022), and Nigeria (2020) [10].

The purpose of this review therefore is to discuss the current issue on emergence of *An. stephensi* with respect to Africa, to help promote awareness of the vector, consequently creating systems to minimize its spread within the society.

Emergence of *Anophelis stephensi* in Africa

Although native to Asia, An. stephensi has lately invaded several African nations, including Sudan (2019), Djibouti (2012), Ethiopia (2016), and Somalia (2019) [11-14]. Given the high risk and heavy cost of malaria in Africa, where the majority of cases, more than 94% of the 229 million predicted cases worldwide in 2019 were reported from the continent, most of the cases occurring in sub-Saharan Africa [15]. In 2020, Gombe State University researchers, in partnership with the Nigeria Institute of Medical Research and the National Malaria Elimination Program (NMEP), discovered *Anopheles stephensi* for the first time in the Biliri Local Government Area of Gombe State, Nigeria [16]. Recently, *An. stephensi* has been discovered in Ghana (2023) also, making it the second West African country with the report of the vector [17]. This suggests a shift in the geographical distribution of *An. stephensi* within the African region, as well as a call for urgent response to tackle the menace of the vector to prevent further spread. This knowledge also reveals the capability of *An. stephensi*' to spread from region to region. Using PCR and ITS2 region sequencing, the presence of *An. stephensi* mosquitoes was confirmed in Ghana [18], outcome, which underlines how urgently more surveillance is needed, as well as reaction plans, to slow the spread.

Seeing the global relevance of the emergence of *An. stephensi* in Africa, the World Health Organization (WHO) has issued a warning regarding the invasion and spread of *An. stephensi* throughout the region. They are requesting that national malaria management programs, along with their partners in high-risk areas, exercise caution and enhance their monitoring systems to identify and manage this invasive mosquito species early on [9].

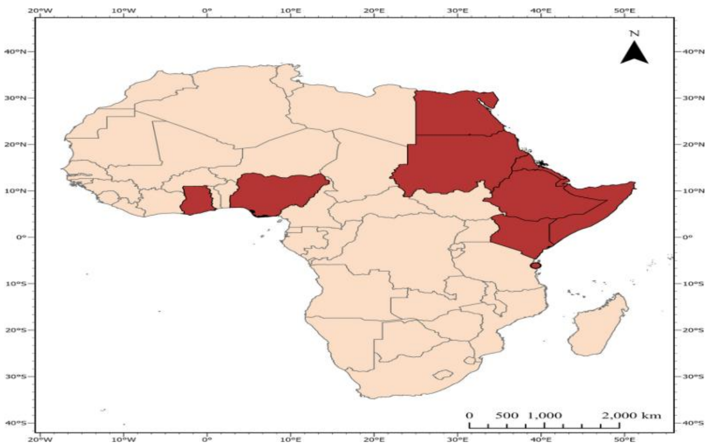

Figure 1: Africa's verified *Anopheles stephensi* distribution by nation. Red-shaded countries have at least once proven the existence of An. stephensi[19].

Biology and Characteristics of *Anophelis stephensi*

Some of the important details regarding the biology and traits of *An. stephensi* are noted below:

Figure 2: Image of *Anopheles stephensi* showing the Maxillary palpus with pale bands; MPlp5 entirely pale; erect head scales broad, white on vertex and dark brown laterally and posteriorly[20].

An. stephensi is native to the Indian subcontinent and the Middle East, but has recently invaded eastern Africa, posing a threat to urban malaria control [21]. According to Ghosh et al. [21] and Khan et al., [22], *An. stephensi* has three biological forms, type, intermediate and mysorensis, that differ in the number of ridges on their eggs and their vectorial competence, these were recognized as sibling species, denoted as Species A, Species B, and Species C, in that order [21, 22]. A study by Vipin et al. [23] showed that while mysorensis is a poor vector (extremely zoophilic) and restricted to rural regions and is sensitive to *Plasmodium vivax* (VK210B), the type biological form is an effective malaria vector in urban areas. There is very little evidence available about the intermediate biological form's capacity to spread, with reports from rural and peri-urban areas [24].

There is currently little knowledge on the population genetics of *An. stephensi* according to Gholizadeh et al. [25]. The typical molecular markers employed in the identification of *An.*

stephensi include the mitochondrial oxidases subunit 1 and 2 (COI and COII), ribosomal internal transcribed spacer 2 (rDNA-ITS2), and domain-3 (D3) loci. However, none of these indicators have been able to accurately distinguish between the biological forms of *An. stephensi* [21, 26]. On the other hand, it has lately been demonstrated that the intron I sequences of Odorant-binding protein 1 may serve as a genetic marker for distinguishing between distinct *An. stephensi* complex members [24]. Precise recognition, understanding the spatial distribution, and monitoring the population dynamics of cryptic species within the *An. stephensi* complex are of significant importance for human health. This is because it directly influences strategies for vector control and disease management [21]. Additionally, *An. stephensi* is highly anthropophilic, meaning that it prefers to feed on humans rather than animals [27].

Habitat Preferences

Thomas et al. [28] reported that *An. stephensi* mosquito species can breed in various aquatic habitats, both natural and artificial. It prefers to breed in containers that hold clear or clean water, such as water tanks, cisterns, pots, and barrels. It is capable of tolerating high salinity levels and breed in water that is as salty as sea water [29]. In urban areas, it mainly breeds in overhead tanks and ground level water tanks, which contribute to its vectorial capacity and malaria transmission [28].

Life Cycle

The life cycle of *An. stephensi* has two phases: aquatic and terrestrial [30]. The aquatic phase consists of the eggs, larvae, and pupae stages, while the terrestrial phase consists of the adult stage only. The eggs are laid on the surface of water in various habitats, such as artificial containers, irrigation canals, stream pools, and stream margins [29]. The eggs hatch into larvae, which feed on organic detritus and microorganisms in the water1. The larvae undergo four instars before transforming into pupae, which are the resting and non-feeding stage before adult emergence [28]. The adult mosquitoes are active at night and feed on blood from humans or animals, depending on their biological form and habitat [20, 30]. Balkew et al. [31] reported that the female mosquitoes need a blood meal to produce eggs, and they can also transmit malaria parasites to humans if they are infected. The adult lifespan of *An. stephensi* can range from a few days to several weeks, depending on environmental factors and availability of hosts [28].

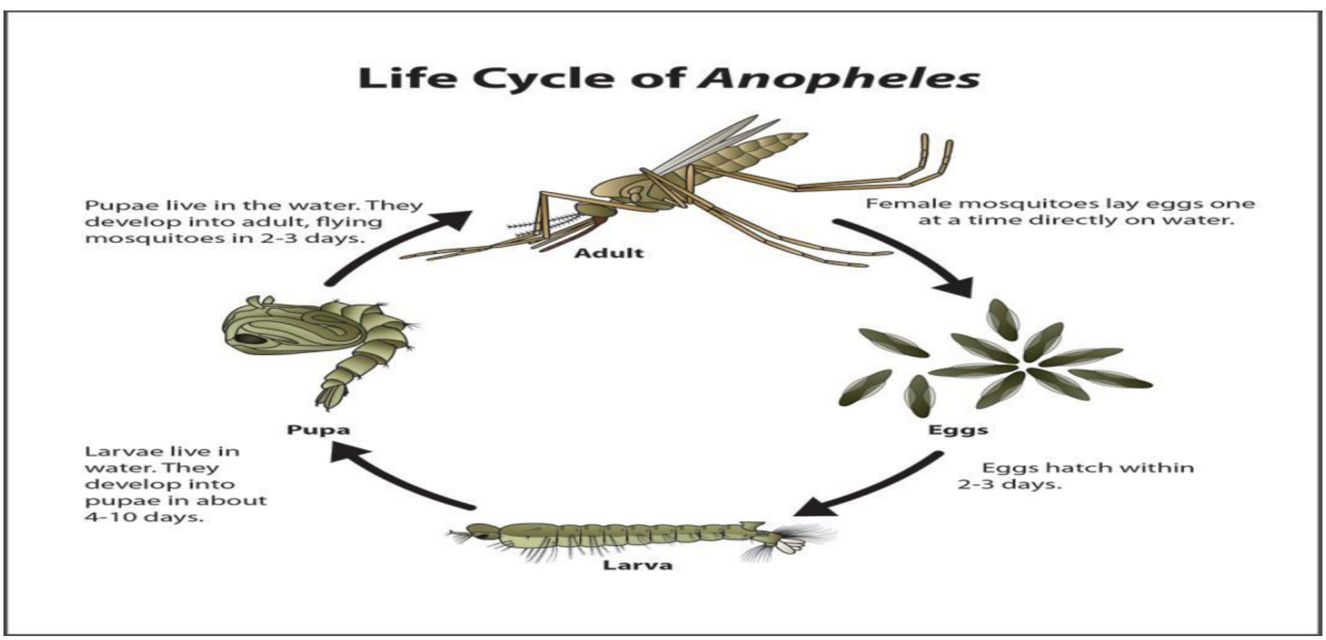

FIGURE 3: LIFE CYCLE OF ANOPHELES SPECIES MOSQUITOES [38]

Behavior And Feeding Patterns

According to some studies, *An. stephensi* has the following characteristics: it prefers to rest and feed in cattle sheds than human dwellings [32]. It is noted to be strongly zoophilic, meaning it feeds mainly on animals rather than humans. It is active at night and exhibits increased flight velocity and host-seeking behavior in response to carbon dioxide [33]. It can be infected with both Plasmodium falciparum and Plasmodium vivax, the two most common malaria parasites that infect humans. *An. stephensi* is capable of altering its feeding and infection patterns depending on the levels of serotonin in the blood meal [33].

Spread in Africa

Current Geographic Distribution

As at the time of this research, *An. stephensi*' current range according to extends to Somalia (2019), Ethiopia (2016), Sudan (2016), Eritrea (2022), and beyond to Yemen (2021), Kenya (2022), Ghana (2022), and Nigeria (2020) [34].

Factors Contributing to Spread

There are several factors that contribute to the spread of *An. stephensi*, a new and invasive malaria vector in Africa and southern Asia. Some of these factors are highlighted below.

Climate change: *An. stephensi* can survive in a wide range of temperatures and humidity levels, and may benefit from the increased rainfall and warmer conditions caused by climate change [2, 40].

Urbanization: *An. stephensi* is well adapted to urban and man-made environments, where it can breed in artificial water containers, such as tanks, barrels, wells, and animal shelters [35]. Urbanization also increases human population density and mobility, which facilitate the transmission and spread of malaria [9].

Lack of surveillance and control: *An. stephensi* is often overlooked or misidentified by the existing vector surveillance and control systems, which are mainly focused on the traditional malaria vectors in Africa and southern Asia [9, 35]. Moreover, *An. stephensi* is resistant to multiple insecticide classes, posing challenges to its control.

Global trade and travel: *An. stephensi* may have been introduced to new regions through the movement of goods and people, especially through the maritime routes connecting the Horn of Africa, the Arabian Peninsula, and South Asia [9, 35].

Water Storage Practices: *An. stephensi* prefers breeding in clean and relatively clear water. Improper water storage practices, such as open water containers or inadequate sealing of water storage tanks, can create favorable breeding conditions. People storing water in and around their homes may unknowingly provide suitable habitats for mosquito larvae [36].

Vector Resistance: In some cases, the mosquitoes may develop resistance to insecticides commonly used in vector control efforts. This resistance can lead to the failure of insecticide-based control measures, allowing the mosquito population to thrive and spread [37].

Vector Control and Management

Based on the study conducted by Mnzava et al. [12], some of the possible interventions and tools to control *An. stephensi* include: environmental management to reduce breeding sites, indoor residual spraying and larviciding to kill adult and larval mosquitoes, use of insecticide-treated nets and repellents to protect people from mosquito bites, genetic modification or gene drive to reduce the reproductive capacity or vector competence of *An. stephensi*, and the use of **Wolbachia infection** to reduce the susceptibility of the vector (*An. stephensi*) to malaria parasites.

Integrated vector management, or IVM, which is a methodical approach to making the most use of available resources for vector control has been suggested by Beier et al., [41]. It entails choosing and putting into practice suitable vector control techniques, including as biological control, insecticide-treated nets, indoor and outdoor residual spraying, and larval source management, based on the local environment and proof of their efficacy and cost-effectiveness. Further management of *An. stephensi* requires increasing collaboration among different sectors across the African continent and beyond, strengthening surveillance through employing techniques like the molecular methods to track the vector spread and distribution, improved data sharing among countries, regions and partners to aid rapid detection, reporting, and response, guidance development and research prioritization to learn more about the biology, ecology, genetics, and vectorial competence of *An. stephensi*, as well as on the effectiveness, cost-effectiveness, and acceptability of various vector control interventions, such as larval source management, indoor and outdoor residual spraying, insecticide-treated nets, and biological control [12, 16].

The table below summarizes the features which distinguishes *An. stephensi* from other *An. species*, making it a matter of urgent concern particularly in Africa at the moment.

Comparison Between *An. stephensi* and other *Anopheles Species*

Table 1: Comparison between *An. Stephen* and other *Anopheles species* with respect to breeding, prevalence, management, parasite being transmitted, lifespan and control measure

Species	Breeding	Prevalence	Management	Parasite Transmission	Lifespan	Control
An. stephensi	Uses human-made containers or cisterns with clean water; adapts to cryptic habitats and high temperatures [12].	Detected in 7 countries in the Horn of Africa; predicted to spread to many other African cities and put 126 million people at risk [2].	Requires inter- and intra-sectoral collaboration; urban planning and environmental management; community engagement and behavior change [12, 16].	Can transmit both *P. falciparum* and *P. vivax;* has high vectorial capacity and competence; can be infected by diverse *Plasmodium* species [12, 40].	Has a longer lifespan than most African vectors; can survive up to 30 days in laboratory conditions [12].	Needs integrated vector management; larval source management; indoor and outdoor residual spraying; insecticide-treated nets; biological control [2, 41]
An. arabiensis	Prefers natural habitats such as pools, ponds, or marshes; breeds in sunlit or shaded water [42]	Widely distributed in sub-Saharan Africa; co-exists with other vectors in many areas [34]	Requires entomological surveillance and monitoring; genetic and molecular tools; operational research [44].	Mainly transmits P. falciparum; has low vectorial capacity and competence; can be infected by *P. vivax* and *P. ovale* [12].	Has a shorter lifespan than *An. gambiae* and *An. funestus*; can survive up to 15 days in laboratory conditions [12]	Can be controlled by insecticide-treated nets; indoor residual spraying; larval source management; genetic manipulation [43].
An. gambiae	Prefers small, temporary, and sunlit water collections; breeds in puddles, hoof prints, or discarded containers [44].	Extensive throughout sub-Saharan Africa; coexists in many places with various vectors [34]	Requires entomological surveillance and monitoring; genetic and molecular tools; operational research [44].	Principally spreads P. *falciparum;* possesses a high vectorial competence and capability; can also host *P. vivax* and *P. ovale* [12]	Has a shorter lifespan than *An. funestus*; can survive up to 20 days in laboratory conditions [12]	Can be regulated by insecticide-treated nets; indoor residual spraying; larval source management; genetic manipulation [42].
An. funestus	Prefers permanent water bodies with vegetation; breeds in swamps, rice fields, or irrigation canals [44].	Substantially dispersed throughout sub-Saharan Africa; frequently coexists with other vectors [34].	Requires entomological surveillance and monitoring; genetic and molecular tools; operational research [44].	Mainly transmits *P. falciparum;* has high vectorial capacity and competence; can be infected by *P. vivax* and P. *ovale* [12]	Has a longer lifespan than *An. gambiae* and *An. arabiensis*; can survive up to 25 days in laboratory conditions [12].	Can be controlled using insecticide-treated nets; indoor residual spraying; larval source management; biological control [46].

Conclusion

The emergence of *Anopheles stephensi*, a potent malaria vector, in Africa poses a significant threat to ongoing malaria control efforts, particularly given its resistance to commonly used insecticides. The spread of this mosquito species in Africa is influenced by factors such as climate change, urbanization, global trade, and water storage practices. Recognizing the importance of effective surveillance and control measures, including environmental management, insecticide use, and genetic modification, becomes crucial to mitigate the impact of *An. stephensi* and reduce the burden of malaria in affected regions.

Recommendation

In light of the alarming spread of *An. stephensi* across Africa and its threat to malaria control efforts, urgent action is recommended. Strengthening surveillance and control measures, especially in urban areas, is imperative. Increased investment in research to understand the mosquito's behavior, genetics, and distribution will inform targeted interventions. Implementing a comprehensive vector control strategy, including environmental management, insecticide use, and genetic modification, is crucial. Collaborative efforts between health authorities, researchers, and communities are vital to effectively curb the spread of *An. stephensi* and mitigate the associated malaria risks in Africa.

References

1. Valenzuela JG, Francischetti IM, Pham VM, Garfield MK, Ribeiro JM. Exploring the salivary gland transcriptome and proteome of the *Anopheles stephensi* mosquito. Insect Biochemistry and Molecular Biology. 2003;33(7):717-32.
2. World Health Organization (WHO). WHO initiative to stop the spread of *Anopheles stephensi* in Africa. 2023. Available from: https://www.who.int/publications/i/item/WHO-UCN-GMP-2022.06. Accessed January 23, 2024.
3. Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J. A global index representing the stability of malaria transmission. The American Journal of Tropical Medicine and Hygiene. 2004;70(5):486-98.
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 Tropica. 2018;188:180-186.
5. 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. Parasitology Research. 2019;118(3):725-732.

6. 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 Tropica. 2014;139:39-43.
7. Gayan-Dharmasiri AG, Perera AY, Harishchandra J, Herath H, Aravindan K, Jayasooriya HTR, Ranawaka GR, Hewavitharane M. First record of *Anopheles stephensi* in Sri Lanka: a potential challenge for prevention of malaria reintroduction. Malaria Journal. 2017;16(1):326.
8. Nolen S. An Invasive Mosquito Threatens Catastrophe in Africa. The New York Times. 2023. Available from: https://www.nytimes.com/2023/09/29/health/mosquitoes-stephensi-malaria-africa.html. Retrieved January 23, 2024.
9. World Health Organization (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. Available from: https://iris.who.int/handle/10665/326595. Accessed January 23, 2024.
10. WHO. Malaria Threats Map. Geneva, World Health Organization. 2021. Available from: https://apps.who.int/malaria/maps/threats/. Accessed January 23, 2024.
11. Ahmed A, Khogali R, Elnour M-AB, Nakao R, Salim B. Emergence of the invasive malaria vector *Anopheles stephensi* in Khartoum State, Central Sudan. Parasites Vectors. 2021;14:511.
12. Mnzava A, Monroe AC, Okumu F. *Anopheles stephensi* in Africa requires a more integrated response. Malaria Journal. 2022;21:156.
13. Hawaria D, Kibret S, Zhong D, Lee M-C, Lelisa K, Bekele B, Birhanu M, Mengesha M, Solomon H, Yewhalaw D, Yan G. First report of *Anopheles stephensi* from southern Ethiopia. Malaria Journal. 2023;22:373.
14. Ali S, Samake JN, Spear J, Carter ET. Morphological identification and genetic characterization of *Anopheles stephensi* in Somaliland. Parasites Vectors. 2022;15:247.
15. WHO. World malaria report 2020. WHO. 2020. Available from: https://cms.who.int/publications-detail-redirect/9789240015791. Accessed January 28, 2024.
16. The PMI VectorLink. Strengthening Local Capacity for the Surveillance of *Anopheles stephensi* in Nigeria. [Internet]. 2023. Available from: https://pmivectorlink.org/2023/02/24/strengthening-local-capacity-for-the-surveillance-of-anopheles-stephensi-in-nigeria/#:~:text=Anopheles%20stephensi%20was%20first%20detected,Malaria%20Elimination%20Program%20(NMEP). Accessed January 23, 2024.
17. BBC News Pidgin. Ghana confirm invasion of new mosquito breed wey be 'stubborn'. [Internet]. 2023. Available from: https://www.bbc.com/pidgin/articles/c3grgv4w9emo. Accessed January 28, 2024.
18. Afrane AY, Abdulai A, Mohammed RA, Akuamoah-Boateng Y, Owusu-Asenso MC, Sraku KI, Yanney AS, Malm K, Lobo FN. First detection of *Anopheles stephensi* in Ghana using molecular surveillance. bioRxiv. 2023;
19. David P, Alexander P, Yvonne-Marie L. Implications of Climate Change and *Anopheles stephensi* Liston in Africa: Knowledge Gaps and Lessons from History. Current Tropical Medicine Reports. 2023;10:1-11.
20. Walter Reed Biosystematics Unit. *Anopheles stephensi* species page. [Internet]. 2017. Available from: https://wrbu.si.edu/vectorspecies/mosquitoes/stephensi. Accessed January 25, 2024.
21. Ghosh C, Kumar N, Kushwah RBS, Soumya M, Joshi GS, Ramanjini KC, Alalamath T, Srinivasan S, Subramani S, Kumar S, Swain S. Enrichment of phenotype among biological forms of *Anopheles stephensi* Liston through establishment of isofemale lines. Parasites Vectors. 2023;16:79.
22. Khan J, Gholizadeh S, Zhang D, Wang G, Guo Y, Zheng X, Wu Z, Wu Y. Identification of a biological form in the *Anopheles stephensi* laboratory colony using the odorant-

binding protein 1 intron I sequence. PLoS One. 2022;17(2):e0263836.
23. Vipin, Dube M, Gakhar SK. Genetic differentiation between three ecological variants ('type', 'mysorensis' and 'intermediate') of malaria vector *Anopheles stephensi* (Diptera: Culicidae). Insect Science. 2010;17:335-343.
24. Firooziyan S, Dinparast Djadid N, Gholizadeh S. Speculation on the possibility for introducing *Anopheles stephensi* as a species complex: preliminary evidence based on odorant binding protein 1 intron I sequence. Malaria Journal. 2018;17(1):366.
25. Gholizadeh S, Firooziyan S, Ladonni H, Hajipirloo HM, Djadid ND, Hosseini A, Raz A. The *Anopheles stephensi* odorant binding protein 1 (AsteObp1) gene: a new molecular marker for biological forms diagnosis. Acta Tropica. 2015;146:101-113.
26. Mishra S, Sharma G, Das MK, Pande V, Singh OP. Intragenomic sequence variations in the second internal transcribed spacer (ITS2) ribosomal DNA of the malaria vector *Anopheles stephensi*. PLoS One. 2021;16(6):e0253173.
27. Ryan JS, Lippi AC, Villena CO, Singh A, Murdock CC, Johnson RL. Mapping current and future thermal limits to suitability for malaria transmission by the invasive mosquito *Anopheles stephensi*. bioRxiv. 2022;12(15):520598.
28. Thomas S, Ravishankaran S, Justin JA, Asokan A, Mathai TM, Valecha N, Thomas BM, Eapen A. Overhead tank is the potential breeding habitat of *Anopheles stephensi* in an urban transmission setting of Chennai, India. Malaria Journal. 2016;15:274.
29. Manouchehri AV, Javadian E, Eshighy N, Motabar M. Ecology of *Anopheles stephensi* Liston in southern Iran. Tropical and Geographical Medicine. 1976;28(3):228-232. Available from: https://europepmc.org/article/MED/1006792.
30. Alomar AA, Alto WB. Asian malaria mosquito, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). [Internet]. 2022. Available from: https://entnemdept.ufl.edu/Creatures/AQUATIC/Asian_malaria_mosquito_Anopheles.htm. Accessed January 25, 2024.
31. Balkew M, Mumba P, Yohannes G, Abiy E, GetachewD, Yared S, Worku A, Gebresilassie A, Tadesse GF, Gadisa E, Esayas E, Ashine T, Ejeta D, Dugassa S, Yohannes M, Lemma W, Yewhalaw D, Chibsa S, Teka H, Murphy M, Yoshimizu M, Dengela D, Zohdy S, Irish S. An update on the distribution, bionomics, and insecticide susceptibility of *Anopheles stephensi* in Ethiopia, 2018–2020. Malaria Journal. 2021;20:263.
32. Briggs AM, Hambly MG, Simão-Gurge RM, Garrison SM, Khaku Z, Van Susteren G, Lewis EE, Riffell JA, Luckhart S. *Anopheles stephensi* Feeding, Flight Behavior, and Infection with Malaria Parasites are Altered by Ingestion of Serotonin. Frontiers in Physiology. 2022;13:911097.
33. Thomas S, Ravishankaran S, Justin NAJA, Asokan A, Mathai MT, Valecha N, Montgomery J, Thomas BM, Eapen A. Resting and feeding preferences of *Anopheles stephensi* in an urban setting, perennial for malaria. Malaria Journal. 2017;16(1).
34. Emiru T, Getachew D, Murphy M, Sedda L, Ejigu AL, Bulto GM, Byrne I, Demisse M, Abdo M, Chali W, Elliott A, Vickers NE, Aranda-Díaz A, Alemayehu L, Behaksera WS, Jebessa G, Dinka H, Tsegaye T, Teka H, Chibsa S, Mumba P, Girma S, Hwang J, Yoshimizu M, Tadesse GT. Evidence for a role of *Anopheles stephensi* in the spread of drug- and diagnosis-resistant malaria in Africa. Nature Medicine. 2023;29:3203–3211.
35. Al-Eryani SM, Irish SR, Carter TE, Lenhart A, Aljasari A, Montoya FL, Awash AA, Mohammed E, Ali S, Esmail AM, Hussain A, Amran GJ, Kayad S, Nouredayem M, Adam AM, Azkoul L, Assada M, Baheshm AY, Eltahir W, Hutin JY. Public health impact of the spread of *Anopheles stephensi* in the WHO Eastern Mediterranean Region countries in Horn of Africa and Yemen: need for integrated vector surveillance and control. Malaria Journal. 2023;22:187.
36. Bloom J. How to Prevent Mosquitoes from Breeding. WikiHow. [Internet]. Available from: https://www.wikihow.life/Prevent-Mosquitoes-from-Breeding. Retrieved January 26, 2024.

37. Roll Back Malaria Partnership to End Malaria. Consensus statement for *Anopheles stephensi* in Africa. [Internet]. Available from: https://endmalaria.org/sites/default/files/RBM%20Consensus%20Statement%20for%20An%20stephensi%20FINAL%2003022023.pdf. Retrieved January 26, 2024.
38. Centers for Disease Control and Prevention (CDC). Life Cycle of *Anopheles species* Mosquitoes. [Internet]. Available from: https://www.cdc.gov/mosquitoes/about/life-cycles/anopheles.html. Accessed January 26, 2024.
39. Rodriguez AM, Hambly MG, Jandu S, Simão-Gurge R, Lowder C, Lewis EE, Riffell JA, Luckhart S. Histamine Ingestion by *Anopheles stephensi* Alters Important Vector Transmission Behaviors and Infection Success with Diverse *Plasmodium Species*. Biomolecules. 2021;11(5):719.
40. Mustafa MSEK, Jaal Z, Abu Kashawa S, Mohd Nor AS. Population genetics of *Anopheles arabiensis*, the primary malaria vector in the Republic of Sudan. Malaria Journal. 2021;20:469.
41. Beier JC, Keating J, Githure JI, Macdonald BM, Impoinvil ED, Novak JR. Integrated vector management for malaria control. Malaria Journal. 2008;7(Suppl 1):S4.
42. Perugini E, Guelbeogo WM, Calzetta M, Manzi S, Virgillito C, Caputo B, Pichler V, Ranson H, Sagnon N'F, della Torre A, Pombi M. Behavioural plasticity of *Anopheles coluzzii* and *Anopheles arabiensis* undermines LLIN community protective effect in a Sudanese-savannah village in Burkina Faso. Parasites Vectors. 2020;13:277.
43. Orondo PW, Nyanjom SG, Atieli H, Githure J, Ondeto MB, Ochwedo OK, Omondi JC, Kazura WJ, Lee M-C, Zhou G, Zhong D, Githeko KA, Yan G. Insecticide resistance status of *Anopheles arabiensis* in irrigated and non-irrigated areas in western Kenya. Parasites Vectors. 2021;14:335.
44. Zogo B, Koffi AA, Alou LPA, Fournet F, Dahounto A, Dabiré KR, Baba-Moussa L, Moiroux N, Pennetier C. Identification and characterization of *Anopheles spp*. breeding habitats in the Korhogo area in northern Côte d'Ivoire: a study prior to a Bti-based larviciding intervention. Parasites Vectors. 2019;12:146.
45. Olusi TA, Simon-Oke IA, Akeju AV. Composition, habitat preference and seasonal variation of malaria vector larval and pupa stage in Akure North Local Government Area of Ondo State, Nigeria. Bulletin of the National Research Centre. 2021;45:87.
46. Ngowo HS, Hape EE, Matthiopoulos J, Ferguson MH, Okumu OF. Fitness characteristics of the malaria vector *Anopheles funestus* during an attempted laboratory colonization. Malaria Journal. 2021;20:148.