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Spatial Variation in *Anopheles gambiae* Complex Species Composition and *Plasmodium* Sporozoite Infection Among Indoor-Resting Mosquitoes in Rural Southwestern Nigeria

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Abstract

Continuous monitoring of mosquito vector distribution and dynamics is critical for effective malaria surveillance and control. However, current entomological data from rural southwestern Nigeria remain scarce. This study characterised indoor-resting mosquito populations and malaria transmission indices in two rural settlements in southwestern Nigeria during the 2022 rainy season. Indoor-resting mosquitoes were collected for three consecutive months in Igbo-Ora and Idere using pyrethrum spray catches and identified to species using standard morphological keys. Morphologically identified *An. gambiae* complex was subsequently differentiated into species by PCR–RFLP, and *Plasmodium falciparum* circumsporozoite protein was detected by ELISA. Among 739 mosquitoes collected, *Anopheles* accounted for 57.2%. Within the *An. gambiae* complex, *An. gambiae sensu stricto* comprised (56.0%) of typed specimens, *An. coluzzii* (31.2%), *An. arabiensis* (10.1%), and interspecific hybrids (2.7%), consistent with the heterogeneous sibling-species structure reported elsewhere in Nigeria. Sporozoite infection was detected only in *An. coluzzii* from Igbo-Ora (4.88%), yielding an overall infection rate of 0.47%, similar to low-to-moderate indoor infection levels observed in other Nigerian settings. Idere showed higher indoor-resting density (0.45 vs. 0.04 mosquitoes/room) and man-biting rate (0.07 vs. 0.01 bites/person/night) than Igbo-Ora, suggesting greater indoor vector–host contact, although such indices may fluctuate with season and local ecology. Marked spatial and temporal variation in species composition and abundance between the two communities mirrors patterns seen in broader Nigerian and West African surveys, where environmental and landscape factors drive fine-scale heterogeneity in vector distributions. These findings provide updated baseline data on *Anopheles* species assemblages and *Plasmodium* infection in rural southwestern Nigeria, underscoring the value of sustained entomological surveillance and site-specific, evidence-based vector control strategies tailored to local transmission dynamics

Keywords: *Anopheles gambiae* complex, malaria vectors, sporozoite infection, indoor residual density, southwestern Nigeria, entomological surveillance

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INTRODUCTION

Vector-borne diseases are a major contributor to global health challenges, responsible for approximately 17% of the infectious disease burden and nearly one million deaths annually (WHO, 2022). Malaria represents the most consequential of these diseases, sustained by intricate

interactions among parasites, mosquito vectors, and human populations. Between 2000 and 2015, intensified efforts to control malaria led to significant reductions worldwide, with a 27% decrease in case incidence and a 60% drop in mortality. These gains were driven largely by the scaling up of vector control measures and the expanded use of artemisinin-based combination therapies (ACTs) (WHO, 2020; Al-Awadhi *et al*., 2021). However, progress has slowed since 2015, with only marginal reductions in incidence, indicating a plateau in control gains (Shi *et al.,* 2023, 2025).

This stagnation reflects emerging challenges, including widespread pyrethroid resistance in vectors and partial artemisinin resistance in *Plasmodium* parasites (Lubanga *et al*., 2024). Despite earlier achievements, these gains remain fragile; the COVID-19 pandemic exposed critical weaknesses in malaria control systems and disrupted ongoing efforts (WHO, 2020; Kouassi *et al*., 2023). Although case fatality rates continue to decline with improved access to diagnosis and treatment (Girgis *et al*., 2022; WHO, 2024), sustained transmission underscores persistent ecological and entomological constraints that limit further progress. Nigeria continues to bear a disproportionate share of the global malaria burden, characterized by perennial transmission cycles across its diverse geographical regions (Awosolu *et al.*, 2021; Oyibo *et al*., 2023; Oyewole *et al*., 2024). The persistent public health challenge is underpinned by the high vectorial capacity of *An. gambiae s.s., An. coluzzii,* and *An. arabiensis*, the three primary members of the *Anopheles gambiae* complex. Due to their remarkable adaptability and efficient transmission dynamics, these species maintain a dominant presence within the entomological landscape of various ecological zones (Awolola *et al*., 2018; Oduola *et al.,* 2022; Adeogun *et al*., 2023; Ebhodaghe *et al*., 2024).

Infections are overwhelmingly caused by *Plasmodium falciparum*, the dominant parasite species in both community-based and clinical studies (Awosolu *et al*., 2020; Abdulraheem *et al*., 2021). To address these challenges, Nigeria's malaria control relies primarily on the mass distribution of insecticide-treated nets (ITNs) as the cornerstone. Guided by the National Malaria Strategic Plan (2020–2025), supplementary measures such as including indoor residual spraying (IRS), larval source management, and chemoprevention, are deployed selectively based on local epidemiological conditions rather than universal mandates (Omojuyigbe *et al*., 2023)

Despite these measures, malaria transmission remains intense in southwestern Nigeria, where seasonal rainfall, poor drainage, and socio-economic vulnerabilities such as substandard housing and limited education sustain high vector densities (Awosolu *et al*., 2021; Ibrahim *et al*., 2022; Simon-Oke *et al*., 2023). A major challenge in malaria control is the increasing mismatch between intervention strategies and the ecological dynamics of vector populations. Widespread insecticide resistance in *Anopheles* populations has reduced the efficacy of major vector control interventions, notably ITNs and IRS (WHO, 2019; Goldstein *et al*., 2019). This decline in efficacy is driven by physiological mechanisms, including target-site mutations and enhanced metabolic detoxification, which enable mosquitoes to survive insecticide exposure (Awolola *et al*., 2018). In parallel, behavioural adaptations, including earlier or outdoor biting, allow vectors to avoid contact with treated surfaces, thereby sustaining residual transmission despite high intervention coverage (Sougoufara *et al*., 2020; WHO, 2022; Musiba *et al*., 2022). Beyond resistance, however, malaria transmission is further

shaped by fine-scale heterogeneity in vector species composition, abundance, and competence for *Plasmodium* infection, which can vary markedly between neighbouring communities (Opondo *et al*., 2016; Mategula & Gichuki, 2023).

In West Africa, intense malaria transmission has historically been sustained by the strongly anthropophilic and endophagic *Anopheles gambiae s.s*. and *An. coluzzii*. These traits promote frequent indoor human biting at night; they have contributed to the high effectiveness of ITNs and IRS for malaria control in the region (Sinka *et al*., 2010; WHO, 2022b; Sanou *et al*., 2021). However, ecological differences and variation in host-seeking behaviour within the *An. gambiae* complex generate pronounced spatial heterogeneity in transmission, even between neighbouring communities (Opondo *et al*., 2016; Akogbeto *et al*., 2018; Takken *et al*., 2024). Increasing evidence also indicates that prolonged use of long-lasting insecticidal nets (LLINs) may promote behavioural shifts toward earlier or outdoor biting, sustaining residual transmission that escapes indoor interventions (Sougoufara *et al*., 2020; Soma *et al*., 2020; Taconet *et al*., 2024; Musiba *et al*., 2022). Together, these dynamics highlight critical misalignment between the spatial complexity of vector ecology and the current reliance on uniform, broad-scale control strategies (Wu et al., 2023). Effective control, therefore, requires continuous, spatially resolved entomological surveillance that captures local variation in vector composition, behaviour, and infection dynamics. Such evidence supports targeted intervention deployment and informed resistance management (Sarangi et al., 2025; Liu *et al*., 2026).

In practice, however, entomological surveillance in many rural Nigerian settings remains limited or outdated (Okiwelu & Noutcha, 2015). In Igbo-Ora, for instance, the most recent published data date back to 2011(Noutcha & Anumudu, 2011), despite substantial ecological and demographic changes. While previous studies in southwestern Nigeria have described mosquito abundance, seasonality, and resistance profiles (Awolola *et al*., 2018; Awosolu *et al*., 2021), few have combined molecular species identification with spatially explicit assessment of Plasmodium sporozoite infection in indoor-resting vectors. As a result, the mechanisms sustaining local transmission remain insufficiently characterised.

This study addresses this gap by examining spatial variation in *Anopheles gambiae* complex species composition, indoor –resting abundance, and *Plasmodium* sporozoite infection among mosquitoes collected from two rural communities in southwestern Nigeria. Guided by an eco-epidemiological framework that links vector composition and infection prevalence to transmission intensity, the study aims to: (i) identify and characterise *Anopheles* species using morphological and molecular methods; (ii) quantify their relative abundance and spatial distribution; and (iii) determine *Plasmodium* sporozoite infection rates. By generating updated, community-level entomological evidence, this work will contribute to a more nuanced understanding of malaria transmission heterogeneity and provide data essential for locally adaptive vector control strategies.

MATERIALS AND METHODS

Entomological Surveys and Sample Processing

Adult *Anopheles* mosquitoes were sampled during the high-transmission rainy season from June to August 2022. Collections were performed using the

pyrethroid spray catch (PSC) technique following standardized World Health Organisation protocols (WHO, 1975), with modifications detailed by Russell *et al*. (2022). Adult mosquito sampling was carried out monthly over a fixed 10-day period, with collections performed between 06:00 and 08:00 h, corresponding to peak indoor resting activity to reduce disturbance and ensure statistical independence. Each household was sampled once per month using the same room where possible, along the sampling months, to permit temporal comparisons. Within each household, one room was selected based on overnight human occupancy, adequate ventilation, and the absence of cooking or food storage activities; bedrooms were prioritised where available. Where selected households were inaccessible, or consent was withdrawn, neighbouring households were recruited following a predefined replacement protocol, with all substitutions recorded. Before spraying, study procedures were explained to household occupants in the local language, and verbal informed consent was obtained. Occupants then vacated the premises temporarily, after which white sheets were spread over the floors to facilitate mosquito collection. Movable items, including furniture and utensils, were removed, while fixed structures such as beds and cupboards were left in place and covered where possible. Doors, windows, and eaves were closed and sealed with cloth or paper to prevent mosquito escape and to ensure effective insecticide action. A commercially available pyrethroid-based aerosol insecticide (Raid™ Multi-Insect Killer; Johnson™ Wax Nigeria Ltd., Lagos, Nigeria; d-trans allethrin 0.10% w/w and permethrin 0.05% w/w) was applied by trained personnel using a standardised technique. Spraying commenced at the ceiling and systematically across walls and indoor surfaces, including furniture, to achieve uniform coverage. Each room was sprayed continuously for 5-10 minutes, with the aerosol can held approximately 30 cm from treated surfaces, using an average volume of 50 mL per 30 m^3 of room space. Immediately after spraying, rooms were sealed by tightly closing all doors and windows, and blocking eaves, vents, and other potential openings with cloth or paper to prevent mosquito escape. A 10–15-minute knockdown period was then observed before re-entry.

Following the 10–15 min knockdown period, mosquitoes were retrieved from the white sheets and surrounding surfaces using stainless steel entomological forceps. Specimens were counted on-site and transferred into labelled Petri dishes lined with Whatman No. 1 filter paper, with a maximum of 10 individuals per dish to prevent overcrowding and damage. Each label recorded the house identification code, date of collection, room type, and specimen count.samples. Samples were transported to the laboratory under cooled conditions within 4 h of collection

Mosquito identification

Mosquitoes were initially sorted by genus and sex (*Anopheles* and *Culex*) under a stereomicroscope (10–40×). Female *Anopheles* were subsequently identified as members of the *An. gambiae* or *An. funestus* complexes. based on wing venation, maxillary palp proportions, and leg morphology, using standard dichotomous keys (Gillies & Coetzee, 1987; Gillett, 1972; Coetzee, 2020). Specimens that could not be reliably distinguished were retained at the An. gambiae complex level (Alves *et al*., 2024). Abdominal condition was classified as unfed, freshly blood-fed, semi-gravid, or gravid according to gut and ovarian characteristics (Assa *et al*., 2023; Woyessa & Yewhalaw, 2025). Individual females were preserved in labelled microcentrifuge tubes

containing silica gel, and stored at −20 °C until molecular analysis (Nabet *et al.,* 2021).

PCR identification of *Anopheles gambiae* Complex

Morphologically identified *Anopheles gambiae* specimens that were preserved individually were subsequently identified to species level using species-specific polymerase chain reaction (PCR) following the protocol of Scott *et al*. (1993). The PCR products were further resolved into *An. gambiae* s.s. and *An. coluzzii* by PCR–restriction fragment length polymorphism (PCR–RFLP) using the *HhaI* restriction enzyme, as described by Fanello *et al*. (2002). Digestion was carried out at 37 °C, after which the resulting amplicons were resolved by agarose gel electrophoresis. Gels were pre-stained with GelRed to enable clear visualization and discrimination of *An. gambiae s.s*., *An. coluzzii*, *An. arabiensis, An. melas*, and *An. quadriannulatus* based on their characteristic banding patterns (Fanello *et al*., 2002; Ikpo *et al.,* 2021; Akeju *et al.,* 2022). Electrophoretic profiles were documented and stored using a digital gel imaging system (Figure 1).

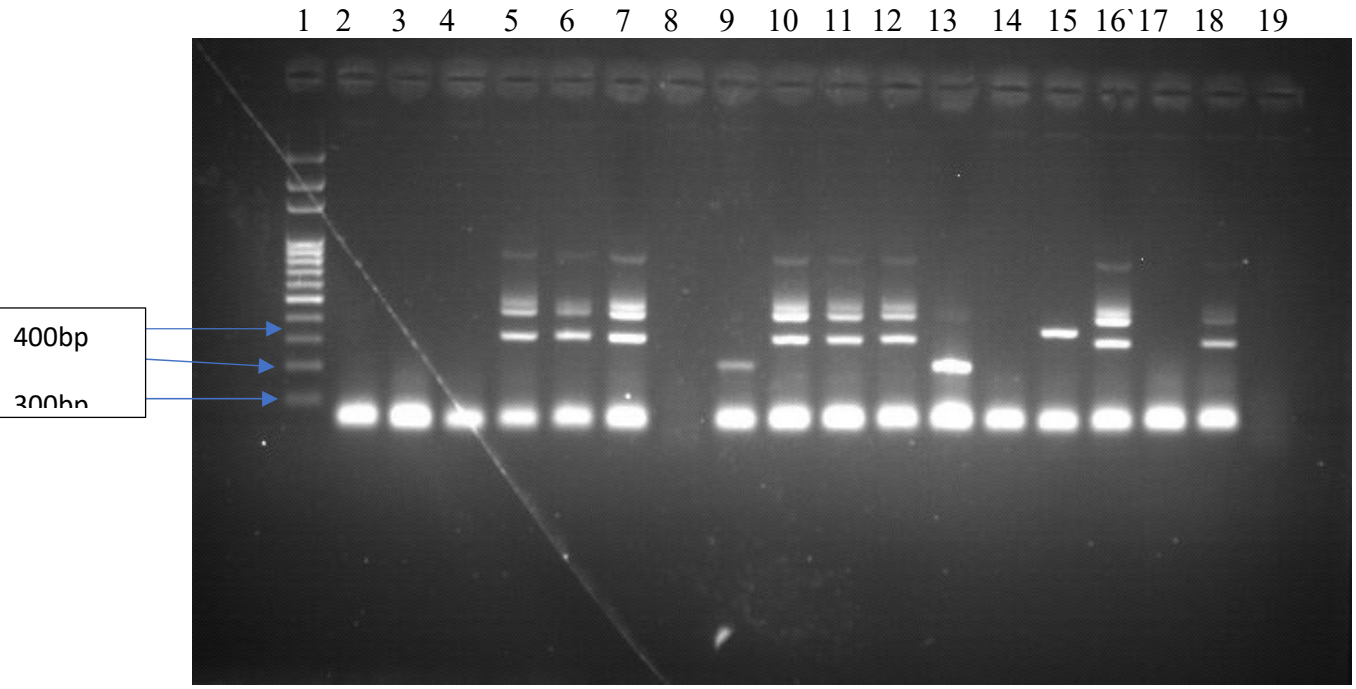

Figure 1: Molecular identification of *Anopheles gambiae* complex species by PCR: agarose gel electrophoresis of amplified products from adult female *Anopheles* mosquitoes.
Lane 1: 100 bp DNA ladder. Lanes 5–7: positive controls for *Anopheles coluzzii* (~400 bp). Lane 9: positive control for *An. gambiae* sensu stricto (~390 bp). Lanes 10–12: field samples identified as *An. coluzzii*. Lane 13: field sample identified as *An. gambiae* s.s. Lane 15: field sample identified as *Anopheles arabiensis* (~315 bp). The remaining lanes contain additional field samples. Species identification was based on expected amplicon sizes following standard *Anopheles gambiae* complex PCR protocols. bp, base pairs.

Statistical Analysis and Entomological Indices

Vector Density and Biting Activity

Indoor Resting Density (IRD) was used to assess the abundance of indoor-resting mosquitoes and is expressed as the average number of female *Anopheles* mosquitoes collected per room per night:

$$IRD = \frac{Nf}{R\,x\,t}$$

where Nf is the total number of female *Anopheles* collected, R represents the number of rooms sampled, and t is the total number of collection nights.

The man-biting rate (MBR), expressed as bites/person/night, was estimated using the density of blood-fed and semi-gravid females:

$$MBR = \frac{N\,x\,HBI}{P\,x\,t}$$

where N is the total number of engorged females, HBI is the Human Blood Index, P is the total number of occupants in the sampled rooms, and t is the number of collection nights.

Using PSC data as a proxy for MBR assumes that mosquitoes collected at dawn fed on occupants of the sampled rooms during the preceding night. This approach provides a practical and ethical alternative to human landing catches, although estimates were interpreted cautiously, given the potential for outdoor or non-human feeding.

Blood Meal Analysis and Infectivity

Human Blood Index (HBI) was the proportion of blood-fed *Anopheles* that had fed on humans:

$$HBI = \frac{n}{N}$$

where n is the number of mosquitoes that tested positive for human blood, and N is the total number of blood-fed mosquitoes examined. Following the criteria of Shililu *et al*. (1998), mixed blood meals containing both human and animal blood were categorised as positive for human blood.

Sporozoite rate was expressed as the percentage of mosquitoes harbouring *Plasmodium* falciparum circumsporozoite protein (CSP) antigens via, ELISA:

$$\text{Sporozoite Rate} = \frac{X}{N} \times 100$$

where X is the number of sporozoite-positive mosquitoes, and N is the total number tested (WHO, 2012).

Behavioral Metrics

To assess the tendency of vectors to rest outdoors after feeding, the Degree of Exophily (DE) was estimated based on PSC collections according to the methodology of Ameneshewa and Service (1993): DE was calculated as

$$DE = \left(1 - \frac{F}{HGG}\right) \times 100$$

Where F represents the number of freshly blood-fed female *Anopheles* mosquitoes, and HGG is the number of half-gravid and gravid females collected indoors. When expressed as a percentage, higher values indicate a greater tendency to leave the house after feeding. This index assumes that mosquitoes remaining indoors progress to later gonotrophic stages, whereas those exiting early are underrepresented among half-gravid and gravid individuals in indoor collections.

Data were subjected to statistical analysis using SPSS software version 25 (IBM Corp., Armonk, NY, USA). Entomological indices, including mosquito abundance, indoor resting density, human blood index (HBI), and man-biting rate (MBR), were summarised using appropriate descriptive statistics. The distribution of continuous variables (e.g., mosquito counts) was evaluated for normality using the Shapiro–Wilk test. Differences between two groups in normally distributed data were evaluated using Student's t-test, but comparisons across multiple groups were done using one-way ANOVA, followed by Tukey's post hoc test. For non-normally distributed data, the Mann–Whitney U test or Kruskal–Wallis test was applied as appropriate. Mosquito count data were

further examined with consideration of their discrete nature. Where overdispersion or deviation from normality was evident, analyses were interpreted cautiously and supported by non-parametric testing where appropriate. The human blood index (HBI) was analysed as a binomial response, defined by the presence or absence of human blood in tested mosquitoes. Logistic regression models were used to estimate the probability of human blood feeding in relation to study site and sampling period.

For the man-biting rate (MBR), the choice of analysis depended on its expression. When treated as a count-based rate (e.g., bites per person per night), group comparisons used parametric or non-parametric tests, as appropriate. When expressed as a proportion, logistic regression was applied in line with the approach used for HBI. All statistical tests were two-tailed, and statistical significance was set at $p<0.05$.

Ethical considerations

The study was approved by the Institutional Ethical Committee of the College of Medicine, University of Ibadan, Nigeria (Protocol number: UI/UCH/23/0170). Study objectives and procedures were explained in Yoruba to community leaders and household heads before data collection. Informed consent was obtained from participating households before the start of the study.

RESULTS

Overall mosquito composition by study site

A total of 739 adult mosquitoes, comprising three genera (*Anopheles*, *Culex*, and *Aedes*), were collected from indoor-resting sites in Idere and Igbo-Ora, with marked differences in species composition between the two communities (Table 1). In Idere, the indoor-resting mosquito population was dominated by *Anopheles* species, with relatively few *Culex* and *Aedes* specimens. In contrast, Igbo-Ora exhibited a more heterogeneous assemblage, where *Culex* species predominated, followed by *Aedes,* while *Anopheles* contributed a smaller proportion of the indoor-resting catch. When data from both sites were combined, *Anopheles* mosquitoes constituted the largest share of collections, whereas *Culex* and *Aedes* contributed comparable proportions. The distribution of mosquito genera showed clear spatial variation between communities, with each site contributing disproportionately to different mosquito taxa (Table 1).

Species Composition and Site-Specific Distribution

Molecular characterization of the *Anopheles gambiae* complex showed that *An. gambiae s.s.* (56.0%) was the dominant species, followed by *An. coluzzii* (31.2%) and *An. arabiensis* (10.1%) . Interspecific hybrids of *An. gambiae* s.s. and *An. coluzzii* were also detected, though at low frequency (2.7%). The distribution of these sibling species exhibited significant spatial heterogeneity between the two study locations (Figure 2). Notably, in Idere, the indoor-resting population was primarily characterized by a high abundance of *An. gambiae s.s.,* with *An. coluzzii* occurring less frequently. A small proportion of specimens could not be resolved to species level and were classified as *An. gambiae/An. coluzzii*, while *An. arabiensis* occurred at low frequency. In contrast, the *Anopheles gambiae* complex in Igbo-Ora was dominated by *An. coluzzii*, with *An. gambiae s.s.* occurring at much lower frequency, while *An. arabiensis* was consistently present. No hybrid forms (*An. gambiae*/*An. coluzzii*) were detected at this site. Overall, the relative dominance of different sibling species varied sharply between communities, indicating pronounced spatial heterogeneity in *An.*

gambiae complex structure across the study area (Figure 2).

Monthly variation in indoor-resting mosquito genera

The relative abundance of indoor-resting mosquito genera varied across the sampling months (Figure 3). Distinct temporal patterns were observed for *Anopheles*, *Culex*, and *Aedes* species during the peak rainy season. In June, the indoor mosquito population was dominated by *Culex* species, while *Aedes* mosquitoes constituted a substantial secondary component. *Anopheles* species represented a comparatively smaller proportion of the total catch during this period. In July, a marked shift in mosquito composition was observed. *Anopheles* species became the predominant indoor-resting mosquitoes, while *Culex* species declined sharply. *Aedes* mosquitoes remained consistently present, contributing a notable proportion of the monthly catch. By August, the relative abundance of mosquito genera became more evenly distributed. *Culex* and *Aedes* species were more frequently encountered than *Anopheles*, which represented the smallest proportion of the indoor-resting mosquito population during this month. Overall, the composition of indoor-resting mosquitoes exhibited clear month-to-month variation, with changes in the dominant mosquito genus observed across the study period (Figure 3).

Indoor-resting *Anopheles* collections

A total of 187 rooms were surveyed over 30 collection nights across the two study sites. Sampling intensity was higher in Igbo-Ora than in Idere, based on the number of rooms visited and nights sampled. Overall, 423 female anopheline mosquitoes were collected, a large proportion of which were engorged. Substantial spatial variation in mosquito abundance was observed. Despite lower sampling effort, Idere contributed the majority of female *Anopheles* collected, whereas Igbo-Ora yielded comparatively few specimens, even though human occupancy was similar between sites. Consequently, indoor-resting density was markedly higher in Idere than in Igbo-Ora. Indoor Resting Density ranged from 0.45 mosquitoes per room per night in Idere to 0.04 mosquitoes per room per night in Igbo-Ora, while the Man Biting Rate was also higher in Idere (0.11 mosquitoes per person per night) than in Igbo-Ora (0.01 mosquitoes per person per night) (Table 2).

Statistical comparisons between the two study sites revealed no significant variation in either indoor-resting density or man-biting rates (Student's t-test, $p > 0.05$\$). While vector activity levels remained comparable across locations, the spatial distribution of engorged females closely mirrored the overall population abundance, with a higher concentration of blood-fed individuals observed in Idere.

Species-specific sporozoite infection rates

A total of 422 female *Anopheles* mosquitoes representing multiple species were screened for *Plasmodium* sporozoite infection across the two study sites. Overall, 2/422 (0.47%) of the mosquitoes tested positive for sporozoites. Sporozoite positivity was exclusively observed in *Anopheles coluzzii* specimens from Igbo-Ora, yielding an infection rate of 4.88% (2/41). In contrast, *An. gambiae s.s., An. arabiensis*, and the *An. gambiae* / *An.coluzzii* hybrids, along with other sampled *Anopheles* species, showed no evidence of *Plasmodium* infection (0%) for each respective group. At the site level, Igbo-Ora recorded 2/79(2.53%) sporozoite-positive mosquitoes, whereas Idere showed no evidence of infection despite a larger sample size (343, 0.0%). These findings indicate a low overall sporozoite rate, with transmission detected only in *An.coluzzi* from Igbo-Ora.

Table 1. Composition of indoor-resting mosquitoes collected in Idere and Igbo-Ora, southwestern Nigeria.

	Mosquito composition N (%)			
Study site	***Anopheles sp***	***Culex sp***	***Aedes sp***	**Total**
Idere	344(82.1)	9 (2.1)	66 (15.8)	419
Igbo-Ora	79 (24.7)	149 (46.6)	92(28.8)	320
Total	**423 (57.2)**	**158 (21.4)**	158**(21.4)**	**739**

N = number of mosquitoes collected; % = percentage contribution of each mosquito genus relative to the total number collected at each study site. Percentages in the "Total" row represent the overall proportion of each genus across both sites.

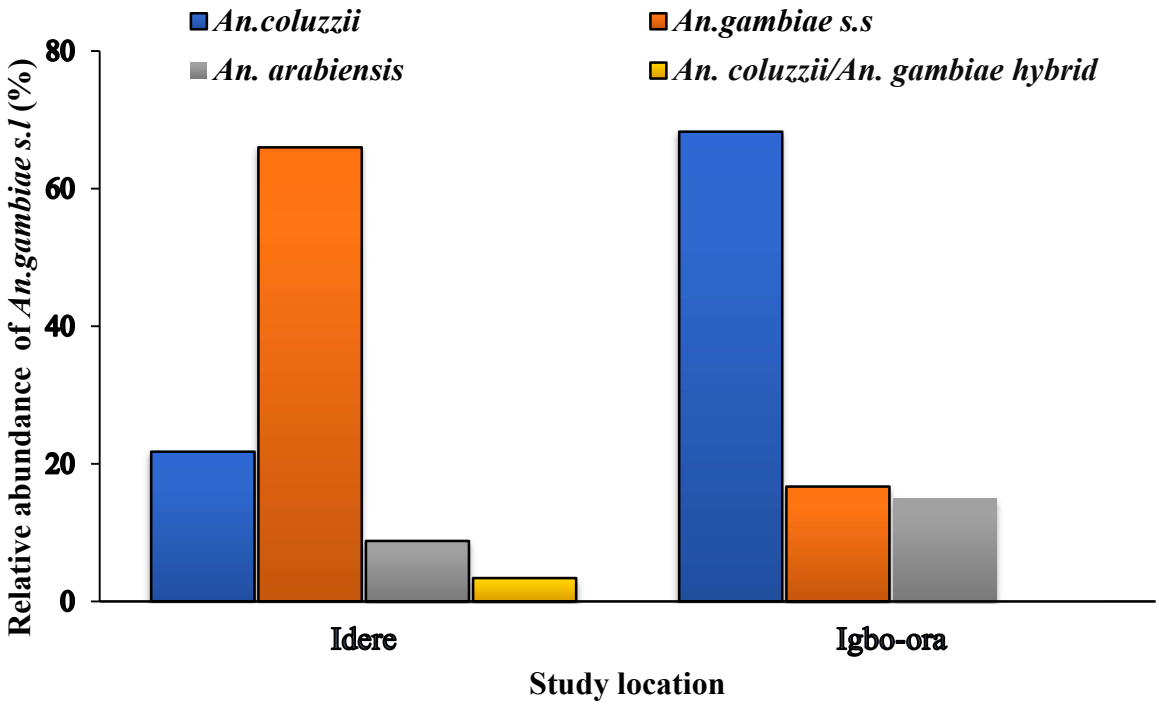

Figure 2. Relative frequency (%) of *Anopheles gambiae* complex sibling species collected indoors in Idere and Igbo-Ora, southwestern Nigeria. Bars represent the proportion of each sibling species among molecularly identified *An. gambiae* complex mosquitoes at each site. Percentages were calculated within study sites. Abbreviation: *An*., *Anopheles*

Table 2. Indoor-resting *Anopheles* mosquito collections and entomological indices in Idere and Igbo-Ora

Site	Rooms surveyed (n)	Collection nights (n)	Human occupants (n)	*Anopheles* Collected (n)	Blood-fed females (n)	HBI	IDR (mosquito/room/night).	MBR Bites/person/night
Idere	76	10	253	344	274	0.64	0.45	0.07
Igbo-Ora	111	20	248	79	67	0.65	0.04	0.01
Total	187	30	501	423	341	0.64	0.08	0.01

HBI, human blood index (proportion of blood-fed mosquitoes with human blood); IRD, indoor resting density (number of female *Anopheles* per room per night); MBR, man-biting rate (bites / person / night).

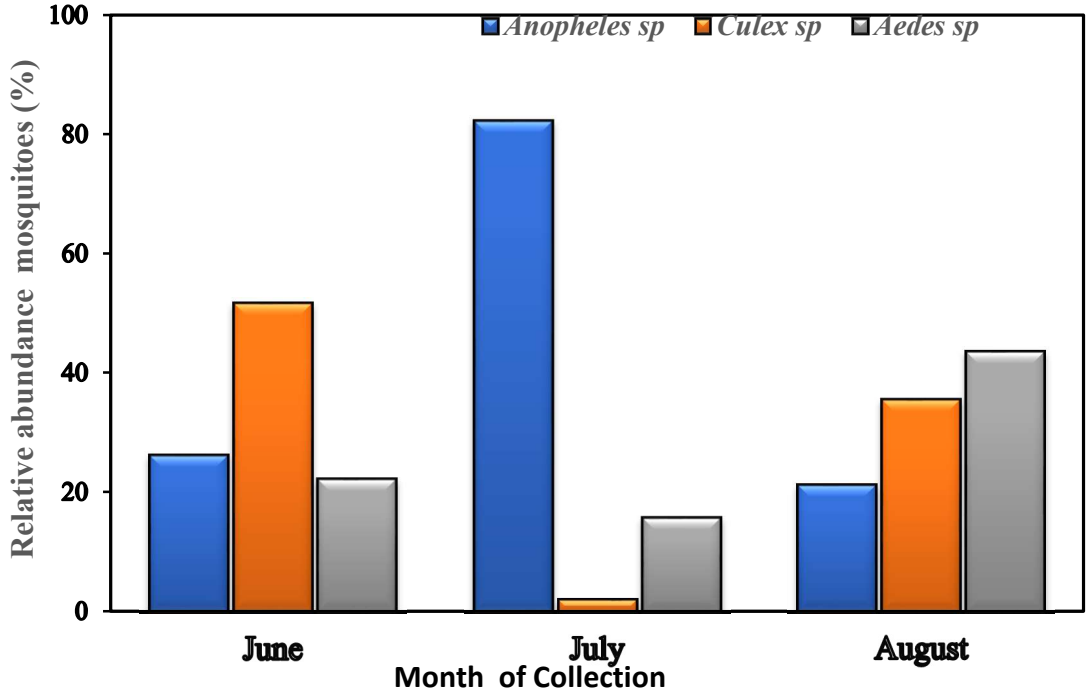

Figure 3. Monthly variation in the relative abundance (%) of indoor-resting mosquito genera collected between June and August in the study area. Bars represent the relative frequency of *Anopheles*, *Culex*, and *Aedes* species per month. Percentages were calculated within each month. Abbreviations: *sp,* species

Table 3: Detection of CSP antigen in *An. gambiae, An. coluzzii An.gambiae/An.coluzzii* hybrid *An. arabiensis* and other anophelines in Idere and Igbo-Ora communities

	An. gambiae			*An. coluzzii*			*An. gambiae/coluzzii hybrid*			*An.arabiensis*			Other species			Overall Total	
Site	Tested	Positive	SPR (%)	Tested	Positive	SPR (%)	Tested	Positive	SPR (%)	tested	positive	SPR (%)	tested	positive	SPR (%)	Test	SPR (%)
Idere																	0
Igbo-Ora	156	0	0	52	0	0	8	0	0	21	0	0	106	0	0	343	2.53
Total	10	0	0	41	2	4.88	0	0	0	9	0	0	19	0	0	79	
	166	0	0	93	2	2.15	8	0	0	30	0	0	125	0	0	422	0.47

Sporozoite positivity (SPR) is expressed as the percentage of mosquitoes testing positive for *Plasmodium* circumsporozoite protein among those tested for each species

DISCUSSION

Understanding mosquito vector composition, spatial distribution, and infection status is essential for designing effective, locally adapted malaria control strategies. In this study, we found substantial spatial and temporal heterogeneity in *Anopheles gambiae* complex populations and *Plasmodium* infection patterns between two neighbouring rural communities in southwestern Nigeria, with important implications for transmission dynamics and vector control.

Mosquito species composition and temporal dynamics

The occurrence of *Anopheles*, *Culex*, and *Aedes* mosquitoes reflects the diversity of larval habitats typical of rural southwestern Nigeria during the rainy season (Fagbohun *et al*., 2020). Mosquito composition varied over time, with *Culex* predominating in June (46.6%), *Anopheles* in July (65.2%), and a more balanced distribution in August. These patterns likely follow rainfall-driven changes in habitat availability and persistence that differentially favour each genus of mosquito (Oduola *et al*., 2013; Sattler *et al.,* 2005). *Anopheles* species comprised the majority of collections (57.2%), in line with previous observations from similar settings (Okwa *et al*., 2009; Oduola *et al*., 2013). However, marked spatial differences emerged between sites. Culicine mosquitoes were significantly more abundant in Igbo-Ora (46.6%) but rare in Idere (2.1%), where *Anopheles* predominated. This contrast likely reflects underlying ecological differences, with Igbo-Ora supporting more organically enriched or stagnant water bodies suitable for *Culex* breeding, whereas Idere provides transient, sunlit habitats favourable for *Anopheles* proliferation. The high culicine density observed in Igbo-ora also highlights a broader public health concern, as it may increase the risk of transmission of other mosquito-borne pathogens besides malaria. The relatively high indoor proportion of *Aedes* mosquitoes (21.4%) is noteworthy given their typically exophilic behaviour in West Africa (Padonou *et al*., 2023). This pattern may reflect their transient indoor resting following blood feeding, favourable indoor microclimates, or the presence of peri-domestic containers that facilitate movement between indoor and outdoor environments (Facchinelli *et al*., 2023). Increased indoor occurrence likely elevates human–vector contact and the risk of arboviral transmission within households, while also indicating that malaria-focused interventions alone may be insufficient. These observations highlight the need for complementary control measures targeting *Aedes* breeding and behaviour, with seasonal conditions during the sampling period likely contributing to the observed distribution. Variations in housing characteristics, sanitation, and human activities likely contribute to the observed differences in mosquito composition (Kaindoa *et al*., 2018). Overall, these findings highlight the importance of local ecological context in shaping mosquito communities and caution against generalising short-term observations across neighbouring areas or transmission periods.

Spatial heterogeneity in sibling species composition

Despite their proximity (~15 km), Idere and Igbo-Ora showed distinct compositions of the *Anopheles gambiae* complex. *Anopheles gambiae* s.s. predominated in Idere (72.9%), whereas *An. coluzzii* was more common in Igbo-Ora (65.8%). This pattern is consistent with established ecological preferences: *An. gambiae* s.s. is typically associated with transient, rain-fed habitats common in agricultural settings, while *An. coluzzii* is

more frequently linked to permanent or human-modified water bodies (Kamdem *et al*., 2012; Tene Fossog *et al*., 2014; Oduola *et al.,* 2021). *Anopheles arabiensis* occurred at lower proportions (10.1% overall; 15.2% in Igbo-Ora) compared with earlier reports from the region (Noutcha and Anumudu, 2011). This reduction may relate to changes in host availability, particularly declining livestock populations, given the species' tendency toward zoophilic feeding (Coluzzii *et al.,* 1979; Onyabe and Conn, 2001; Adebayo *et al*., 2014). A relative increase in anthropophilic species, notably *An. gambiae* s.s. and *An. coluzzii*, may have implications for malaria transmission risk in these communities.

Hybrid forms of *An. gambiae* s.s. × *An. coluzzii* were detected at low frequency (2.7%), confirming their continued presence in rural southwestern Nigeria (Okorie *et al*., 2015). Although infrequent, such hybrids are epidemiologically relevant, as gene flow between sibling species can facilitate the spread of adaptive traits, including insecticide resistance (Norris *et al*., 2015). The restriction of hybrids to Idere suggests localised ecological or behavioural conditions that may promote interbreeding and merit further investigation.

Entomological indices

Indoor-resting density (IRD) and man-biting rate (MBR) were higher in Idere than in Igbo-Ora, although the differences were not statistically significant ($p > 0.05$), indicating substantial variability over the short sampling period. Such fine-scale heterogeneity without clear statistical separation has been reported in similar settings and reflects the dynamic nature of malaria vector populations (Ototo *et al*., 2015; Mmbando *et al*., 2021; Tarekegn *et al*., 2024). The elevated IRD and MBR in Idere likely relate to a greater contribution of endophilic and anthropophilic vectors, which enhance indoor human–vector contact and are often associated with higher transmission potential. However, variability in species composition and behavioural patterns within and between sites can obscure consistent relationships between vector density and transmission (Machani *et al*., 2020; Ebuako *et al*., 2025). Studies from other African settings show that communities dominated by indoor-biting, anthropophilic vectors (e.g. *An. gambiae s.s., An. funestus, An. coluzzii*) typically exhibit higher indoor biting densities and entomological inoculation rates (EIRs) than areas where more exophagic or zoophagic species predominate (Nzioki *et al*., 2023; Akuoko *et al*., 2024; Ebuako *et al*., 2025). However, even in such settings, substantial within-site variability and temporal shifts in species composition remain common (Nzioki *et al*., 2023).

The lack of statistical significance indicates substantial uncertainty in IRD and MBR estimates, likely driven by limited sample size, short sampling duration, and nightly variability in mosquito activity (Mmbando *et al*., 2021; Tarekegn *et al*., 2014; Lamidi *et al*., 2018). Consistent with previous studies, these indices alone provide weak proxies for transmission intensity, particularly in settings with low sporozoite prevalence, outdoor biting, or contributions from secondary vectors (Awosolu *et al*., 2021; Ibrahim *et al*., 2022). Accordingly, vector density and biting rates should be interpreted with caution. Longer-term, seasonally stratified sampling would provide a more robust assessment of site-specific transmission dynamics. These results, therefore, underscore the importance of extended, seasonally resolved surveillance to capture fluctuations in vector populations over time. Combining sporozoite infection data with measures of vector density and human behaviour would strengthen the evidence base for estimating transmission risk

and support the design of context-specific control strategies.

Sporozoite infections: spatial discordance between vector density and parasite prevalence

Sporozoite infections were detected only in *Anopheles coluzzii* from Igbo-Ora (2/41; 4.88%), despite substantially higher Anopheles densities in Idere (344 vs. 79), indicating that transmission is not directly proportional to vector abundance but is shaped by multiple interacting factors. This pattern likely reflects differences in mosquito age structure and survival, with *An. coluzzii* populations more likely to include older individuals capable of completing parasite development (typically 10–14 days; Bruce-Chwatt, 1954). However, higher densities in Idere may consist largely of recently emerged, non-infectious cohorts. Variation in human infectiousness, shaped by treatment access and intervention use, together with species-specific feeding behaviour, may further influence exposure to infectious blood meals (Onyabe *et al*., 2003; Nwuba *et al*., 2008). Methodological constraints should also be considered. The small number of infected mosquitoes (2/422; 0.47%) limits statistical inference, and reliance on indoor-resting collections may underestimate transmission involving outdoor-feeding vectors. Sampling restricted to the rainy season may further overlook temporal variation in sporozoite prevalence (Gerken *et al*., 2025). The overall infection rate observed in this study is lower than earlier reports from Igbo-Ora (Noutcha and Anumudu, 2009), although differences in the study design and timing preclude direct comparison. Together, these findings highlight that malaria transmission reflects interactions among vector composition, mosquito survival, human infectiousness, and intervention coverage rather than density alone.

Implications for vector control

Marked heterogeneity over a short spatial scale indicates that uniform control strategies may be inadequate. In Idere, the dominance of *Anopheles gambiae s.s.* and the relatively high vector densities indicate that indoor-focused interventions remain appropriate. The continued use of ITNs and IRS remains appropriate in this setting, given the strongly endophilic and anthropophilic behaviour of this vector population. In contrast, the mixed vector composition in Igbo-Ora, together with the detection of sporozoite-positive *An. coluzzii*, suggests a need for complementary approaches that address peri-domestic and potentially outdoor transmission. These approaches may include larval source management and targeted environmental sanitation. The presence of *An. gambiae s.s.* × *An. coluzzii* hybrids indicate possible gene flow, underscoring the need for routine monitoring of insecticide resistance.

Study Limitations

This study focused on the rainy season and indoor –resting mosquitoes, which may underestimate the contributions of outdoor –biting vectors and overlook dry-season transmission. The small number of sporozoite-positive mosquitoes (n = 2) limited statistical power. In addition, the absence of concurrent data on human infection and intervention coverage restricted the interpretation of transmission dynamics. Future studies should incorporate longitudinal, multi-season sampling, include outdoor collections, and integrate human parasitological and molecular resistance data to provide a more detailed evaluation of residual transmission.

CONCLUSION

The findings demonstrate substantial spatial and temporal variation in *Anopheles*

gambiae complex composition and *Plasmodium* infection patterns between neighbouring rural communities in southwestern Nigeria. The discrepancy between vector density in Idere and sporozoite infections in Igbo-Ora suggests that transmission intensity is not directly determined by vector abundance alone. Instead, vector species composition and local ecological conditions play a critical role. These results support the need for fine-scale surveillance systems that capture epidemiologically relevant variation in vector populations. In resource-limited settings, reliance on density-based indicators alone may overlook persistent transmission foci, highlighting the importance of locally tailored, species-specific control strategies.

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REFERENCES

Adebayo, A.M., Akinyemi, O.O., Cadmus, E.O. (2014). Ownership and utilisation of insecticide-treated mosquito nets among caregivers of under-five children and pregnant women in a rural community in Southwest Nigeria. *Journal of Preventive Medicine and Hygiene 55*(2), 58.

Abdulraheem, M. A., Ernest, M., Ugwuanyi, I., Abkallo, H. M., Nishikawa, S., Adeleke, M., ... & Culleton, R. (2022). High prevalence of *Plasmodium malariae* and *Plasmodium ovale* in co-infections with *Plasmodium falciparum* in asymptomatic malaria parasite carriers in southwestern Nigeria. *International journal for parasitology*, *52*(1), 23-33.

Alves, G., Troco, A. D., Seixas, G., Pabst, R., Francisco, A., Pedro, C., ... & Lopes, S. (2024). Molecular and entomological surveillance of malaria vectors in urban and rural communities of Benguela Province, Angola. *Parasites & Vectors*, 17(1), 112.

Akeju, A. V., Olusi, T. A., & Simon-Oke, I. A. (2022). Molecular identification and wing variations among malaria vectors in Akure North Local Government Area, Nigeria. *Scientific Reports*, 12(1), 7674

Akogbeto, M., Salako, A., Dagnon, F., Aïkpon, R., Kouletio, M., Sovi, A., & Sèzonlin, M. (2018). Blood feeding behaviour comparison and contribution of Anopheles coluzzii and Anopheles gambiae, two sibling species living in sympatry, to malaria transmission in Alibori and Donga region, northern Benin, West Africa. *Malaria Journal*, 17.

Akuoko, O., Dhikrullahi, S., Hinne, I., Mohammed, A., Owusu-Asenso, C., Coleman, S., Dadzie, S., Kyerematen, R., Boakye, D., Wilson, M., & Afrane, Y. (2024). Biting behaviour, spatio-temporal dynamics, and the insecticide resistance status of malaria vectors in different ecological zones in Ghana. *17*(1), 16.

Al-Awadhi, M., Ahmad, S., & Iqbal, J. (2021). Current status and the epidemiology of malaria in the Middle East region and beyond. *Microorganisms*, 9(2), 338.

Ameneshewa, B., & Service, M. W. (1996). Resting habits of *Anopheles arabiensis* in

the Awash river valley of Ethiopia. *Annals of Tropical Medicine & Parasitology*, 90(5), 515-521.

Assa, A., Eligo, N., & Massebo, F. (2023). Anopheles mosquito diversity, entomological indicators of malaria transmission and challenges of morphological identification in southwestern Ethiopia. *Tropical Medicine and Health*, 51(1), 38.

Awolola T.S., Adeogun A., Olakiigbe A.K., Oyeniyi T., Olukosi Y.A., Okoh H.,Arowolo T., Akila J., Oduola A., Amajoh C.N. (2018). Pyrethroids resistance intensity and resistance mechanisms in *Anopheles gambiae* from malaria vector surveillance sites in Nigeria. PLoS One.2018; 13(12): 1-13 5.

Awolola, T. S., Oduola, A. O., Obansa, J. B., Chukwurar, N. J., & Unyimadu, J. P. (2007). *Anopheles gambiae ss* breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *Journal of Vector Borne Diseases*, 44(4), 241

Awosolu, O.B., Yahaya, Z.S., Haziqah, M.T.F., Simon-Oke, I.A., Fakunle, C. (2020). A cross-sectional study of the prevalence, density, and risk factors associated with malaria transmission in urban communities of Ibadan, Southwestern Nigeria. *Heliyon* 7 (1), e05975.

Awosolu, O. B., Yahaya, Z. S., Farah Haziqah, M. T., Simon-Oke, I. A., Olanipekun, I. T., & Oniya, M. O. (2021). Epidemiology of *falciparum* malaria among residents of some rural and periurban communities in Ekiti State, Southwestern Nigeria. Tropical biomedicine, 38(1), 14–21.

Bruce-Chwatt, L.J. (1954). Problems of malaria control in tropical Africa. *British Medical Journal. 1*(4855), 169–174.

Coetzee, M. (2020). Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malaria journal,* 19(1), 70.

Collins, F.H., Mendez, M.A., Rasmussen, M.O., Mehaffey, P.C., Besansky, N.J., Finnerty, V., (1987). A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. *The American Journal. Tropical Medicine and Hygiene* 37(1), 37–41.

Coluzzi, M., Sabatini, A., Petrarca, V., Di Deco, M.A., (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex, *Transactions of The Royal Society of Tropical Medicine and Hygiene* 73(5), 483-497.

Dahan-Moss, Y., Hendershot, A., Dhoogra, M., Julius, H., Zawada, J., Kaiser, M., ... & Koekemoer, L. L. (2020). Member species of the *Anopheles gambiae* complex can be mis-identified as Anopheles leesoni. *Malaria journal*, 19(1), 89.

Ebenezer, A., Ben, H.I.B., Enaregha, E.B. (2013). Spatial Distribution and Indoor Resting Density of Mosquito Species in the Lowland Rainforest of Bayelsa State, Nigeria. *International Journal of Tropical Medicine* 8(4), 87-91.

Ebuako, A., Owusu-Asenso, C., Abdulai, A., Sabtiu, A., Sraku, I., Akuamoah-Boateng, Y., Appiah-Kwarteng, C., Forson, A., Ayeh-Kumi, P., & Afrane,

Y. (2025). Malaria vector diversity, transmission and insecticide resistance in Island communities along the Volta lake in Southern Ghana. *BMC Infectious Diseases*, 25(1), 904.

Ebhodaghe, F. I., Sanchez-Vargas, I., Isaac, C., Foy, B. D., & Hemming-Schroeder, E. (2024). Sibling species of the major malaria vector *Anopheles gambiae* display divergent preferences for aquatic breeding sites in southern Nigeria. *Malaria Journal*, 23(1), 60.

Erlank, E., Koekemoer, L. L., & Coetzee, M. (2018). The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programmes. Malaria Journal, 17(1), 43.

Ezihe, E.K., Chikezie, F.M., Egbuche, C.M., Nwankwo, E.N., Onyido, A.E., Aribodor, D., Samdi M.L. (2017). Seasonal distribution and micro-climatic factors influencing the abundance of the malaria vectors in south-east Nigeria. *Journal of Mosquito Research* 2017: 7.

Facchinelli, L., Badolo, A., & McCall, P. J. (2023). Biology and behaviour of Aedes aegypti in the human environment: opportunities for vector control of arbovirus transmission. *Viruses*, 15(3), 636.

Fagbohun, I. K., Idowu, E. T., Awolola, T. S., & Otubanjo, O. A. (2020). Seasonal abundance and larval habitats characterization of mosquito species in Lagos State, Nigeria. *Scientific African*, 10, e00656.

Fanello, C., Santolamazza, F., Della Torre, A. (2002). Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCRRFLP. *Medical and Veterinary Entomology* 16(4), 1–4.

Gerken, K. N., Olubowa, R. R., Chiuya, T., Korir, M., Fèvre, E. M., Stringer, A., & Baylis, M. (2025). Seasonal variation in mosquito abundance and environmental predictors in semi-pastoral southern Kenya: implications for endemic Rift Valley fever. *Parasites & Vectors* 19, 1

Gilies, M., Coetzee, M. (1987). A supplement to the *Anophelinae* of Africa south of the Sahara (Afrotropical region) 1987.

Gillet, J.D. (1972). Common Africa Mosquitoes and their Medical Importance. Wilem Heimann Medical Books Ltd, London 1972: 7-33.

Girgis, S.T., Adika, E., Nenyewodey, F.E., Senoo Jnr, D.K., Ngoi, J.M., Bandoh, K., *et al*., (2022). Nanopore sequencing for real-time genomic surveillance of *Plasmodium falciparum*. *bioRxiv*.

Goldstein, C., Boireau, P., Pagès, J.C. (2019). Benefits and limitations of emerging techniques for mosquito vector control. *Comptes Rendus Biologies* 342(7-8), 270-272.

Ibrahim, A.O., Bello, I.S., Shabi, O.M., Omonijo, A.O., Ayodapo, A., Afolabi, B.A. (2022). Malaria infection and its association with socio-demographics, preventive measures, and co-morbid ailments among adult febrile patients in rural Southwestern Nigeria: A cross-sectional study. *SAGE Open Medicine*10, 20503121221117853.

Ikpo, A. U., Nwaorgu, O. C., Irikannu, K. C., Anumba, J. U., Ezemuoka, L. C., & Aniefuna, C. O. (2021). Molecular characterization of Anopheles gambiae sl from three senatorial districts in Enugu State, Southeastern Nigeria. *Journal of Entomology and Zoology Studies*, 9(4), 106-110.

Kaindoa, E. W., Finda, M., Kiplagat, J., Mkandawile, G., Nyoni, A., Coetzee, M., & Okumu, F. O. (2018). Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. *Malaria journal*, 17(1), 298.

Kamdem C., Fossog B.T., Simard F., Etouna J., Ndo C., Kengne P., *et al*., (2012). Anthropogenic habitat disturbance and ecological divergence between incipientspecies of the malaria mosquito *Anopheles gambiae.* PLoS ONE. 7:e39453. doi: 10.1371/journal.pone.0039453.

Kouassi, B.L., Edi, C., Ouattara, A.F., Ekra, A.K., Bellai, L.G., Gouaméné, J., *et al.,* (2023). Entomological monitoring data driving decision-making for appropriate and sustainable malaria vector control in Côte d'Ivoire. *Malarial Journal* 22(1), 14.

Lamidi, B., Naphtali, R., Alo, E., & Oyeniyi, A. (2018). Malaria vector population density and man-biting rate in three selected areas of Taraba State, north-east Nigeria. *Nigerian Journal of Parasitology,* 39(2), 141–147..

Liu, K., Li, P., Fang, L. Q., & Liu, W. (2026). Geospatial epidemiological approaches to the prevention and control of vector-borne diseases: A systematic review. *Infectious Diseases & Immunity*, 6(1), 37-47.

Lubanga, A. F., Bwanali, A. N., Moyo, C., Chumbi, G. D., Matola, Y. M., Nam, H., Kim, L., Park, J., & Chung, J. (2024). Beyond RTS,S malaria vaccine piloting to adoption and historic introduction in sub-Saharan Africa: A new hope in the fight against the vector-borne disease. *Frontiers in Tropical Diseases*, 5.

Machani, M., Ochomo, E., Amimo, F., Kosgei, J., Munga, S., Zhou, G., Githeko, A., Yan, G., & Afrane, Y. (2020). Resting behaviour of malaria vectors in highland and lowland sites of western Kenya: Implication on malaria vector control measures. *PLoS ONE 15(2) e0224718*

Markwalter, C., Lapp, Z., Abel, L., Kimachas, E., Omollo, E., Freedman, E., Chepkwony, T., Amunga, M., McCormick, T., Bérubé, S., Mangeni, J., Wesolowski, A., Obala, A., Taylor, S., & O'Meara, W. (2024). *Plasmodium falciparum* infection in humans and mosquitoes influence natural Anopheline biting behavior and transmission. Nature communications, 15(1), 4626.

Mategula, D., & Gichuki, J. (2023). Understanding the fine-scale heterogeneity and spatial drivers of malaria transmission in Kenya using model-based geostatistical methods. *PLOS Global Public Health,* 3(12), e0002260.

Mmbando, A., Kaindoa, E., Ngowo, H., Swai, J., Matowo, N., Kilalangongono, M., Lingamba, G., Mgando, J.,

Namango, I., Okumu, F., & Nelli, L. (2021). Fine-scale distribution of malaria mosquitoes biting or resting outside human dwellings in three low-altitude Tanzanian villages. *PLoS One, 16(1*), e0245750.

Musiba, R., Tarimo, B., Monroe, A., Msaky, D., Ngowo, H., Mihayo, K., Limwagu, A., Chilla, G., Shubis, G., Ibrahim, A., Greer, G., Mcha, J., Haji, K., Abbas, F., Ali, A., Okumu, F., & Kiware, S. (2022). Outdoor biting and pyrethroid resistance as potential drivers of persistent malaria transmission in Zanzibar. *Malaria Journal, 21*

Nabet, C., Kone, A. K., Dia, A. K., Sylla, M., Gautier, M., Yattara, M., ... & Piarroux, R. (2021). New assessment of *Anopheles* vector species identification using MALDI-TOF MS. *Malaria Journal*, 20(1), 33.

Norris, L.C., Main, B.J., Lee, Y., Collier, T.C., Fofana, A., Cornel, A.J., *et al*.,(2015). Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. Proceedings of the National Academy of Sciences of the United States of America 112(3), 815–820.

Noutcha, M.A.E., Anumudu, C.I. (2009). Entomological indices of *Anopheles gambiae sensu lato* at a rural community in south-west Nigeria. *Journal of Vector Borne Diseases.* 46, 43-51.

Noutcha, M.A.E., Anumudu, C.I. (2011). *Anopheles gambiae* complex: Molecular forms and occurrence of kdr gene in rural southwestern Nigeria. *Nigerian Journal of Entomology* 28: 7-16.

Nwuba, R.I., Omosun, Y.O., Anumudu, C.I., Adoro, S., Odaibo, A.B., Nwagwu, M. (2008). Epidemiology of malaria in children living at Igbo-Ora, Southwestern Nigeria. *Ife Journal. Science* 10(1), 53-58.

Nzioki, I., Machani, M. G., Onyango, S. A., Kabui, K. K., Githeko, A. K., Ochomo, E., ... & Afrane, Y. A. (2023). Differences in malaria vector biting behavior and changing vulnerability to malaria transmission in contrasting ecosystems of western Kenya. *Parasites & vectors*, 16(1), 376.

Oduola, A.O., Obembe, A., Lateef, S.A., Abdulbaki, M.K., Kehinde, E.A., Adelaja, O.J., et al., (2021). Species Composition and Plasmodium falciparum Infection Rates of Anopheles gambiae s.l. Mosquitoes in Six Localities of Kwara State, North Central, Nigeria. *Journal of Applied Sciences and Environmental Management* 25(10), 1801-1806.

Oduola, A.O., Olojede, J.B., Oyewole, I.O., Otubanjo, O.A., Awolola, T.S. (2013). Abundance and diversity of *Anopheles* species (Diptera: Culicidae) associated with malaria transmission in human dwellings in rural and urban communities in Oyo State, Southwestern Nigeria. *Parasitology Research 112*, 3433-3439.

Okiwelu & Noutcha (2015). Challenges and "New Frontiers" in Entomological Training and Research in Nigeria. *Nigerian Journal of Entomology* 31: 63-72.

Okorie, P.N., Ademowo, O.G., Irving, H., Kelly-Hope, L.A., Wondji, C.S. (2015). Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* mosquitoes in Ibadan, Southwest Nigeria.

Medical and Veterinary Entomology 29(1), 44-50.

Okwa, O.O., Akinmolayan, F.I., Carter, V., Hurd, H. (2009). Transmission dynamics of malaria in Nigeria. *Annals of African Medicine* 8(1).

Olatunbosun-Oduola, A., Abba, E., Adelaja, O., Taiwo-Ande, A., Poloma-Yoriyo, K., & Samson-Awolola, T. (2019). Widespread report of multiple insecticide resistance in *Anopheles gambiae sl* mosquitoes in eight communities in southern Gombe, North-Eastern Nigeria. *Journal of Arthropod-Borne Diseases*, 13(1), 50.

Omojuyigbe, J. O., Owolade, A. J. J., Sokunbi, T. O., Bakenne, H. A., Ogungbe, B. A., Oladipo, H. J., & Agughalam, P. I. (2023). Malaria eradication in Nigeria: state of the nation and priorities for action. *Journal of Medicine, Surgery, and Public Health*, 1, 100024.

Onyabe, D.Y., Conn, J.E. (2001). The distribution of two major malaria vectors, *Anopheles gambiae* and *Anopheles arabiensis*, in Nigeria. *Memórias do Instituto Oswaldo Cruz* 96, 1081-1084.

Onyabe, D.Y., Vajime, C.G., Nock, I.H., Ndams, I.S., Akpa, A.U., Alaribe, A.A., *et al*., (2003). The distribution of M and S molecular forms of *Anopheles gambiae* in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 97(5), 605-608.

Opondo, K., Weetman, D., Jawara, M., Diatta, M., Fofana, A., Crombe, F., Mwesigwa, J., d'Alessandro, U., & Donnelly, M. (2016). Does insecticide resistance contribute to heterogeneities in malaria transmission in The Gambia?. *Malaria Journal, 15*

Ototo, E., Mbugi, J., Wanjala, C., Zhou, G., Githeko, A., & Yan, G. (2015). Surveillance of malaria vector population density and biting behaviour in western Kenya. *Malaria Journal, 14:244*

Oyewole, T. A., Mohammed, N. O., Osarenren, B. O., Tijani, M. K., Persson, K. E., & Falade, M. O. (2024). *Plasmodium falciparum* transmission based on merozoite surface protein 1 (msp1) and 2 (msp2) gene diversity and antibody responses in Ibadan, Nigeria. *Parasite Epidemiology and Control*, *26*, e00366.

Oyibo, W., Latham, V., Oladipo, O., Ntadom, G., Uhomoibhi, P., Ogbulafor, N., & Conway, D. J. (2023). Malaria parasite density and detailed qualitative microscopy enhances large-scale profiling of infection endemicity in Nigeria. *Scientific reports, 13*(1), 1599.

Padonou, G. G., Konkon, A. K., Salako, A. S., Zoungbédji, D. M., Osse, R., Sovi, A., & Akogbeto, M. C. (2023). Distribution and Abundance of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Benin, West Africa. *Tropical Medicine and Infectious Disease*, 8(9), 439.

Russell, T.L., Staunton, K., Burkot, T. (2022). Standard Operating Procedure for collecting resting mosquitoes with pyrethrum spray catch. https://protocols.io/view/standard-operating-procedure-for-collecting-restin-b9mur46w

Sanou, A., Nelli, L., Guelbéogo, W., Cissé,

F., Tapsoba, M., Ouédraogo, P., Sagnon, N., Ranson, H., Matthiopoulos, J., & Ferguson, H. (2021). Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors in the Cascades region of Burkina Faso. *Scientific Reports*, 11

Sarangi, S., Bhavana, P., & Padhan, E. K. (2025). Insecticide resistance and management strategies. *Advances in Applied Entomology*; Dash, SS, Pradhan, PP, Sahoo, S., Nag, YK, Sorahia, D., Eds, 184-208

Sattler M.A., Mtasiwa D., Kiama M., Premji Z., Tanner M., Killeen G.F., *et al.*, (2005). Habitat characterisation and spatial distribution of *Anopheles sp*. Mosquito larvae in Dares Salaam (Tanzania) during an extended dry period. *Malarial Journal* 4. doi:10.1186/1475-2875-4-4.

Scott, J.A., Brongdon, W.G., Collins, F.H. (1993). Identification of single specimen of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* 49, 520–9.

Service, M.W. (1970). Identification of the *Anopheles gambiae* complex in Nigeria by larval and adult chromosomes. *Annals of Tropical Medicine and Parasitology* 64, 131-136.

Shi, J., Wang, M., Kakunze, A., Margai, H. C., Hu, H., Feng, Y., ... & Ren, M. (2025). Global Assistance and the Cascade of Malaria Prevention and Control—Sub-Saharan Africa, 2011–2022. China CDC Weekly, 7(18), 620.

Shi, D., Wei, L., Liang, H., Yan, D., Zhang, J., & Wang, Z. (2023). Trends of the global, regional and national incidence, mortality, and disability-adjusted life years of malaria, 1990–2019: An analysis of the Global Burden of Disease Study 2019. *Risk Management and Healthcare Policy*, 16, 1187–1201.

Shililu, J., Maier, W., Seitz, H., Orago, A.(1998). Seasonal density sporozoite rates and entomological inoculation rates of *Anopheles gambiae* and *Anopheles funestus* in the high altitude sugarcane growing zone in western Kenya. *Tropical Medicine and International Health* 3(9), 706–10.

Simon-Oke, A.I., Awosolu, O.B., Odeyemi, O. (2023). Prevalence of Malaria and COVID-19 Infection in Akure North Local Government Area of Ondo State, Nigeria. *Journal of Parasitology Research* 2023: 9529563.

Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., ... & Hay, S. I. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites & vectors*, 3(1), 117.

Soma, D., Zogo, B., Somé, A., Tchiekoi, B., De Sales Hien, D., Pooda, H., Coulibaly, S., Gnambani, J., Ouari, A., Mouline, K., Dahounto, A., Ouédraogo, G., Fournet, F., Koffi, A., Pennetier, C., Moiroux, N., & Dabiré, R. (2020). *Anopheles* bionomics, insecticide resistance and malaria transmission in southwest Burkina Faso: A pre-intervention study. PLoS ONE, 15. https://doi.org/10.1371/journal.pone.0236920.

Sougoufara, S., Ottih, E. C., & Tripet, F. (2020). The need for new vector control approaches targeting outdoor biting Anopheline malaria vector communities. *Parasites & Vectors*, 13(1), 295.

Tarekegn, M., Dugassa, S., Negash, Y., Tekie, H., & Woldehawariat, Y. (2024). A survey of malaria vectors feeding preference, biting site and resting behaviour in the malaria elimination settings of Dembiya District, north-western Ethiopia. *Malaria Journal*, 23(1), 352..

Taconet, P., Soma, D., Zogo, B., Mouline, K., Simard, F., Koffi, A., Dabiré, R., Pennetier, C., & Moiroux, N. (2024). Physiological and behavioural resistance of malaria vectors in rural West Africa: a data mining study to address their fine-scale spatiotemporal heterogeneity, drivers, and predictability. bioRxiv.intervention study. *PLoS ONE, 15*

Takken, W., Charlwood, D., & Lindsay, S. W. (2024). The behaviour of adult *Anopheles gambiae,* sub-Saharan Africa's principal malaria vector, and its relevance to malaria control: a review. *Malaria Journal*, 23(1), 161.

Tene Fossog, B., Ayala, D., Acevedo, P., Kengne, P., Ngomo, A., Mebuy, I., Makanga, B., *et al*., (2014). Habitat segregation and ecological character displacement in cryptic African malaria mosquitoes. *Evolutionary Applications* 8(4), 326–345.

World Health Organisation (1975b). Manual of Practical Entomology in Malaria. Part II. Methods and Techniques. WHO Offset Publication No. 13.

World Health Organisation (2019). High burden to high impact: a targeted malaria response (No. WHO/CDS/GMP/2019.25 Rev. 1). World Health Organisation.

World Health Organisation (2020). World malaria report 2020: 20 years of global progress and challenge Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO

World Health Organisation (2024). World Malaria Report 2024. https://www.who.int/news-room/fact-sheets/detail/malaria

World Health Organization. (2022a). Tool to assess the impact of human resources for health investments on HIV, TB and malaria services and health outcomes.

World Health Organization. (2022b). WHO Guidelines for malaria, 31 March 2022 (No. WHO/UCN/GMP/2022.01 Rev. 1). World Health Organization.

Woyessa, D., & Yewhalaw, D. (2025). Anopheles mosquito fauna, blood meal sources and transmission intensity from high and moderate malaria endemic areas of Ethiopia. *Scientific Reports,* 15(1), 10636.

Wu, S. L., Henry, J. M., Citron, D. T., Mbabazi Ssebuliba, D., Nakakawa Nsumba, J., Sánchez C, H. M., & Smith, D. L. (2023). Spatial dynamics of malaria transmission. *PLOS Computational Biology,* 19(6), e1010684.

Yusuf, R.(2021). Socio-Economic Status and Prevalence of *P. falciparum* Infection in Symptomatic and Asymptomatic

Individuals in Parts of Kaduna Metropolis, Kaduna State, Nigeria. *Nigerian Journal of Microbiology* 35(2), 5693-5704.

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