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ORIGINAL RESEARCH ARTICLE

Molecular identification of sibling species in the *Anopheles gambiae* complex using PCR in Bauchi State, Nigeria

Aliyu Abdulhamid Omar[1], Auwal Alhassan Barde[2], Usman Alhaji Mohammed[3] and Umar Aliyu[4]
[1]Department of Science Laboratory Technology, Sa'adu Zungur University, Bauchi State, Nigeria
[2]Department of Biological Sciences Abubakar Tafawa Balewa University Bauchi, Nigeria
[3]Department of Environmental Health, Federal University of Health Sciences, Azare, Bauchi State, Nigeria
[4]Department of Biological Sciences, Sa'adu Zungur University, Bauchi State, Nigeria.

ABSTRACT

Malaria vector control programs in Bauchi State, Nigeria, rely on accurate identification of mosquito species. This study aimed to determine the composition of the *Anopheles gambiae* sibling species complex in the region. Using Pyrethroid spray collection, 6935 adult mosquitoes were collected, and 1000 female *Anopheles gambiae* complex mosquitoes were analyzed using Polymerase Chain Reaction (PCR)-based molecular identification. Results showed that *Anopheles gambiae coluzzii* and *Anopheles gambiae arabiensis* are the predominant malaria vectors in the study area, comprising 65% and 30% of the analyzed samples, respectively, while other species made up 5%. These findings are consistent with studies from similar ecological zones in northern Bauchi State and other parts of Nigeria. Notably, our results differ from those reported in the Sudan Savannah region, where *Anopheles gambiae s.s.* is more prevalent, highlighting the importance of molecular identification in understanding the complex dynamics of malaria transmission in the region.

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INTRODUCTION

Malaria is a major public health problem in Nigeria, accounting for 60% of outpatient attendance in health facilities and 30% of all hospital admissions (Yahuza et al., 2024a; Yahuza et al., 2024b). The disease is responsible for an estimated 249 million cases in 2022 alone, with around 233 million (around 94%) in the WHO African Region, and Nigeria alone accounting for about 63 million cases, clearly demonstrating significant economic implications (Venkatesan, 2024). Also in 2022, malaria was responsible for approximately 608,000 deaths worldwide, equating to a mortality rate of 14.3 deaths for every 100,000 people at risk. Over half of these fatalities were concentrated in only four nations: Nigeria is the top, accounting for 31% (188,480) of global deaths, followed by the Democratic Republic of the Congo (12%), Niger (6%), and Tanzania (4%) (Venkatesan, 2024).

The spread of Plasmodium parasites—the causative agents of malaria—occurs primarily through the bite of a female Anopheles mosquito that has previously fed on an infected host (Saab et al., 2025; Guttery et al., 2022). Geographically, malaria remains endemic across broad swaths of Africa, Latin America, and parts of the Caribbean, as well as throughout much of Asia, including South Asia, Southeast Asia, and the Middle East (Ridpath & Wallender, 2025). Beyond mosquito-borne transmission, the disease can also be acquired through non-vector routes, albeit less commonly. These include blood transfusions (Foko et al., 2025; Owusu-Ofori et al., 2013), the sharing of contaminated needles (Alavi et al., 2010), organ transplantation (Velasco et al., 2017), or mother-to-child transmission during pregnancy or childbirth (Poespoprodjo et al., 2011; Menendez & Mayor, 2007). Furthermore, there have been documented instances of malaria spreading via contaminated medical equipment or shared medication vials, highlighting the importance of strict infection control practices in healthcare settings (Ridpath & Wallender, 2025).

In Nigeria, malaria is a major cause of infant mortality and is the only insect-borne parasite disease comparable in impact to the World's major killer transmissible diseases: diarrhea, acute respiratory infections, tuberculosis, and AIDS (Dasgupta et al., 2022). The disease causes great misery to sufferers and adversely affects the social and psychological well-being of individuals, families, and the nation at large (Mezieobi et al., 2025). Despite efforts to

Correspondence: Aliyu Abdulhamid Omar. Department of Science Laboratory Technology, Sa'adu Zungur University, Bauchi State, Nigeria. ✉ aliyuaomar@sazu.edu.ng.
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control malaria, the disease burden is still on the rise, and some estimate that the number of cases will continue to increase in the coming years without the development of new control methods (Sands, 2025; Li et al., 2025).

Given the persistently high malaria burden in Bauchi State and the year-round transmission patterns documented over several decades (Haruna et al., 2024; Kurmi et al., 2024; Abubakar et al., 2024; Magaji & Mahmud, 2025; Yahaya et al., 2023), effective vector control requires more than a generalized approach. The Anopheles gambiae complex, which comprises morphologically indistinguishable sibling species with distinct ecological behaviors, feeding preferences, and roles in malaria transmission (Zianni et al., 2013; Bass et al., 2007; Walsh, 2023; Pombi et al., 2017), presents a unique challenge. For instance, *Anopheles gambiae sensu stricto* is typically highly endophilic and anthropophilic (Takken & Knols, 1999), whereas *Anopheles gambiae arabiensis* exhibits greater behavioral plasticity, including a tendency to feed outdoors and on animals (Lyimo et al., 2012), potentially allowing it to evade indoor-based interventions such as insecticide-treated nets and indoor residual spraying (Wanjala et al., 2015). Failure to differentiate these cryptic species can lead to misinterpretation of transmission dynamics and ineffective deployment of control resources. Therefore, this study aimed to apply Polymerase Chain Reaction (PCR)-based molecular identification to determine the composition and relative abundance of sibling species within the Anopheles gambiae complex in Bauchi State, Nigeria. Accurate species-level identification is essential for understanding local transmission ecology, predicting the impact of existing control measures, and guiding the development of targeted, evidence-based strategies to reduce malaria burden in this high-transmission region.

METHODOLOGY

2.1 Study area

Bauchi state

Bauchi State occupies a total land area of 49,119 km^2 (18,965 sq mi), representing about 5.3% of Nigeria's total land mass, and is located between latitudes 9° 3' and 12° 3' north and longitudes 8° 50' and 11° east (BSG, 2025). The state is bordered by seven states: Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east, and Kaduna to the west. Bauchi State is one of the states in the northern part of Nigeria that spans two distinctive vegetation zones, namely, the Sudan savannah and the Sahel savannah (BSG, 2025).

Sudan savannah of the state

The Sudan savanna vegetation type covers the southern part of the state. Here, the vegetation gets richer and richer towards the south, especially along water sources or rivers, but generally the vegetation is less uniform and grasses are shorter than what grows even further south, that is, in the forest zone of the middle belt, this part of the state, is mountainous as a result of the continuation of the Jos Plateau (Ariko et al., 2024; BSG 2025). The vegetation types here are conditioned by the climatic factors, which in turn determine the amount of rainfall received in the area (Tama et al., 2025). For instance, rainfall ranges from 1,300 millimeters (51 in) per annum in this part of the state. Consequently, rains start earlier in this part of the state and become heaviest and last longer; they start in April, with the highest recorded amount of 1,300 millimeters (51 in) per annum. This pattern is because in the West Africa sub-region, rains generally come from the south as the southwestern wind carries them (Maidabino et al., 2025; BSG, 2025).

Sahel savannah of the state

The Sahel type of savannah, also known as semi-desert and generally sandy, vegetation, becomes manifest from the beginning of this part of the state as one moves from the state's south to its north. This type of vegetation comprises isolated stands of thorny shrubs. There is therefore a progressive dryness towards the north, culminating in the desert condition in the far north (extreme sahel savannah) and rainfall here is only 700 millimetres (28 in) per annum (BSG, 2025). In contrast to the Sudan savannah, the Sahel savannah part of the state receives rainfall late, usually around June or July, and records the highest rainfall of 700 millimeters (28 in) per annum. (BSG, 2025).

General overview

The weather in the south and the north is nearly the same, and it is humidly hot during the early part of the rainy season in the Sudan; the hot, dry, and dusty weather lingers into the Sahel (Ariko et al., 2024). In addition to rainfall, Bauchi State is watered by a number of rivers. They include the Gongola and Jama'are rivers (Abubakar Sadiq et al., 2014). The Gongola River crosses Bauchi State in Tafawa Balewa Local Government Area in the south and in Kirfi and Alkaleri Local Government Areas in the eastern part of the state, while the Jama'are River cuts across a number of Local Government Areas in the northern part of the state (Abubakar Sadiq et al., 2014). Moreover, a substantial part of the Hadeja-Jama'are River basin lies in Bauchi State (Kimmage & Adams, 1992), which, along with various fadama (floodplain) areas in the state, provides suitable land for agricultural activities (Abubakar Sadiq et al., 2014). These are further supported by a number of dams meant for irrigation and other purposes. These include the Gubi Dam (Sallau et al., 2022) and Tilde-Fulani dams. There are also lakes, such as Maladumba Lake in Misau Local Government Area (Godwin et al., 2024; Auwal et al., 2020), that provide the necessary conditions for agriculture.

Local Government Areas and Communities Selected/Visited During the Research

Nine Local Government Areas (three communities from each Local Government) were selected from Bauchi State as the study areas (Figure 1). The selection of the Local Governments and the communities was based on the previous record of malaria cases in the state. Local Governments with higher reported malaria cases were selected, as listed in the Table 1.

Table 1: Selected Local Government Areas and Communities During the Research

Sudan savannah	Sahel savannah
Bauchi LGA	**Katagum LGA**
L/Katagum, Yalwa, Tirwun	Azare. Chinade, Madara
Dass LGA	**Gamawa LGA**
Dass, Shalgwantar,Wandi	Gamawa, Udubo, Raga
Kirfi LGA	**Jama'are LGA**
Kirfi, Bara, Guyaba	Jama are, D/Jeji, Galdimari
Ganjuwa LGA	**Misau LGA**
K/Madaki, Miya, Soro	Misau, Hardawa, Zadawa
Dambam LGA	**Dambam LGA**
Jalam	Dambam, Dagauda,

Fig 1: LGAs of sampling

2.2 Adult Mosquito collection and storage

Adult mosquitoes from the study area were collected throughout the study period (June 2019 – Dec, 2021) using the pyrethrum spray catch/collection (PSC) method as described by Service (1993). Selected rooms for mosquito collection were those in which at least one person had slept the night prior to collection. Before spraying, all occupants and easily removable objects were removed from the rooms, and the doors and windows were closed. The floor of each room was completely covered with a clean, white bedsheet. A pyrethrum-based insecticide (0.1–0.2% pyrethrum in kerosene) was then sprayed throughout the room, with particular attention paid to potential escape routes, such as doors, windows, and eaves. After spraying, the room was left closed for approximately 10–15 minutes. The spray sheet was then carefully folded from the sides toward the center of the room, removed from the room, and taken outside. Mosquitoes that had fallen onto the sheet were collected using forceps and placed into petri dishes. The collected mosquito samples were transported to the research laboratory in the Department of Biological Sciences at Abubakar Tafawa Balewa University, Bauchi, for further processing. For molecular analysis, specimens were stored dry using silica gel.

2.3 Morphological identification of *Anopheles gambiae s.l.* mosquitoes

The collected *Anopheles* mosquitoes were identified to species level as members of the *Anopheles gambiae* species complex using the morphological identification key of Gillies & Coetzee (1987). The morphological characters examined included: presence or absence of lateral abdominal tufts of hair, completely colored tarsal segments, speckled legs, three-banded palps, pale interruptions on the third main dark area of the first wing vein, and palps as long as the proboscis.

2.4 Molecular identification

DNA extraction

Genomic DNA was extracted from individual mosquitoes using a modified version of the method described by Collins et al. (1987). Briefly, each mosquito was homogenized in 100 µl of grinding buffer (0.1 M NaCl, 0.1 M Tris-HCl, 0.05 M EDTA, 0.5% SDS) and incubated at 65°C for 30 minutes. The homogenate was then centrifuged at 12,000 rpm for 5 minutes, and the supernatant containing the DNA was transferred to a clean microcentrifuge tube. To prevent cross-contamination, forceps were wiped clean between handling different specimens.

PCR amplification

The extracted DNA was used as a template for PCR amplification using species-specific primers as described by Scott et al. (1993). The PCR reaction mixture (25 µl total volume) consisted of 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 unit of *Taq* DNA polymerase, and 2 µl of template DNA. The PCR cycling conditions were: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. All PCR reactions were performed using a thermal cycler (Bio-Rad Laboratories, USA).

Agarose gel electrophoresis

PCR products were analyzed by electrophoresis on a 2% agarose gel prepared in 1× TBE buffer (Tris-borate-EDTA), as described by Sambrook & Russell (2001). Briefly, 0.50 g of agarose was dissolved in 50 ml of 1× TBE buffer by heating in a microwave until completely dissolved. The solution was cooled to approximately 50°C, and 10 µl of ethidium bromide (10 mg/ml) was added. The gel was poured into a gel mould with a comb placed approximately 1.5 cm from the end to create wells, and allowed to polymerize for 25 minutes.

After polymerization, the comb was carefully removed, and the gel was placed in an electrophoresis tank containing 1× TBE buffer, with the wells closest to the negative electrode. For each sample, 5 µl of PCR product was mixed with 2 µl of loading dye and loaded into individual wells. A 100 bp DNA ladder (5 µl) was loaded into the first well as a size reference. Electrophoresis was performed at 120 V for 35 minutes.

Visualization and documentation

Following electrophoresis, the gel was carefully removed and placed on a UV transilluminator. DNA bands were visualized by ethidium bromide staining under ultraviolet light. The gel was photographed using a gel documentation system (Helcam Polaroid Camera). DNA amplification success was indicated by the presence of fluorescent bands at the expected sizes. All gel images were labeled, and the gel was subsequently disposed of in a sealed polythene bag as biohazard waste.

Species identification

Species identification was performed using species-specific PCR assays, where diagnostic fragment sizes were used to distinguish members of the *Anopheles gambiae* complex, following Scott et al. (1993) and Santolamazza et al. (2008). *Anopheles arabiensis* was identified by a 315 bp fragment, while *Anopheles coluzzii* was identified by a 479 bp fragment.

RESULTS AND DISCUSSION

Table 2: Number of Adult Mosquitoes and Indoor Resting Densities across the LGAs in the study area.

LGAs	No. of rooms visited	No. of Adult Mosquitoes Collected
Bauchi	30	1200
Dass	30	865
Kirfi	30	601
Misau	30	701
Ganjuwa	30	648
Dambam	30	506
Katagum	30	742
Gamawa	30	911
Jamaare	30	761
Total	**270**	**6935**

A total of 6935 adult mosquitoes were collected from the study areas (Table 2). The results showed that houses with open eaves and no windows had a higher risk of indoor resting mosquitoes (Spitzen et al., 2025). The indoor occurrence of mosquitoes could be attributed to factors such as indoor microclimate, cooking, sleeping, and tethering livestock inside residential houses, which increase indoor temperature and provide access to blood meal sources (Paaijmans & Thomas, 2011; Service, 1963; Ekoko et al., 2019). Table 3 shows the number of adult mosquitoes collected and the composition of anopheles gambiae mosquitoes according to lgas of the study area.

The collected mosquitoes exhibited endophilic behavior, with *Anopheles gambiae colluzii* and *Anopheles gambiae arabiensis* being the predominant malaria vectors. Indoor-resting mosquitoes are estimated to transmit malaria earlier than those resting outdoors. The abundance of indoor-resting mosquitoes increased rapidly at the onset of rainfall, consistent with the observations of Oduola et al. (2021).

Bauchi State and Nigeria at large have a high burden of malaria (Adejoro, 2025; Magaji et al., 2025). Several mosquito collection techniques are routinely used in the malaria parasite vector control and related programs (Mashatola et al., 2025; Kosgei et al., 2024). Taxonomy that utilizes morphological features has long been the standard method for identifying various mosquito species. However, this morphological identification is challenging when there are limited experts and/or when external characters are damaged due to improper specimen handling (Lobo et al., 2015).

Table 3: Number of Adult Mosquitoes Collected and the Composition of *Anopheles gambiae* mosquitoes according to LGAs of the study area.

LGAs	Adult mosquitoes collected	Number of *Anopheles gambiae*	Number of blood-fed female *An gambiae*	Number of *An gambiae coluzzii*	Number of *An gambiae arabiensis*
Bauchi	1200	401	140	52	80
Dass	865	267	120	21	92
Kirfi	601	197	50	22	28
Misau	701	331	100	65	35
Ganjuwa	648	298	100	60	33
Dambam	506	303	180	121	59
Katagum	742	399	140	29	110
Gamawa	911	478	80	20	60
Jamaare	761	167	90	33	57
TOTAL	**6935**	**2841**	**1000**	**423**	**554**

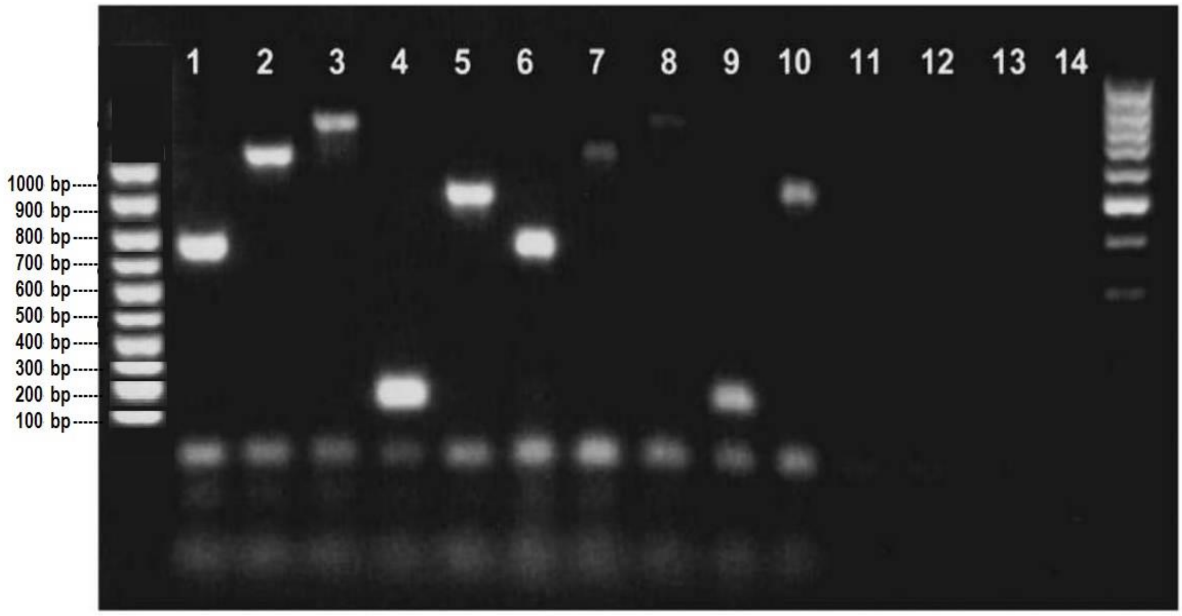

Figure 2: Agarose gel electrophoresis of PCR products for molecular identification of members of the *Anopheles gambiae* species complex.

Table 4: Number of Adult Mosquitoes Collected and the Composition of *Anopheles gambiae* mosquitoes in the different ecological zones of the study area.

Ecological zone	Adult mosquitoes collected	Number of *Anoheles gambiae*	Number of blood fed female *An gambiae*	Number of *An gambiae coluzzii*	Number of *An gambiae arabiensis*
Sudan savannah	4015	1494	510	220	268
Sahel savannah	2920	1347	490	203	286

In this study, a total of 6935 adult mosquitoes were collected using pyrethroid spray collection as adapted from Service (1993), similar to the work of Barde et al. (2019) in the Katagum area of Bauchi State, which also identified Anopheles gambiae s.s. and Anopheles arabiensis as the predominant sibling species. The results of White and Rosen's (1973) work in Kaduna State, Nigeria, also show a similar species composition, although they reported a higher prevalence of Anopheles gambiae s.s. (86-91%) compared to the present study, likely reflecting differences in ecological zones or changes in species distribution over time.

Polymerase chain reaction for the identification of the sibling species composition of *Anopheles gambiae* s.l. was conducted on the 1000 sampled female *Anopheles gambiae* complex mosquitoes from the study areas (Figure 2). Results obtained show that *Anopheles gambiae coluzzii* and *Anopheles gambiae arabiensis* are the predominant members of the *Anopheles gambiae* complex and are the major malaria vectors in the study area. This is a similar result to that obtained in research conducted in the Katagum area of Bauchi State, Nigeria, although that study reported *Anopheles gambiae s.s.* and *Anopheles arabiensis* rather than *An. coluzzii* (Barde et al., 2019). This also agrees with the work of Barde et al. (2019) in the north central part of Nigeria, where their results show *An. gambiae s.s.* and *An. arabiensis*, collected indoors, is the major malaria vector in the area. These findings also agree with the results of research conducted by Onyabe and Conn (2001) on the distribution of *Anopheles* species across Nigeria's ecological zones, as well as with the findings of Ibrahim et al. (2025) in rural southwestern Nigeria, which reported *An. gambiae s.s.* (56%), *An. coluzzii* (31.2%), and *An. arabiensis* (10.1%) coexist in the same communities. Findings of Onyabe and Conn (2001) also show that *An. gambiae coluzzii* and *An. arabiensis* coexist over much of their range. Similar research was conducted in southwestern Nigeria by Ibrahim et al. (2025), and results indicated that even in communities near Ibadan, *An. arabiensis* is an important malaria vector alongside *An. coluzzii* and *An. gambiae s.s.* Vector species with a relatively broad host range, such as *Anopheles arabiensis*, are thought to be better able to persist in areas with high insecticide use.

In contrast, *An. coluzzii* were caught in higher abundances in pyrethrum spray catches (PSC) because this method is intended to collect mosquitoes that feed and rest indoors (endophagic and endophilic). However, Onyabe and Conn (2001) reported that the range of An has been extended. *arabiensis* in Nigeria as a malaria vector prevailing in arid zones and also in some forest zones.

Similar studies to this one were conducted in Burkina Faso. Amara et al. (2025) reported that in Bobo-Dioulasso, three species of the *Anopheles gambiae* complex, including *An. arabiensis* (highest), *An. coluzzii*, and *An. gambiae* (lowest), predominates with *Plasmodium falciparum* infections detected year-round. In western Burkina Faso, Kouadio et al. (2025) found that among the *An. gambiae* s.l. complex, *An. coluzzii* was the dominant species, followed by *An. arabiensis*, with seven *Anopheles* species recorded overall and substantial residual malaria transmission persisting despite widespread use of long-lasting insecticidal nets. A nationwide study across Burkina Faso's three climatic zones by Badolo et al. (2025) revealed that *An. coluzzii* predominated in the Sahelian and Sudano-Sahelian zones, while *An. gambiae* s.s. was most frequent in the Soudanian zone, highlighting marked spatial heterogeneity in vector composition. Another similar study was conducted by Moss et al. (2024) on the Bijagós Archipelago, Guinea-Bissau, where results indicated that in addition to *Anopheles gambiae coluzzii* and *Anopheles gambiae arabiensis*, *Anopheles melas* constituted a substantial proportion of the malaria vectors in those areas. In Uganda, Mwesige et al. (2025) investigated sibling species composition in indoor residual spraying (IRS) and non-IRS districts of Lira and Kole, northern Uganda. In the non-IRS district, *An. gambiae* s.s. dominated the vector population, followed by *An. funestus* and *An. arabiensis*. In contrast, in the IRS district, *An. funestus* became the predominant species, followed by *An. gambiae* s.s. and *An. arabiensis*, demonstrating how vector control interventions can alter species composition. In northern Ghana, Zong et al. (2024) reported that *An. gambiae* s.s. was the most abundant species, while *An. arabiensis* was the least observed. Beyond Africa, similar patterns of *Anopheles* species composition and transmission dynamics have been documented in South America, but unlike the *An. gambiae* complex in Africa, where multiple sibling species coexist and hybridize, *An. darlingi* populations in South America show greater genetic differentiation across geographic distances, suggesting that local adaptation plays a more prominent role in vectorial capacity (Moss et al., 2025).

The current findings, like many others (Etang et al., 2016; Sougoufara et al., 2017; Ajayi et al., 2025), show an insignificant difference in the resting behavior as well as the vectorial capacity of *Anopheles gambiae coluzzii* and *Anopheles gambiae arabiensis* in the study areas. These results indicate that most malaria vectors are exophagic and endophilic.

The Sudan Savannah region had higher numbers of adult mosquitoes, *Anopheles gambiae* complex, blood-fed female *Anopheles gambiae*, and *Anopheles gambiae coluzzii* compared to the Sahel Savannah region (Table 4). This finding is consistent with Akpan et al. (2019), who reported a higher abundance of *Anopheles gambiae* in the Sudan Savannah compared to the Sahel Savannah.

Conclusion

In conclusion, this study highlights the importance of molecular identification of malaria vectors in Bauchi State, Nigeria. Our findings reveal that Anopheles gambiae coluzzii and Anopheles gambiae arabiensis are the predominant malaria vectors in the study area, with varying abundance across ecological zones. The indoor-resting patterns of these vectors suggest that housing characteristics may influence their presence indoors. Based on these findings, improving housing structures to prevent mosquito entry could help reduce malaria transmission in the region. These results have implications for targeted vector control strategies and highlight the need for further research on the relationship between housing and malaria transmission

Recommendations

1. **Mosquito-proof houses**: Implement measures to construct mosquito-proof houses, such as installing window screens, door nets, and sealing open eaves, to reduce indoor resting mosquitoes.
2. **Targeted vector control**: Implement strategies such as indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) in areas with high malaria transmission.
3. **Public education**: Educate communities on the importance of mosquito-proofing their homes and using personal protective measures, such as bed nets and repellents.
4. **Surveillance and monitoring**: Establish a surveillance and monitoring system to track mosquito populations and malaria transmission in the region.

5. **Integrated vector management**: Implement an integrated vector management approach that combines multiple control methods, such as environmental management, biological control, and chemical control.

6. **Further research**: Examine the dynamics of malaria transmission in the region and evaluate the effectiveness of control measures.

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