JJBS

Volume 18, Number 1,March 2025
ISSN 1995-6673
Pages 145 – 154
https://doi.org/10.54319/jjbs/180116

Jordan Journal of Biological Sciences

First Report of *Plasmodium* Infectivity and Dynamics of *Anopheles* Mosquito species in Gombe State, Northeastern, Nigeria.

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Received: April 16, 2024; Revised: September 27, 2024; Accepted: October 4, 2024

Abstract

This study was conducted between January and December 2022 in eight communities in Gombe State, North-East Nigeria. The study aimed at assessing the prevalence of *Plasmodium falciparum* sporozoites in *Anopheles gambiae* s.l. and examining the heterogeneity of *Anopheles* mosquito populations. To collect data, Indoor Pyrethrum Spray Catch (PSC) was employed in three randomly selected houses per community for adult mosquito collection. Additionally, a standard dipper was used to collect immature *Anopheles* stages in a stream, puddles, trailer park and soakaway from thirty (30) breeding sites of eight communities, which were then reared to adulthood in the laboratory. Morphological identification was carried out on all collected mosquitoes, and *An. gambiae* s.l. specimens were further validated using standard PCR protocol to distinguish sibling species. Out of the 3, 837 emerged adults, *An. gambiae* s.l. was the predominant species, constituting 58.33% (2, 238 samples), while *An. rufipes* was the least prevalent at 1.36% (52 samples). Although no significant difference in *Anopheles* composition was observed among the eight study communities ($p > 0.05$, $F = 0.0129$), a significant difference was found between the individual *Anopheles* species ($p < 0.05$, $F = 9.10$). The results indicate that, Adult *An. gambiae* s.l. was distributed across all eight communities, with a Shannon Weiner diversity index of 1.896 and Dominance of 0.1649. Notably, *An. rufipes* exhibited the highest Evenness value of 0.8516 among Anopheline species. Molecular identification of 91 *Anopheles gambiae* s.l. sub-samples revealed that 26.37% were *An. gambiae* s.s, 39.56% were *An. coluzzii*, and 3.30% were *An. arabiensis.* An overall prevalence of 11.00% (2 out of 18 blood-fed female *Anopheles gambiae* s.l.) for *P. falciparum* was established, though limited to one study community. Understanding the diversity, distribution, abundance, and infectivity of *Anopheles* mosquitoes is crucial for effective malaria control and elimination efforts in Gombe State and Nigeria as a whole.

Keywords: *Anopheles,* communities, composition, diversity, *P. falciparum,* Gombe State.

1. Introduction

Mosquitoes are dipteran flies, similar in appearance with other flies belonging to the family Culicidae. The most important genera of man biting mosquitoes are *Anopheles, Aedes, Culex, Mansoni, Psorphora, Haemagogus* and *Sabethes* (Medeiros-Sousa *et al.,* 2015). *Anopheles* are the most widely distributed mosquito species in Africa, and they are also found in the temperate, tropical and subtropical world except the polar region and altitude above 2000 meter (Ekedo and Ukpai, 2020). They are highly anthropophilic and sometime zoophilic. The genus *Anopheles* contains over 500 species globally, and over 140 described species of the genus have been reported in Africa, out of which eight (8) species are known to be efficient vectors of malaria (Coetzee, 2020; Escobar *et al.,* 2020). Of these Global *Anopheles* mosquitoes, approximately 60 to70 are vectors of human malaria while 41 are the most dominant malaria vectors (Silva *et al.,* 2014; WHO, 2019). Previous studies have reported thirty (30) *Anopheles* species in Nigeria. However, recent studies from insecticide resistance monitoring and longitudinal surveillance supported by Global Fund and PMI have reported eleven (11) *Anopheles* species across five ecological zones of Nigeria (NMCP, 2020). The Gombe State vector control sentinel site have recorded seven (7) *Anopheles* species (Adeogun *et al.,* 2023) and few of these species have been linked to malaria transmission in Nigeria (Okorie *et al.,* 2011) although, their composition, diversity and vectorial capacity might vary over time.

Anopheles gambiae s.l. complex has nine sibling species that look morphologically similar but genetically and behaviourally may be distinct species that vary in their ability to transmit malaria parasites (Coetzee, 2020). The

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sibling species may also vary in terms of feeding habit, distribution, habitat preference, behavior, biting habits as well as vectorial competence(Weeraratne *et al.,* 2017) which makes species identification and distribution vital towards operational control and elimination of malaria (Umar and Ndams, 2022). In addition, *An. gambiae* is known to exist in two distinct species; *An. coluzzii* and *An. gambiae* s.s. representing a distinct species belonging to *Anopheles gambiae* complex (Coetzee, 2020). However, the sibling species are indistinguishable morphologically and their identification is important for effective control intervention (Wahedi *et al.,* 2021).

The female *Anopheles* mosquito transmits pathogens of the genus *Plasmodium; P. vivax, P. malariae, P. ovale, P. falciparum* and *P. knowlesi* which have been documented to cause malaria in humans. Malaria in particular is one of the most severe public health problems worldwide (WHO, 2020) accounting for the death of over 600 thousand people mostly under five aged children and pregnant women every year (WHO, 2023). Nigeria accounted for 31.3% of the global malaria death with an estimated 97 million cases annually (WHO, 2023).

Plasmodium falciparum is the principal malaria agent followed by *P. malariae* in Nigeria and sub-Saharan African (FMoH, 2015). They account for 98% of all malaria cases including severe malaria and parasitization of the placenta in pregnancy leading to pre-mature birth and abortions (FMoH, 2015). Generally, Gombe State accounted for over 1 million malaria cases in 2021 amounting to 2.0% of all malaria cases in Nigeria (WHO, 2022). Although, evidence of malaria vector resistance has been reported in the Gombe State (Oduola *et al.,* 2019; Ahmed-yusuf *et al.,* 2020), there is not enough data to make evidence-based decisions on malaria vector control. This study aimed at providing information on the identity, diversity and *Plasmodium falciparum* infection of *Anopheles gambiae* s.l. in eight communities in Gombe State. This is required to design and implement appropriate malaria vector control and management program in Gombe State.

2. Materials and Methods

2.1. Study Area

Gombe State is situated in the North-Eastern region of Nigeria, spanning latitudes 9°30'00" to 12°30'00" N and longitudes 8°45'00" to 11°45'00" E of the Greenwich Meridian. Covering an area of 18,768 km², it had a projected population of 3,545,032 as per the 2006 census. The State experiences an annual rainfall of 850mm and a temperature of 30 °C, with Sudan Savannah vegetation. It undergoes two distinct seasons: the dry season (November – May) and the rainy season (June – October), with an average rainfall of 850mm. Approximately, 60% of the population is engaged in agriculture, with others involved in business and civil service.

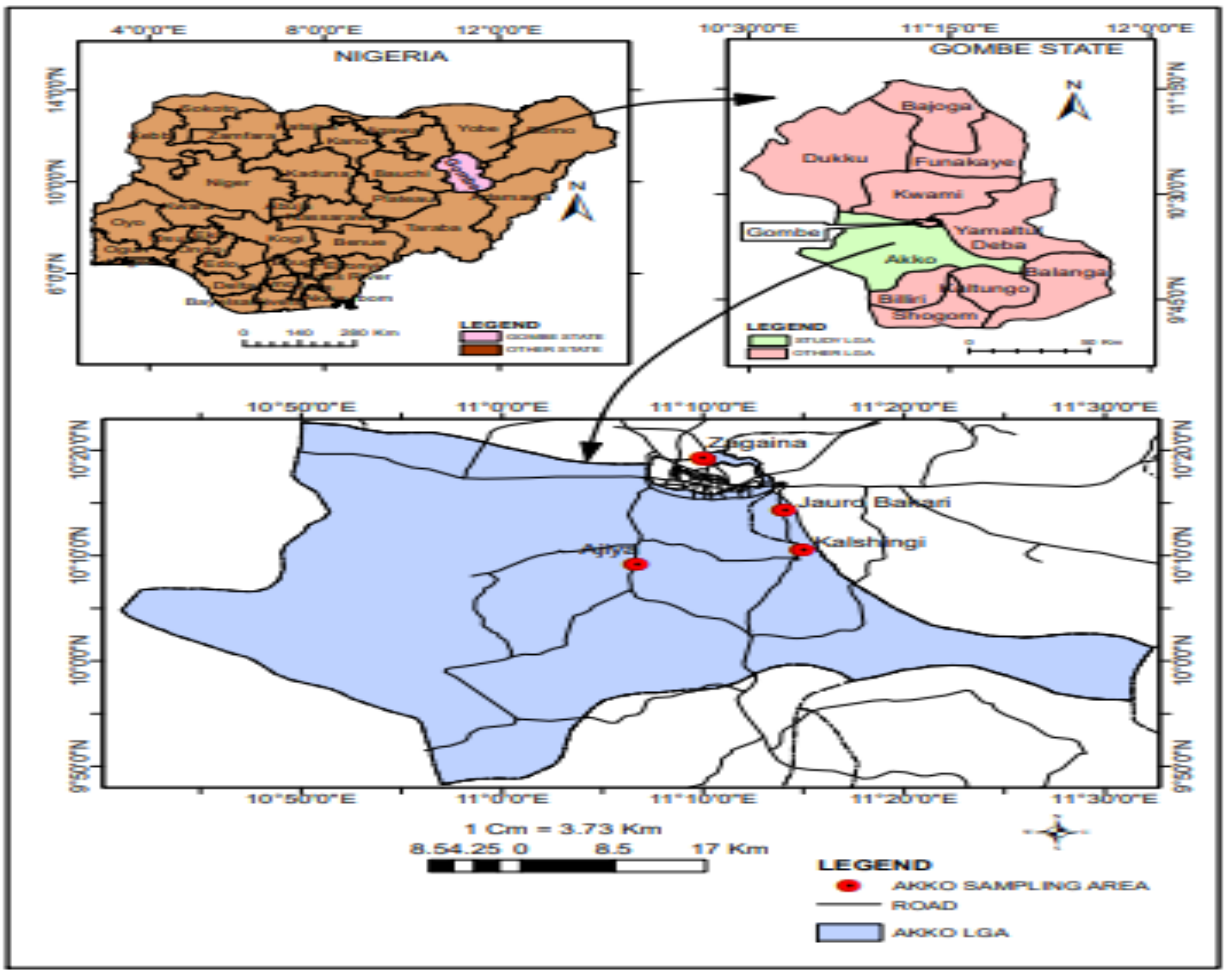

Figure1. Map of Akko LGA sampling communities (Source: ZASTAL Kashere)

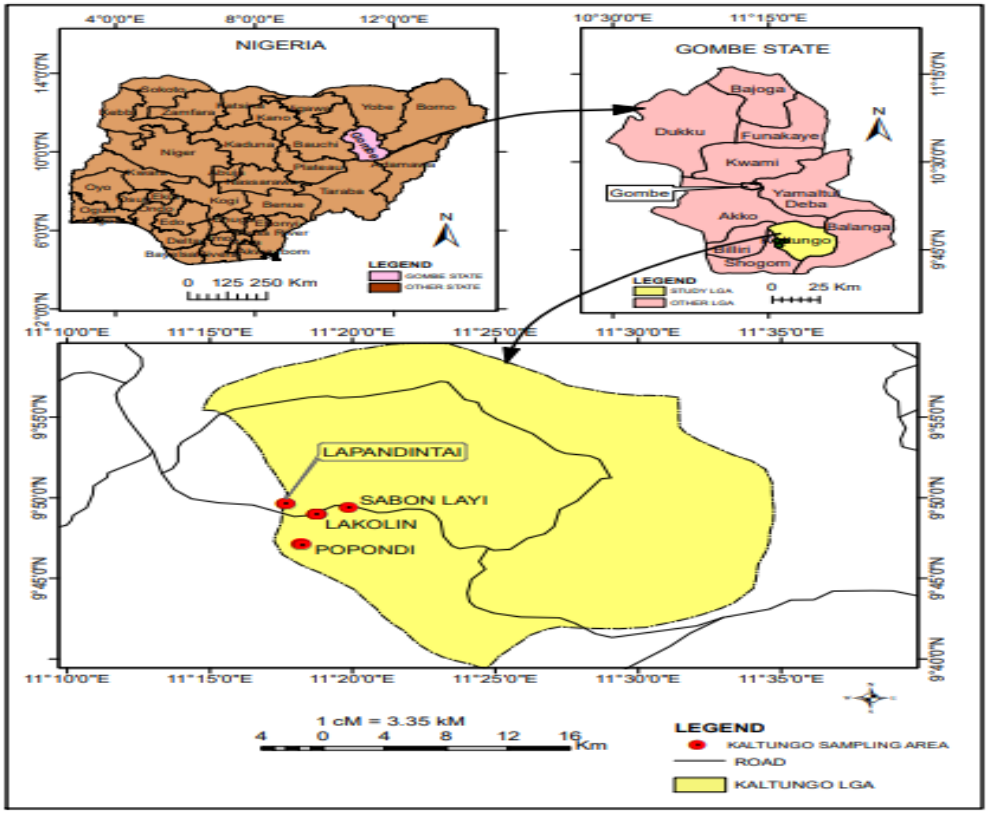

Figure 2. Map of Kaltungo LGA sampling communities (Source: ZASTAL Kashere)

2.2. Ethical Considerations and Consent

No ethical clearance was required for this study. Consent from community leaders was obtained before larval collection through advocacy visits. Additionally, household consent was secured before indoor mosquito collection using pyrethrum spray techniques. All procedures adhered to the 1964 Declarations of Helsinki during the World Medical Assembly (WMA) Finland.

2.3. Study Design

Purposive sampling selected two Local Government Areas (LGA); Akko and Kaltungo based on mosquito breeding habitats, farming activities, LLINs use history, geographical location, zone, and epidemiological importance. Eight communities were randomly chosen, including four semi-urban; Sabon layi, Lapandintai in Kaltungo, Zagaina, Ajiya Quarters in Akko and four rural; Popandi, Lakolin in Kaltungo, Jauro Bakari, Kalshigi in Akko LGA respectively (Fig 1&2).Their coordinates are as follows: Ajiya quarters: 10.152312°N and 11.114179°E; Kalshingi: 10.103902°N and 11.248228°E; Jauro Bakari:10.144859°N and 11.135512°E; Zagaina: 10.191044°N and 11.105690°E in Akko (Figure 1) while Lakolin: 9.831042°N and 11.338485°E; Lapandintai: 9.822407°N and 11.331450°E; Popondi: 9.471161°N and 11.312323°E; SabonLayi: 9.816280°N and 11.311228°E communities in Kaltungo LGA (Figure 2). Immature *Anopheles* mosquitoes were collected from temporary and semi-permanent breeding habitats, and Pyrethrum Spray collection was conducted in two rooms per three houses in each community for adult collection.

2.4. Collection and Rearing of Immature Anopheles Mosquitoes

Larvae and pupae were collected from eight study communities across 200m radius in Akko and Kaltungo LGAs of Gombe, Nigeria, using standard dipping methods. Breeding sites coordinates were recorded using GPS software. Collected larvae were transported to Gombe State malaria control sentinel laboratory, pooled, and reared until adult emergence. Larvae were fed with yeast extract and Carbin biscuit, while emerged adults were maintained under controlled conditions.

2.5. Adult Indoor Mosquito Collection

Pyrethrum Spray Catch and Prokopack aspirator collections were carried out in three randomly selected houses per community from January to December, 2022. Eighteen (18) blood-fed female *Anopheles* mosquitoes were collected, and their DNA was extracted for *Plasmodium falciparum* infection rate determination using modified direct PCR techniques of Echeverry *et al.* (2016).

2.6. Morphological Identification of Anopheles Species

Morphological identification was performed on all reared and collected *Anopheles* using Coetzee's, (2020) morphological keys. Morphological characteristics were observed using an LCD digital microscope, and identified *Anopheles gambiae* complex specimens were stored for molecular study (Fig. 3a).

2.7. Molecular Characterization of Anopheles gambiae s.l.

Genomic DNA was extracted from 91 individual mosquitoes using the Livak method. The extracted DNA was used for PCR analysis to identify sibling species of the

An. gambiae complex. Molecular identification of sub-species was conducted using PCR techniques proposed by Santolamazza *et al*. (2008). Presence or absence of bands was visualized through gel electrophoresis.

2.8. Plasmodium falciparum sporozoite Infection of Indoor Blood-Fed Anopheles

DNA extraction was performed on 18 blood fed female *Anopheles* mosquitoes collected through Pyrethrum Spray and Prokopack. *Plasmodium falciparum* infection was determined through PCR analysis from blood fed *An. gambiae* s.l collected in Zagaina community using a modified direct PCR technique of Echeverry *et al*. (2016).

All molecular investigations were conducted at Bayero-Wellcome Trust Laboratory, Department of Biochemistry, Bayero University Kano, Nigeria.

3. Results

3.1. Diversity and composition of Anopheles mosquito species

The composition of *Anopheles* mosquito species based on the morphological identification revealed four (4) different species of Anopheline mosquitoes; *An. gambiae* s.l. (Fig. 3a), *An. pretoriensis* (Fig. 3b), *An. maculipalpis* (Fig. 3c) and *An. rufipes* (Fig. 3d). *An. gambiae* s.l accounted for 2,238 (58.33%) of the 3,837 adults raised from larvae collections (Table 1). *An. gambiae* s.l was the most abundant species followed by *An. pretoriensis* with 1,218 (31.74%), *An. maculipalpis* with 257 (6.70%) and the least were *An. rufipes* 52 (1.36%). The distribution of *Anopheles* populations varied significantly (P<0.05) across the study communities. Out of the 2,238 (58.33%), *An. gambiae* s.l, 496 (22.16%) were found to be dominant in Zagaina with no other *Anopheles* species recorded, whereas Popondi had the least but with preponderance of *An. pretoriensis* 372 (30.54%). Sabon Layi had abundance of *An. maculipalpis* 108 (42.02%) compared with other communities. The finding from this work recorded zero *An. maculipalpis* and *An. rufipes* in Jauro Bakari, Kalshigi, Ajiya quarters and Zagaina communities of Akko LGA. In contrast, Lakolin, Lapandintai, Popandi and Sabon layi communities of Kaltungo LGA recorded the presence of *An. maculipalpis* and *An. rufipes* with Lapandintai having the most *An. rufipes*, 26 (50%) and Sabon layi having the least 5 (9.62%). Significant difference was observed between the reared *Anopheles* mosquitoes collected from all the communities (p<0.05, F=9.10), whereas no significant difference was observed among the total *Anopheles* reared to adulthood across the study communities (p>0.05, F=0.0129). The Shannon Weiner diversity index revealed that, *Anopheles gambiae* s.l was highly diverse when compared to other Anopheline species (1.896) and it dominated in all the eight communities (0.1649). The Evenness of *An. rufipes* was high (0.8516) compared to *An. pretoriensis* (0.7569), *An. maculipalpis* (0.8508) and *An. gambiae* s.l (0.8328) occurring in seven, four and three communities respectively (Table 2).

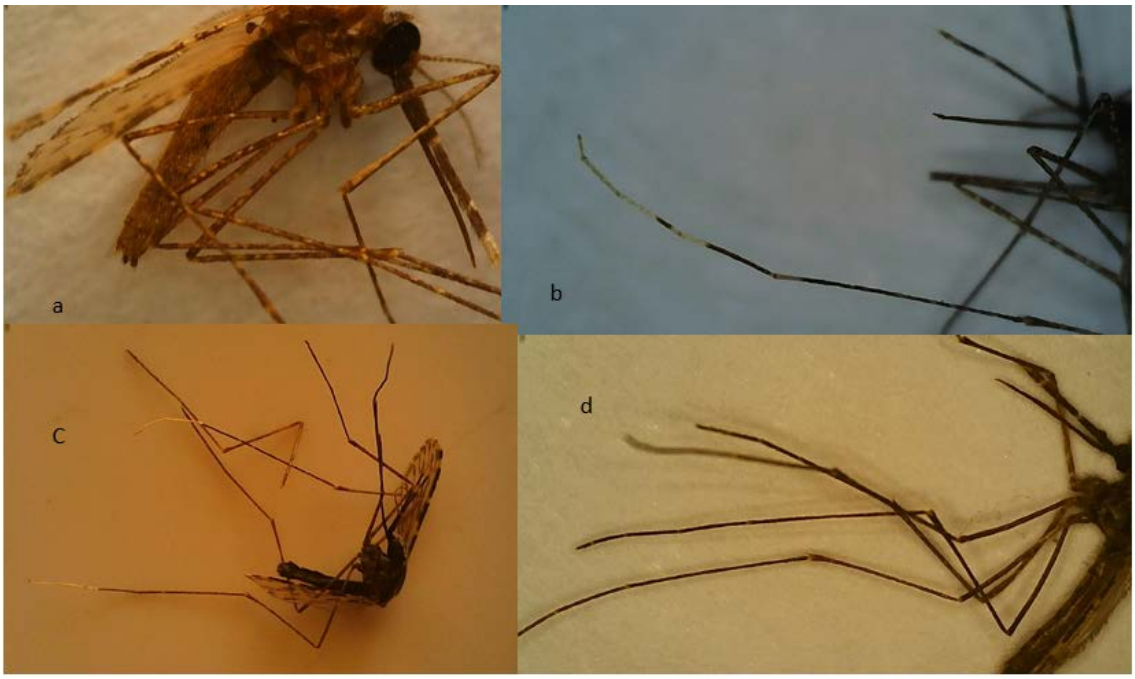

Figure 3: Morphological identification of *Anopheles* mosquitoes from eight study community in Gombe State. A = *Anopheles gambiae* s.l. b = *Anopheles pretoriensis* leg c = *Anopheles maculipalpis*, d = *Anopheles rufipes*

Table 1: Distribution and composition of members of *An. gambiae* s.l in the eight study communities

Communities	*An. gambiae*s.l. *n(%)*	*An. pretoriensis n(%)*	*An. Maculipalpis n(%)*	*An. Rufipes n(%)*	Unidentified n(%)	Overall n(%)
Ajiya quarters	440(19.66)	26(2.13)	0(0)	0(0)	0(0)	466(12.14)
JauroBakari	421(18.81)	59(4.84)	0(0)	0(0)	1(2.78)	481(12.54)
Kalshingi	340(15.19)	68(5.58)	0(0)	0(0)	2(5.56)	410(10.69)
Lakolin	220(9.83)	183(15.02)	90(35.02)	0(0)	5(13.89)	493(12.85)
Lapandintai	87(3.89)	341(28.00)	24(9.34)	26(50)	7(19.44)	485(12.64)
Popondi	46(2.06)	372(30.54)	35(13.62)	21(40.38)	18(50)	533(13.89)
Sabonlayi	188(8.40)	169(13.88)	108(42.02)	5(9.62)	3(8.33)	473(12.33)
Zagaina	496(22.16)	0(0)	0(0)	0(0)	0(0)	496(12.93)
Total	2238(58.33)	1218(31.74)	257(6.70)	52(1.36)	36(0.94)	3837(100)

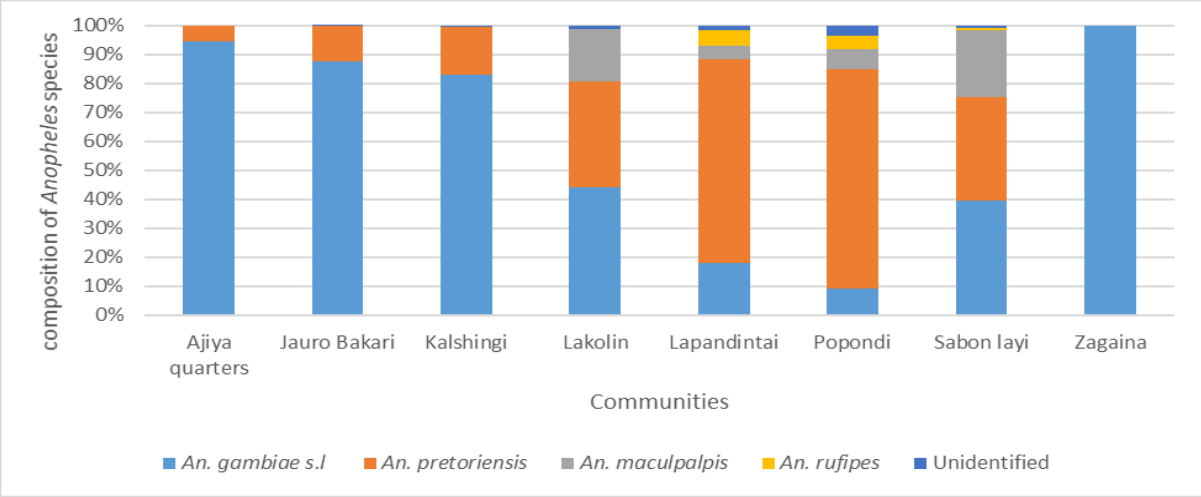

Figure 4. Species composition of *Anopheles* mosquitoes from the eight communities of the two LGAs of Gombe State, Nigeria

Table 2. Diversity indices of identified *Anopheles* mosquitoes across the sampling community

Species	Number of species	Dominance	Shannon (H)	Evenness (E)
An. gambiae s.l.	2238	0.1649	1.896	0.8328
An. pretoriensis	1218	0.2194	1.667	0.7569
An. maculipalpis	257	0.3265	1.225	0.8508
An. rufipes	52	0.4223	0.9379	0.8516

3.2. Molecular identification of the sibling species of Anopheles gambiae

Out of 2,238 *An. gambiae* complex, 91 were selected at random and molecularly identified. The molecular identification showed that *An. coluzzii* (Fig. 7) was abundant in Zagaina and *An. gambiae s.s* (Fig. 5) was predominant in Lakolin while *An. arabiensis* (Fig. 6) was found in Sabon Layi and Jauro Bakari (Table 3).

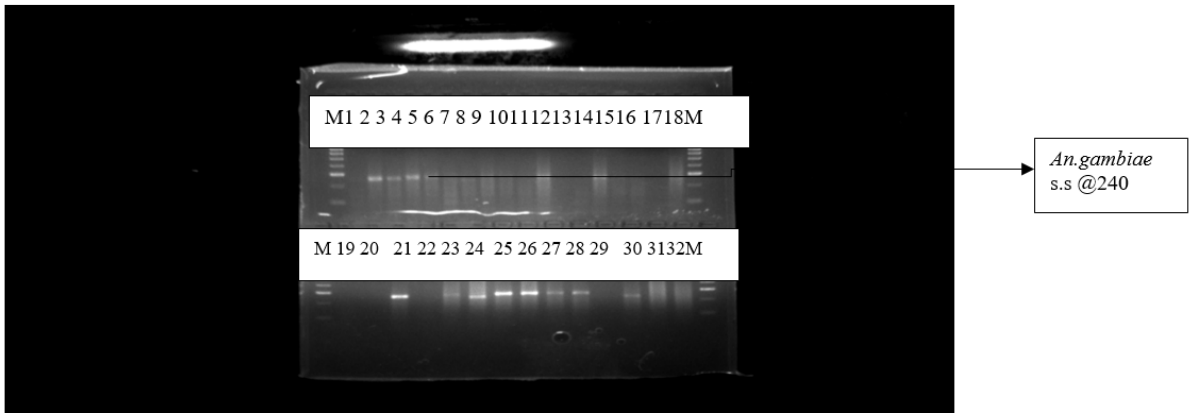

Figure 5: Agarose gel 3% for distinguishing *An. gambiae* s.l. after PCR with primers (SINE200F and SINE200R). Lane M= 100 bp hyper ladder visible at 300 bp. Positive samples show band size of 470bp to authenticate *An. coluzzii*, 240bp = *An. gambiae s.s* and 220bp = *An. arabiensis*. Lane 1= Negative control, 2-5, 7-9,11, 14, 18, 21, 23-28, 30-32 = *An. gambiae s.s.*, Lane 6 , 10, 12-13, 15-17, 19-20, 22, 29= not amplified.

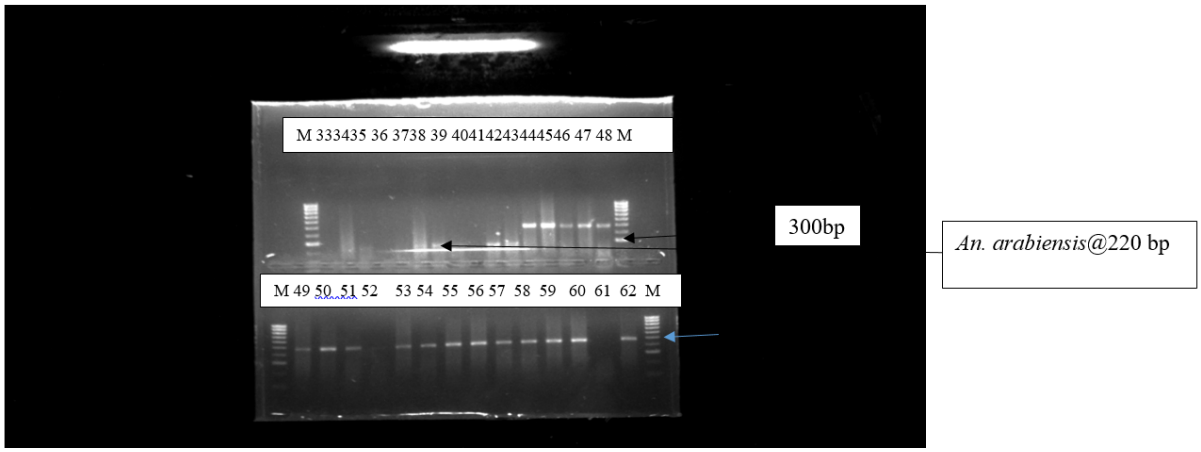

Figure 6: Agarose gel 3% for distinguishing *An. gambiae* s.l. after PCR with primers (SINE200F and SINE200R). Lane M= 100 bp hyper ladder visible at 300 bp. Positive samples show band size of 470bp to authenticate *An. coluzzii*, 240bp = *An. gambiae s.s* and 220bp = *An. arabiensis*. Lane 33 = negative control, Lane 34 = *An. arabiensis*, 38-39, 42-43 = *An. gambiae s.s.*, 44-60, 62 = *An. coluzzii*, 35-37, 40-41, 61, = not amplified.

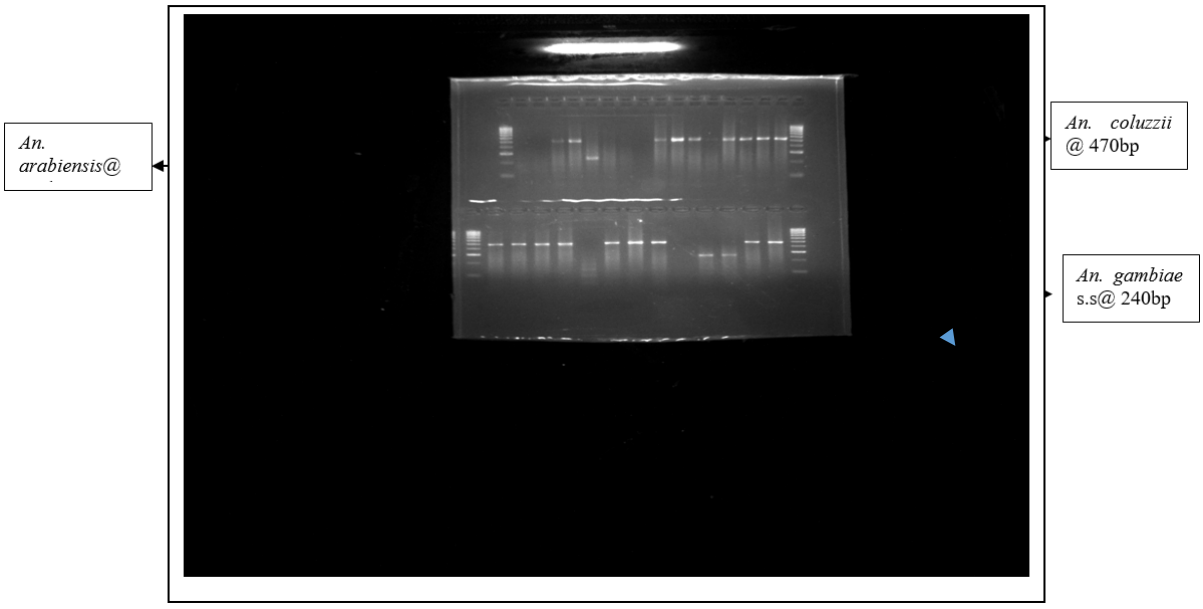

Figure 7: Three percent Agarose gel resolving *An. gambiae* s.l. after PCR with primers (SINE200F and SINE200R). Lane M= 100 bp hyper ladder visible at 300 bp. Postive samples show band size of 470bp to authenticate *An. coluzzii*, 240bp = *An. gambiae s.s* and 220bp = *An. arabiensis*. Lane 63 = Negative control; Lane 67 = *An. arabiensis*, Lane 88-89 = *An. gambiae* s.s. Lane 65-66, 71-82, 84-86, 90-91= *An. coluzzii*. Lane 64, 68-70, 83, 87 = not amplified.

Table 3. Distribution and Species composition of members of *An. gambiae* s.l in the eight study communities in 2 LGAs of Gombe State

				Species identified (%)			
LGA	Location	No examined (N)	No. PCR positive (%)	*An. Arabiensis* n(%)	*An. gambiae* s.s n(%)	*An. coluzzii* n(%)	Not amplified n(%)
	Ajiya quarters	12	11(91.67)	0(0)	0(0)	11(91.67)	1(8.33)
	Jaurobakari	15	9(60)	1(6.6)	0(0)	5(53.33)	6(40)
Akko	Kalshingi	12	12(100)	0(0)	0(0)	12(100)	0(0)
	Zagaina	09	7(77.78)	0(0)	2(22.22)	5(55.56)	2(22.22)
	Popondi	09	7(77.78)	0(0)	7(77.78)	0(0)	2(22.22)
Kaltungo	Sabonlayi	12	6(60)	2(16.67)	4(33.33)	0(0)	6(60)
	Lakolin	11	8(72.73)	0(0)	8(72.73)	0(0)	3(27.27)
	Lapandintai	11	3(27.27)	0(0)	3(27.27)	0(0)	8(72.73

3.3. Plasmodium falciparum Sporozoites Infectivity of Indoor Blood Fed Anopheles gambiae s.l.

A total of 18 blood fed *An. gambiae* s.l were collected from Zagaina and Ajiya quarters out of which 2 were positive for *P. falciparum* corresponding to 11% of all the samples collected (Fig. 8) from Zagaina. No blood fed *An. gambiae* s.l was found in all other study communities.

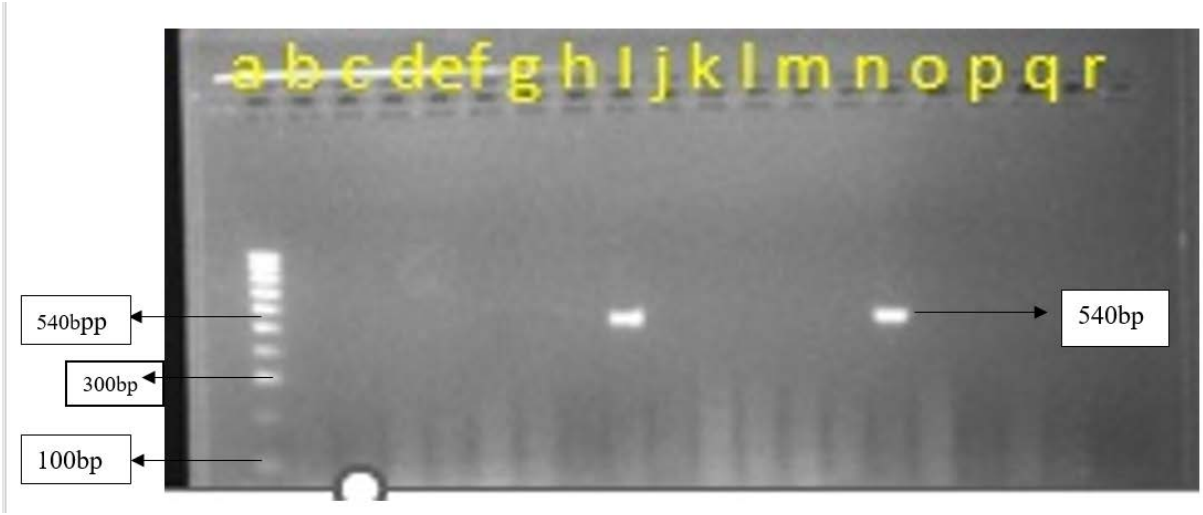

Figure 8: PCR bands of indoor collected blood fed mosquito from Zagaina tested for *P. falciparum* using COX1 gene. a = 100 bp hyper ladder, i and n = 540bp sporozoite for *P. falciparum.*

4. Discussion

The distribution of *Anopheline* mosquito from our findings revealed four (4) different species; *An. gambiae s.l., An. pretoriensis, An. maculipalpis* and *An. rufipes*. Similarly, Adeogun *et al.* (2023) reported seven *Anopheline species; An. salbaii, An. kingi, An. machardyi, An. pretoriensis, An. maculipalpis, An. stephensi* and *An. gambiae* s.l. from mosquito surveillance sites in 2020 though, in six LGAs of Gombe State among which one of the present study LGA (Akko) was inclusive. *An. gambiae* s.l dominated in six of the eight study communities with a percentage composition of 58.33% of the total collection. Similar results were obtained in Kontogora, Niger State (Garba, 2023), and from overall mosquito collection in Nigeria (60%) (Okorie *et al.*, 2011). Likewise, *Anopheles gambiae* complex constitute 98% of all the *Anopheles* collection for vector surveillance and insecticide resistance monitoring activities in Nigeria (NMCP, 2020; Adeogun *et al.*, 2023). The difference observed could be due to variation in the month of *Anopheles* larvae collection and year of the study. However, our study covered the whole year compared to their studies which was done during the rainy season when the vector population were in abundance though, the collection method was the same. The abundance and diversity of *An. gambiae* s.l. over *An. pretoriensis, An. maculipalpis* and *An. rufipes* is suggestive of anthropophilic behavior of *Anopheles gambiae* complex resulting into vectorial competence. It is also likely due to rainfall pattern coupled with relative humidity in the study sites. *An. gambiae* complex are the only *Anopheles* mosquitoes found in Zagaina (Evenness = 0) whereas two or more *Anophelines* were recorded in other communities. The preponderance of *Anopheles gambiae* s.l. is a result of its close proximity to residential houses and the suitability of the breeding water bodies. Findings from our work revealed the preponderance of secondary vectors; *An. pretoriensis, An. maculipalpis* and *An. rufipes* in Kaltungo study community. Similarly, Abba *et al.* (2024) reported the preponderance of secondary malaria vectors in Southern Gombe. However, our result is contrary to findings of Adeogun *et al.* (2023) who reported less than 2% in 12 out of 36 states of Nigeria. The abundance of *An. coluzzii* as the major malaria vector in the study area is not surprising as it was previously

reported to be approximately 98% of all the *Anopheles gambiae* complex collections in Yemaltu Deba LGA, Gombe State (Ahmed-yusuf *et al*., 2020), Kano (Ibrahim *et al*., 2019; Safiyanu *et al*., 2019; Ononamadu *et al*., 2020), South West (Omotoyo *et al*., 2022) and two vegetation zones of North Eastern Adamawa State that share boundary with the study location (Wahedi *et al*., 2020). Similarly, Ibrahim *et al*. (2023) observed *An. coluzzii* as the major malaria vector in the Sahelio-Sudanian region of Northern Nigeria, Niger, Cameroon and Chad. Our finding was also supported by the work of Adeogun *et al.* (2023) who reported the presence of all the sibling species of *An. gambiae* complex across Nigeria and *An. coluzzii* dominating most of Northern Nigeria. Efa *et al*. (2022) also reported *An. coluzzii* as a dominant species in Elobowa Southern Cameroon and Ellibou Southern Cote divore (N'Dri *et al*., 2023). Our findings do not corroborate previous works reporting *An. gambiae s.s* as the major malaria vector in Nigeria and sub-Saharan Africa (Awolola *et al*., 2009; Oduola *et al*., 2012). *An. coluzzii* established mostly in Akko LGA that is neighboring Gombe metropolis. Similarly, *An. coluzzii* were known to dominate metropolitan area of Lagos (Omotayo *et al*., 2022) which gives them the name Urban-mosquito and their establishment is likely due to high resistance to pyrethroids insecticides and other synthetic pesticide (Wahedi *et al*., 2021). However, Oduola *et al*. (2019) previously reported preponderance of *An. gambiae* s.s. in Southern Gombe which is similar to present study. Previous studies have reported dominance of *An. gambiae* s.s. in Anambra and Katsina States (Irikannu *et al*., 2019; Umar and Ndams, 2022) as opposed to zero record of *An. gambiae* s.s in Sudan Savannah of Northern Nigeria (Ibrahim *et al*., 2014). The establishment of *An. gambiae* s.s is possible due to their preference for breeding in both permanent and temporary water bodies, Sunlit, found in both rainy and dry seasons likewise in areas within tense application of agrochemical pesticides (Defo-talom and Zeukeng, 2021). In this study, the *An. gambiae* s.s and *An. coluzzii* established in two separate ecological zone of Gombe with sympatric population of *An. arabiensis* agrees with a recent study conducted in twelve (12) States of Nigeria (Adeogun *et al*., 2023). The rapid expansion of *An. coluzzii* among the sibling species in Nigeria specifically Gombe might be as a result of its adaptability to various ecological zones as well as climatic conditions. The co-habitation of *An gambiae* s.l. sibling species within a given habitat might threaten the recent achievement gained in malaria vector control programme in malaria endemic countries (Tokponnon *et al*., 2023). This change in *An. gambiae* complex composition has implications on malaria vector management and control methods. It calls for the application of both indoor and outdoor vector control methods in order to minimize the expansion of *An. coluzzii* species population although no evidence of outdoor biting of *An. coluzzii* was documented.

This study is the first documented report of *P. falciparum* in *An. gambiae* complex from Gombe State with a prevalence rate of 11% compared to low sporozoites rate of 6.9% previously detected in *An. coluzzii* from neighbouring community of Adamawa State (Wahedi*et al*., 2020). However, previous work by Rice *et al*. (2022) reported higher prevalence of *P. falciparum* (35%) in a University campus in Zaria. Conversely, low sporozoites infection in *An. gambiae* s.s. was established in Southern Nigeria (Irikannu *et al*., 2019; Obembe, 2023). Similarly, Altahir *et al*. (2022) reported 6.9% prevalence in *An. arabiensis* from Sudan while Mbewe *et al*. (2022) found low 0.16% sporozoite infection rate in two study areas in Malawi and Stephen *et al*. (2022) established 1.06% *Plasmodium* in *An. coustani* out of 376 blood fed *Anopheles* mosquito collected in coastal Kenya. Additionally, Moin-Vaziri *et al*. (2022) reported *Plasmodium* infection rate of 1.25 in *An. stephensi* from Afghanistan in contrast to study from South East, Nigeria where no *Plasmodium* was detected in all *Anopheles* mosquito species collected indoor and outdoor (Oduwole *et al*., 2020). This is similar to finding in Chad where all 147 wild type blood fed *An. coluzzii* (Ibrahim *et al*., 2019), and *An. stephensi* from Iran (Moin-Vaziri *et al*., 2022) had no *P. falciparum* which could be due to active malaria vector control programme in Chad and Iran respectively. The low prevalence of *P. falciparum* observed could be as a result of mass distribution campaign of LLINs which kill, repels and prevent mosquito from taking blood when correctly used during sleeping hours. It was concluded from this study that *An. coluzzii* is the main malaria vector in Gombe contrary to earlier reports by Okorie *et al*. (2011) and Oduola *et al*. (2012).

5. Conclusion

The study established *An. gambiae* s.l. as the dominant malaria vector with sympatric populations of secondary vectors; *An. pretoriensis, An. maculipalpis* and *An. rufipes.* The presence of three sibling species; *An. gambiae* s.s, *An. arabiensis, An. coluzzii* co-habiting together and preponderance of *An. coluzzii* was also established. *An. coluzzii* is now becoming a major malaria vector in Gombe State due to speciation within the complex hence, vector surveillance is paramount. For the first time prevalence of *P. falciparum* sporozoite in *An. coluzzii* was established in Gombe State. Therefore, the State and National malaria elimination programme should focus more toward the control of both primary and secondary malaria vectors although; the latter is yet to be incriminated in malaria transmission in Gombe State.

6. Conflicts of Interest

The authors declare that they have no conflicts of interest.

7. Author's contribution

Authors A.B.S., I.S.N., S.G.J. and N.P.C. performed the field work, morphological identification, molecular analysis and developed the manuscript. Author E.A. and K.P.Y. analyzed the data. All authors proved read the manuscript and gave approval.

8. Funding

The study was funded by Tertiary Education Trust Fund, Nigeria through the TET Fund Scholarship for Academic Staff (TSAS) awarded to the corresponding author.

Acknowledgements

The authors sincerely thank the Gombe State Malaria vector surveillance laboratory, Gombe State University and Bayero Wellcome Research Unit, Department of Biochemistry, Bayero University Kano for working space to conduct molecular study.

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