

First report of heterogeneity and sympatry of malaria vectors in Southern Gombe, Northeastern Nigeria: its implications for malaria vector control

Ezra Abba^{1*}, Pukuma Micah Sale², Adedapo Adeogun³, Kennedy Poloma Yoriyo¹, Abdulmalik Bala Shuaibu¹, Olukayode James Adelaja⁴ and Omotayo Ahmed Idowu³

1. Zoology Department, Faculty of Science, Gombe State University, Gombe, Nigeria

2. Department of Zoology, Faculty of Life Science, Modibbo Adama University, Yola, Nigeria

3. Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria

4. Entomology Unit, Department of Zoology, University of Ilorin, Nigeria.

Corresponding author: ezra.abba@gmail.com, +2348080426350

Running title: Heterogeneity and sympatry of malaria vectors in Southern Gombe

Abstract

A survey of the malaria vectors in an area is a critical component of an effective vector control strategy. This study aimed to investigate the malaria vectors in four communities of Southern Gombe, Northeastern Nigeria. A total of 3200 adult female *Anopheles* reared from larvae in the four communities from two Local Government Areas (LGAs) in Southern Gombe were identified. *Anopheles pretoriensis* were dominant 1662 (51.9%) followed by *An. gambiae* sl 868(27.1%), *An. maculipalpis* 267(8.3), *An. rufipes* 252(7.9) and *An. coustani* 10(0.3%), and the least were *An. pharoensis* 6(0.2%). The remaining 135(4.3%) were unidentified. Of the 262 *An. gambiae* sl identified by species-specific PCR method, 135(51.5%) were found to be *An. coluzzi*, 60(22.9%) *An. gambiae* and only 1(0.4%) was *An. arabiensis* whereas hybrid constitutes 16(6.1%). To determine significant differences in species composition, the results from the four study sites were pooled together. *Anopheles pretoriensis* was significantly different from all the other species identified ($p < 0.0001$ - 0.0454). *An. gambiae* sl. was significantly different from *An. coustani* and *An. pharoensis* ($p = 0.0258$, 0.0249 respectively). There was no significant difference between *An. maculipalpis*, *An. rufipes*, *An. coustani* and *An. pharoensis* ($p = 0.9261$ - >0.9999). There was a significant difference between the number of species identified as *An. coluzzi* and *An. arabiensis* ($p = 0.0025$). But there was no significant difference between *An. coluzzi* and *An. gambiae* ($p = 0.1212$). The heterogeneity and sympatry of *Anopheles* species observed is a threat to malaria control as the secondary vectors have behaviour that evades the current vector control interventions. It is expedient to re-strategize the vector control interventions in the study area.

Key-words: Malaria vector, Vector control, Gombe, *Anopheles*

1. Introduction

Malaria is a life-threatening disease caused by *Plasmodium* parasites (Campos et al., 2023). Globally, there were an estimated 249 malaria cases in 2022. This shows a rise of 5 million malaria cases compared to the 2021 reported cases. The 2022 cases were from 85 countries with endemic

malaria, and the *Sub-Saharan African* Region recorded the majority of this increase. With 233 million of the 2022 cases, the *Sub-Saharan African* continent accounted for around 94% of the global total cases. Nigeria accounted for 27% of the global total cases carrying a greater percentage than any other country (WHO, 2023). In Nigeria, malaria accounts for up to 60% of all outpatient visits to healthcare facilities and 30% of all hospital hospitalizations. Malaria is thought to be responsible for 25% of baby fatalities, 30% of child deaths, and 11% of maternal deaths. According to estimates, this disease costs Nigeria's economy ₦132 billion annually (approximately US\$167,300) in lost wages due to treatment expenses, preventative measures, wasted manpower, etc (National Malaria Elimination Programme & Federal Ministry of Health Nigeria, 2014). The bulk of malaria cases in *Sub-Saharan Africa* is a consequence of the widespread of *An. gambiae* sl across the continent (Sinka et al., 2010).

Malaria is known to be spread by *Anopheles* mosquitoes. The geographic distribution of these species varies, and they can be categorized as primary vectors – those that are most efficient in transmitting the parasite in a given region or secondary vectors – those that are of secondary significance in the epidemiology of the disease. The secondary vectors are sometimes disregarded, although research has shown that they may continue to transmit malaria when primary vector populations are reduced or eradicated (Antonio-Nkondjio et al., 2006; Braack et al., 2020; Lobo et al., 2015). *Anopheles gambiae* sl, *An. funestus* group and other secondary vectors have been widely reported as important vectors of malaria parasites in *Sub-Saharan Africa* (Bedasso et al., 2022; Braack et al., 2020; Epopa et al., 2019; Nelly Armanda Kala-Chouakeu et al., 2022; Nkya et al., 2022; Olatunbosun-Oduola et al., 2019; Saili et al., 2023; Sinka et al., 2010; Tabue et al., 2017). *Anopheles gambiae*, *An. coluzzi* and *An. arabiensis* were all incriminated in malaria transmission in Nigeria, (Awolola et al., 2018; Olatunbosun-Oduola et al., 2019; Wahedi et al., 2021). Other Anopheline species reported in Nigeria are *An. funestus*, *An. moucheti*, *An. melas*, *An. lesoni*, *An. coustani*, *An. pharoensis*, *An. rufipes*, *An. nili*, *An. squamosus*, *An. pretoriensis*, and *An. longipalpis* (Adeogun et al., 2023; Awolola et al., 2005; Ebenezer et al., 2014; Garba et al., 2017; National Malaria Control Programme, 2020; Oyewole et al., 2007, 2010; PMI, 2021).

There have been cases where the presumed secondary malaria vectors have been shown to play a significant role in the transmission of malaria. The contribution of these vectors were majorly outdoor (Goupeyou-Youmsi et al., 2020; Joseph et al., 2013; Tabue et al., 2017), though they were also incriminated in indoor transmission (Saili et al., 2023). The outdoor feeding and resting habits of secondary malaria vectors and their frequent contact with humans infected with *Plasmodium* species might elude vector control interventions (Ciubotariu et al., 2020).

Limited data on malaria vectors is available in Southern Gombe and there is paucity of up-to-date community-specific data on the vectors in the region. A crucial part of the epidemiological research of malaria is the species-level identification of individual vectors within the same morphologically identical taxon. It is the first step in developing a strategic control programme and will aid in the incrimination of the malaria vectors in Gombe state. Though there were other national entomological surveillance of malaria vectors in Nigeria as reported by (Adeogun et al., 2023), however, this study is the first report of community-specific data on the co-existence of both primary and alternate vectors of malaria in Southern Gombe, Northeastern Nigeria. This has a tremendous implication in the control of malaria vectors which currently mainly targets the mosquitoes biting humans indoors.

2. Materials and methods

Study Area

The study was carried out in four communities of Southern Gombe. Gombe state is located in the North-eastern part of Nigeria and has a land mass of 20,265 km² area. The state experiences both dry and wet seasons commencing between November - March and April – October respectively. With wide-open grassland that dries out during the dry season, the vegetation is typical of the Sudan Savanna. Gombe State is surrounded by Yobe State to the north, Adamawa and Taraba States to the south, Borno State to the east, and Bauchi State to the west. Most of the residents of Gombe South are farmers producing both food and cash crops. Yam, tomato, cassava, sorghum, maize, millet, cowpea, and peanut are some of its food crops, while cotton is grown mostly for export. They also keep goats, cattle, sheep, and horses and practice the traditional crafts of weaving and dyeing cotton. Along with keeping cattle, goats, sheep, and horses, they also engage in traditional crafts like weaving and cotton dying. Gombe State has a malaria prevalence of 30% (National Malaria Control Programme, 2020b). The GPS coordinates of the study communities are Awai (Lat. 9.902760⁰, Long. 11.214893⁰), Kentengereng (Lat. 9.883385⁰, Long. 11.228432⁰), Talasse (Lat. 9.973737⁰, Long. 11.669845⁰), Putoki (Lat. 9.839087⁰, Long. 11.677787⁰) (Figure 1).

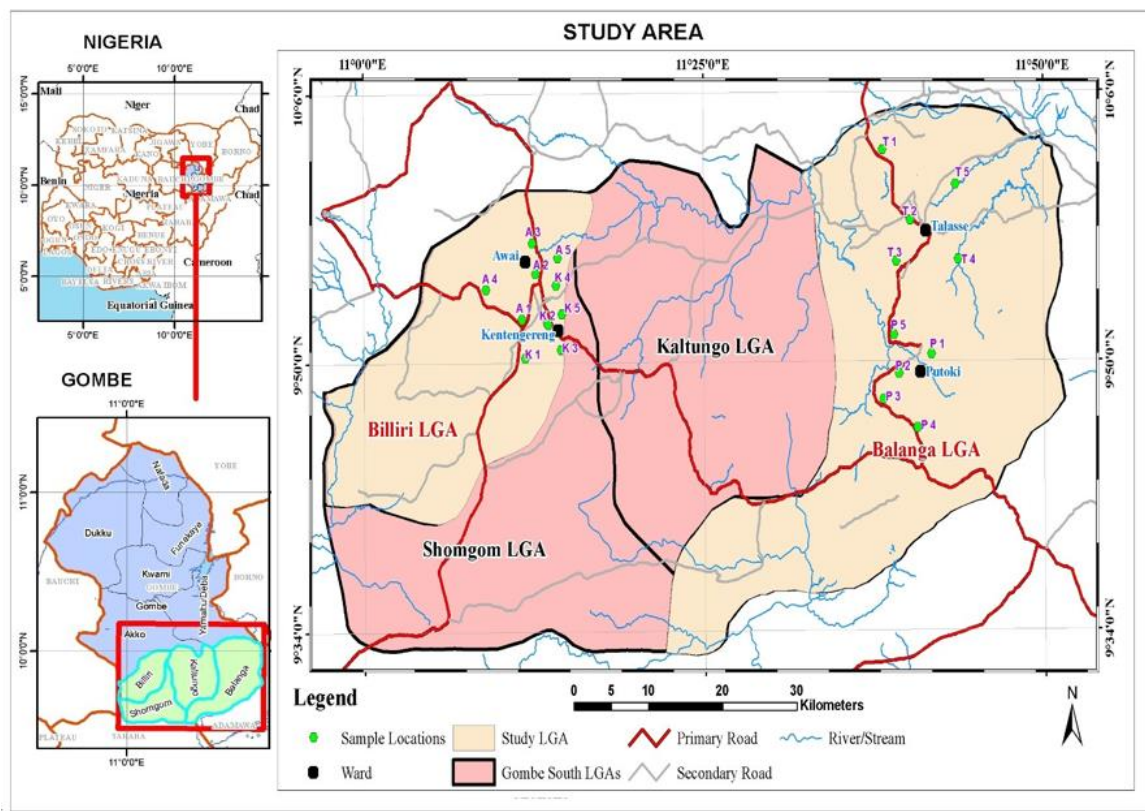


Figure 1: Map of Study Area Showing Selected LGAs and Sample Locations in Southern Gombe

Study design

The study LGAs and communities were chosen based on ease in finding *Anopheles* mosquito breeding sites and preliminary studies conducted (unpublished data) using the Randomized sampling method. Breeding sites were sampled based on specific peculiarities/characteristics that *Anopheles* mosquito breeding sites possess as well as a good coverage of the community.

Collection and rearing of *Anopheles* mosquitoes

Between April and December 2022, mosquito larvae were collected simultaneously from natural development sites such as pools, ditches, streams, rice fields, and brick pits using dippers in each of the four study localities. The coordinates of the study communities and breeding sites were established using Global Positioning System (GPS). Larvae were transferred to the Gombe state sentinel insectary at the Gombe State University in small, clear containers. The larvae were then transferred to plastic trays (5cm x 27cm x 36cm) to an approximate depth of 2cm and each tray was then covered with nets labelled to reflect and labelled appropriately according to (WHO, 2016) protocol. The larvae were kept in trays at 25-28°C and 76±5% relative humidity and fed with 200mg of ground fish food and yeast powder once every morning until their emergence into adults. The adults were then transferred to adult cages.

Morphological Identification of *Anopheles* mosquitoes

The morphological identification key (Coetzee, 2020) focusing on the Abdomen, legs, maxillary palps and wings was used in the identification. In all, 800 female *Anopheles* mosquitoes were randomly selected from the adults that emerged from each community and were morphologically identified.

Molecular Identification of Sibling Species of *An. gambiae* s.l Using Standard Polymerase Chain Reaction (PCR).

For the discrimination of *An. coluzzi* and *An. gambiae* (formerly M and S molecular forms respectively), atleast 80 samples were randomly picked from the morphologically identified *An. gambiae* complex, but in other communities that have less than 80 in total, the available samples were used. The combined *An. gambiae* complex and ribosomal DNA type assay was used (Wilkins et al., 2006a). This technique for differentiating the *Anopheles gambiae* complex is based on species-specific single nucleotide polymorphisms (SNPs) in the intergenic spacer region (IGS). The technique is supplemented with primers to simultaneously reveal the type of ribosomal DNA. To boost specificity, these additional primers also include intentional mismatches (Intentional Mismatch Primers (IMPs)).

Genomic DNA (gDNA) was extracted from the whole body of each mosquito using spin column DNA extraction kit (Jena Bioscience). Molecular identification of these mosquito samples was carried out using multiplex PCR as described by (Wilkins et al., 2006). Seven primers including IMP-UN (Universal), AR-3T (*Anopheles arabiensis*), GA-3T (*Anopheles gambiae* s.s), ME-3T (*Anopheles merus*), QD-3T (*Anopheles quadriannulatus*), IMP-S1 (*Anopheles gambiae*) and IMP-M1 (*Anopheles coluzzii*) were used for molecular characterization of *Anopheles gambiae* s.l sibling species.

A reaction mixture of 12.5 µl PCR reaction was prepared for each sample. The mixture contains 5.5 µl of double distilled water, 2.5µl of mastermix (5X; Solis Biodyne), 0.5 µl of each of IMP-UN, ME-3T, AR-3T, QD-3T, GA-3T, IMP-S1 and IMP-M1 (0.4 µM) as well as 1.0µl of DNA template. PCR cycle conditions was carried out in a BIO-RAD T100 thermocycler included: initial denaturation at 95 °C for 5 mins, 35 cycles of 95 °C for 30 sec, 59.2 °C for 30 sec, 72 °C for 30sec and final extension at 72°C for 5 mins with 4°C hold. Thereafter, a 1.5% agarose gel stained with ethidium bromide was used to run 10 µl of the amplified samples. Primers created fragments of 463 *An. gambiae* s.l, 387 *An. arabiensis*, 333 *An. coluzzi* (M-form) and 221 *An. gambiae* (S-form) when viewed in the gel documentation machine (Figure 3).

The primer sequences are as follows:

IMP-UN	5' GCTGCGAGTTGTAGAGATGCG 3'
AR-3T	5' GTGTTAAGTGTCTTCTCCGTC 3'
GA-3T	5' GCTTACTGGTTTGGTCGGCATGT 3'
ME-3T	5' CAACCCACTCCCTTGACGATG 3'
QD-3T	5' GCATGTCCACCAACGTAAATCC 3'
IMP-S1	5' CCAGACCAAGATGGTTCGCTG 3'
IMP-M1	5' TAGCCAGCTCTTGTCCACTAGTTTT 3'

Data Analysis

The number of mosquitoes collected (mosquito genera and *Anopheles gambiae* s.l. sibling species) were compared across the four study communities and two LGAs using Analysis of Variance (ANOVA) to determine the level of significance ($p < 0.05$) using Statistical Package for Social Sciences (SPSS) version 22 software (Chicago, IL, USA).

3. Result/discussion

A total of 3200 female *Anopheles* mosquitoes were identified from the four different communities in Southern Gombe (Kentengereng, Awai, Putoki and Talasse), Northeastern Nigeria. Overall, *An. pretoriensis* was significantly different compared to the other *Anopheles* species identified ($p = < 0.0001 - 0.0454$) and more abundant with 1662 (51.9%) followed by *An. gambiae* s.l. 868 (27.1%) which was significantly different compared to *An. coustani* and *An. pharoensis* ($p = 0.0258, 0.0249$ respectively). The number of other *Anopheles* species encountered as well as their respective percentages were *An. maculipalpis* 267 (8.3%), *An. rufipes* 252 (7.9%), *An. coustani* 10 (0.3%) and *An. pharoensis* 6 (0.2%) and there was no significant difference between their abundance ($p = 0.9261$ to > 0.9999). However, a total of 135 (4.3%) of the mosquitoes collected were unidentified. *An. pretoriensis* was the dominant species in Kentengereng (46.9%); Putoki (47.1%) and Talasse (81.8%). Out of 1600 mosquitoes identified morphologically from Billiri LGA, *An. pretoriensis* dominates with 631 (39.4%) while the least was *An. pharoensis* 4 (0.3%). Again, *An. pretoriensis* was dominant in Balanga LGA constituting 1031 (64.4%) and *An. pharoensis* was the least 2 (0.0%) (Figure 2).

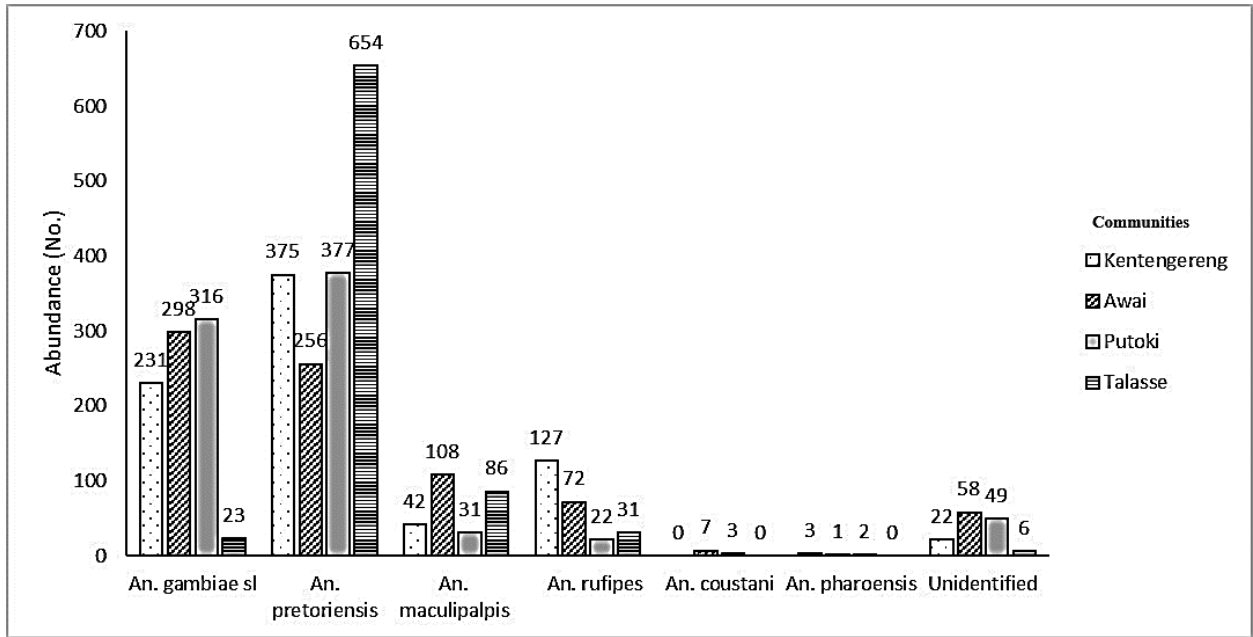


Figure 2: Morphological Identification of female *Anopheles* mosquitoes in four communities of Southern Gombe

Table 1: PCR Molecular Identification of Sibling species of *Anopheles gambiae* sl. in Southern Gombe

LGA's	Communities	Number Assayed (N)	Species composition				
			<i>An. gambiae</i>	<i>An. coluzzi</i>	<i>An. arabiensis</i>	Hybrid	No Amplification
Billiri	Kentengereng	82	27(32.9)	35(42.7)	0(0.0)	1(1.2)	19(23.2)
	Awai	84	19(22.6)	52(61.9)	1(1.2)	1(1.2)	11(13.1)
Balanga	Putoki	81	13(16.0)	38(46.9)	0(0.0)	11(13.6)	19(23.5)
	Talasse	15	1(6.7)	10(66.6)	0(0.0)	3(20)	1(6.7)
	Total	262	60(22.9)	135(51.5)	1(0.4)	16(6.1)	50(19.1)

N= Total number of *Anopheles gambiae* sl, Numbers in brackets represent percentages

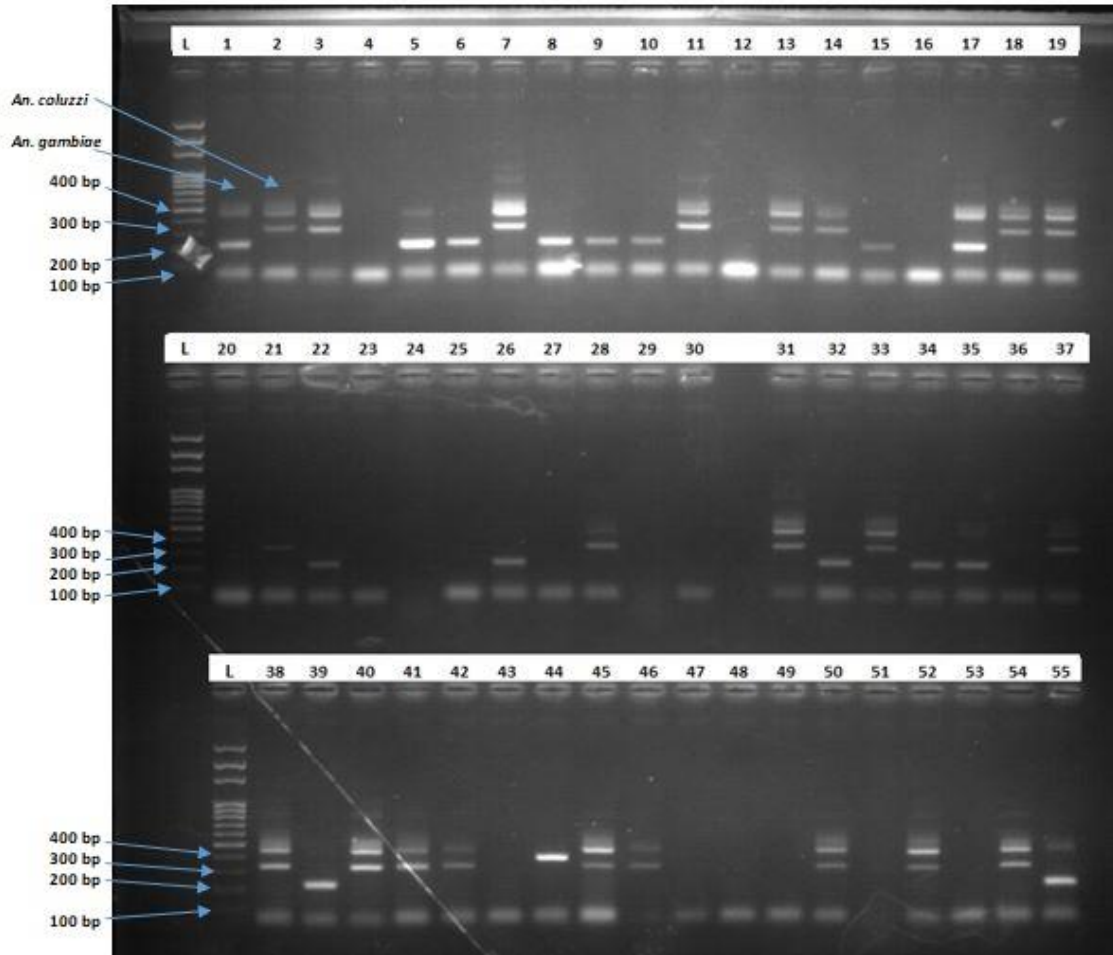


Figure 3: PCR Gel Picture for the molecular identification of *Anopheles gambiae* sl sibling species. Fragments of 463 = *An. gambiae* s.l.; 333 = *An. coluzzi* (M-form) (Lane 2) and 221 *An. gambiae* (S-form) (Lane 1).

The result is at variance with previous studies, where they all showed the dominance of *An. gambiae* sl over other *Anopheles* mosquitoes (Garba et al., 2017; Olatunbosun-Oduola et al., 2019; Wahedi et al., 2020; Yoriyo et al., 2014). Olatunbosun-Oduola et al. (2019) collected samples in Gombe state from the same LGAs with this study and found a varying result. Though their sampling locations are not the same with this study, it is thought that the species diversity seen in this study might be a result of the larger coverage of this study. In this study, a total of 20 sampling locations were used for the sampling. The habit of most previous studies is that once the expected number of sample sizes is reached (even if it is within a single point) collections are usually discontinued and analyzed. However, as a result of the design of this work, more breeding sites were sampled and pooled together to have an average of several breeding sites within the community. Secondly, the difference might be a result of differences in communities. Therefore, it could be rightly established that *An. pretoriensis* can be found in abundance in these communities. There was a report of the emergence of secondary malaria vectors such as *An. coustani*, *An. pharoensis* and *An. rufipes* in Northeastern Nigeria as reported by (Garba et al., 2017). However, these species were not found to be dominant over the primary vectors as reported in the present study. Even though the scope of this study does not include the incrimination of the vectors for malaria transmission, however, these secondary vectors were incriminated for malaria transmission previously (Antonio-Nkondjio et al., 2006; Assa et al., 2023; Bedasso et al., 2022; Goupeyou-Youmsi et al., 2020;

Joseph et al., 2013; PMI, 2021; Saili et al., 2023; Tabue et al., 2017). This opens up a new conversation in policy making especially in Gombe state, as these secondary species mainly rest and feed outdoors (Bedasso et al., 2022). In other parts of *Sub-Saharan Africa*, *Anopheles rufipes*, *An. coustani*, and *An. pretoriensis* have all been discovered to harbour *P. falciparum* sporozoites and all of these species were detected in Southern Gombe during our investigations, often as with *An. pretoriensis* and *An. rufipes*. Malaria transmission has been attributed to secondary malaria vectors as being significant. This is because they frequently exhibit outdoor resting and feeding habits which could lengthen the period that malaria transmission continues after primary vectors have been decreased by indoor control methods (Afrane et al., 2016).

Since the majority of secondary vectors rest outdoor, existing management strategies do not address outdoor biting. Some of the variables that could affect the conversion of secondary vectors to primary vectors include the high rate of insecticide usage in vector control, climate change, and extraordinary changes in land use (Afrane et al., 2016; Sinka, 2013). For instance, (Lafferty & Mordecai, 2016) reported that climate changes could lead to modification of vector behaviour thereby affecting their distribution and eventually their ability to transmit diseases. Insecticide resistance is thought to impact greatly on malaria vector competence (Suh et al., 2023), hence giving the secondary vectors the advantage of being competent vectors when the primary vector populations are depleted. Many environmental conditions can directly impact vector behaviour or pathogen transmission, which in turn can affect the occurrence and transmission of vector-borne diseases. By changing the life cycles of vectors or reservoirs, these factors may also indirectly affect vector-borne diseases, ultimately affecting their distribution and abundance (Caminade et al., 2019). Surprisingly, in only in Awai, one of the four study communities, *An. gambiae* s.l. were found to be dominant. This agrees with the findings of Olatunbosun-Oduola et al. (2019). Overall, 262 *An. gambiae* s.l. were identified by the species-specific PCR method, where 135(51.1%) were found to be *An. coluzzi*, 60(22.9%) *An. gambiae* and only 1(0.4%) was *An. arabiensis*. There was a significant difference between the number of species identified as *An. coluzzi* and *An. arabiensis* ($p = 0.0025$). But there was no significant difference between *An. coluzzi* and *An. gambiae* ($p = 0.1212$). Hybrid constitute 16(6.1%) while 50(19.1%) samples did not amplify. Out of 166 mosquitoes identified from Billiri LGA, *An. coluzzi* constitutes the majority 87(52.4%) followed by *An. gambiae* 46(27.7%). Hybrid was only 2(1.2%) while the least was *An. arabiensis*. *An. coluzzi* was also the dominant species in Balanga LGA constituting 50% of the total collection while *An. gambiae* and Hybrid each numbered 14(14.6%). No *An. arabiensis* was found in Balanga LGA. (Table 1). The predominance of *An. coluzzi* is noted in this study. All these species were noted as the principal malaria vectors in Nigeria (Adeogun et al., 2023; Adeogun et al., 2019; PMI, 2021). The findings of (Ebenezer et al., 2014; Ebenezer et al., 2012; Olatunbosun-Oduola et al., 2019) were contrary where they reported the predominance of *An. gambiae* against the findings of this study.

4. Conclusion

For the first time, both primary (*An. gambiae*, *An. coluzzi* and *An. arabiensis*) and secondary malaria vectors (*An. pretoriensis*, *An. rufipes*, *An. maculipalpis*, *An. coustani* and *An. pharoensis*) were found in sympatry in communities of Southern Gombe, Northeastern Nigeria. These secondary vectors are known mainly to be exophilic and exophagic. However, the current malaria vector control strategies in Nigeria target endophilic and endophagic mosquitoes. This development poses a big threat to the control of malaria vectors and may reverse gains made so far. Therefore, it is necessary that the National Malaria Elimination Program re-strategizes its policy to meet the

current challenge. Larviciding among other alternatives could be considered to target multiple mosquito species irrespective of the adult's feeding and resting behaviour.

Author's contribution

Authors E.A., A.B.S. and P.M.S. performed the fieldwork, and morphological identification and developed the manuscript. Author A.A. performed the molecular analysis and authors K.P.Y., O.A.I. and O.J.A. performed the data analysis. All authors read the final version of the manuscript and approved it.

Funding

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

Conflict of interests

The authors declare that there is no conflict of interest.

Acknowledgement

Many thanks to the field assistant, Mr. Titus Bellison Haske who assisted in the larval collection. Our gratitude goes to the Gombe Sentinel Insectary at Gombe State University for providing the workspace. We also appreciate the entire staff of the molecular entomology laboratory at the Nigerian Institute of Medical Research, Yaba, Lagos for the molecular analysis.

References

- Adeogun, A., Babalola, A. S., Okoko, O. O., Oyeniyi, T., Omotayo, A., Izekor, R. T., Adetunji, O., Olakiigbe, A., Olagundoye, O., Adeleke, M., Ojianwuna, C., Adamu, D., Daskum, A., Musa, J., Sambo, O., Adedayo, O., Inyama, P. U., Samdi, L., Obembe, A., ... Salako, B. (2023a). Spatial distribution and ecological niche modeling of geographical spread of *Anopheles gambiae* complex in Nigeria using real time data. *Scientific Reports*, 13(1), 1–18. <https://doi.org/10.1038/s41598-023-40929-5>
- Adeogun, A. O., Popoola, K. O. K., Olakiigbe, A. K., & Awolola, S. T. (2019). Distribution of Members of the *Anopheles Gamibiae* s.l. In Oyo State, South West Nigeria. *Pan African Journal of Life Sciences*, 3(1), 138–144. [https://doi.org/10.36108/pajols/9102/30\(0140\)](https://doi.org/10.36108/pajols/9102/30(0140))
- Afrane, Y. A., Bonizzoni, M., & Yan, G. (2016). Secondary Malaria Vectors of Sub-Saharan Africa: Threat to Malaria Elimination on the Continent? In *Current Topics in Malaria* (pp. 474–490). <https://doi.org/10.5772/65359>
- Antonio-Nkondjio, C., Kera, C. H., Simard, F., Awono-Ambene, P., Chouaibou, M., Tchuinkam, T., & Fontenille, D. (2006). Complexity of the malaria vectorial system in Cameroon: Contribution of secondary vectors to malaria transmission. *Journal of Medical Entomology*, 43(6), 1215–1221. [https://doi.org/10.1603/0022-2585\(2006\)43\[1215:COTMVS\]2.0.CO;2](https://doi.org/10.1603/0022-2585(2006)43[1215:COTMVS]2.0.CO;2)

308 Assa, A., Eligo, N., & Massebo, F. (2023). Anopheles mosquito diversity, entomological indicators
309 of malaria transmission and challenges of morphological identification in southwestern
310 Ethiopia. *Tropical Medicine and Health*, 51(1), 1–8. [https://doi.org/10.1186/s41182-023-](https://doi.org/10.1186/s41182-023-00529-5)
311 00529-5

312 Awolola, T. S., Adeogun, A., Olakiigbe, A. K., Oyeniyi, T., Olukosi, Y. A., Okoh, H., Arowolo,
313 T., Akila, J., Oduola, A., & Amajoh, C. N. (2018). Pyrethroids resistance intensity and
314 resistance mechanisms in *Anopheles gambiae* from malaria vector surveillance sites in
315 Nigeria. *PLoS ONE*, 13(12), 1–13. <https://doi.org/10.1371/journal.pone.0205230>

316 Awolola, T. S., Oyewole, I. O., Amajoh, C. N., Idowu, E. T., Ajayi, M. B., Oduola, A., Manafa, O.
317 U., Ibrahim, K., Koekemoer, L. L., & Coetzee, M. (2005). Distribution of the molecular forms
318 of *Anopheles gambiae* and pyrethroid knock down resistance gene in Nigeria. *Acta Tropica*,
319 95(3), 204–209. <https://doi.org/10.1016/j.actatropica.2005.06.002>

320 Bedasso, A. H., Gutto, A. A., Waldetensai, A., Eukubay, A., Bokore, G. E., Kinde, S., Gemechu,
321 F., Debebe, Y., Aklilu, M., Tasew, G., Massebo, F., Teshome, A., Kebede, T., Abdulatif, B.,
322 Sisay, A., Solomon, H., & Kweka, E. J. (2022). Malaria vector feeding, peak biting time and
323 resting place preference behaviors in line with Indoor based intervention tools and its
324 implication: scenario from selected sentinel sites of Ethiopia. *Heliyon*, 8(12).
325 <https://doi.org/10.1016/j.heliyon.2022.e12178>

326 Braack, L., Bornman, R., Kruger, T., Dahan-moss, Y., Gilbert, A., Kaiser, M., Oliver, S. V., Cornel,
327 A. J., Lee, Y., Norris, D. E., Coetzee, M., Brooke, B., & de Jager, C. (2020). Malaria vectors
328 and vector surveillance in Limpopo province (South Africa): 1927 to 2018. *International*
329 *Journal of Environmental Research and Public Health*, 17(11), 1–21.
330 <https://doi.org/10.3390/ijerph17114125>

331 Caminade, C., McIntyre, K. M., & Jones, A. E. (2019). Impact of recent and future climate change
332 on vector-borne diseases. In *Annals of the New York Academy of Sciences* (Vol. 1436, Issue
333 1, pp. 157–173). Blackwell Publishing Inc. <https://doi.org/10.1111/nyas.13950>

334 Campos, M., Patel, N., Marshall, C., Gripkey, H., Ditter, R. E., Crepeau, M. W., Toilibou, A.,
335 Amina, Y., Cornel, A. J., Lee, Y., & Lanzaro, G. C. (2023). Population Genetics of *Anopheles*
336 *pretoriensis* in Grande Comore Island. *Insects*, 14(1), 1–8.
337 <https://doi.org/10.3390/insects14010014>

338 Ciubotariu, I. I., Jones, C. M., Kobayashi, T., Bobanga, T., Muleba, M., Pringle, J. C., Stevenson,
339 J. C., Carpi, G., & Norris, D. E. (2020). Genetic diversity of *Anopheles coustani* (Diptera:
340 Culicidae) in malaria transmission foci in southern and central africa. *Journal of Medical*
341 *Entomology*, 57(6), 1782–1792. <https://doi.org/10.1093/jme/tjaa132>

342 Coetzee, M. (2020). Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae).
343 *Malaria Journal*, 19(1), 1–20. <https://doi.org/10.1186/s12936-020-3144-9>

344 Ebenezer, A., Noutcha, A. E. M., Agi, P. I., Okiwelu, S. N., & Commander, T. (2014). Spatial
345 distribution of the sibling species of *Anopheles gambiae* sensu lato (Diptera: Culicidae) and

346 malaria prevalence in Bayelsa State, Nigeria. *Parasites and Vectors*, 7(1), 1–6.
 347 <https://doi.org/10.1186/1756-3305-7-32>

348 Ebenezer, A., Okiwelu, S. N., Agi, P. I., Noutcha, M. A. E., Awolola, T. S., & Oduola, A. O. (2012).
 349 Species composition of the *Anopheles gambiae* complex across eco-vegetational zones in
 350 Bayelsa state, Niger Delta region, Nigeria. *Journal of Vector Borne Diseases*, 49(3), 164–
 351 167.

352 Epopa, P. S., Collins, C. M., North, A., Millogo, A. A., Benedict, M. Q., Tripet, F., & Diabate, A.
 353 (2019). Seasonal malaria vector and transmission dynamics in western Burkina Faso. *Malaria*
 354 *Journal*, 18(1), 1–13. <https://doi.org/10.1186/s12936-019-2747-5>

355 Garba, LC. , Oyieke, FA. , Owino. EA., Mwansat, GS. , Houmsou, RS. , Darda, E., & Chintem, D.
 356 (2017). Species Diversity and Relative Abundance of Anopheline Vectors of Malaria on the
 357 Highlands of Mambilla Plateau Northeast, Nigeria. *Journal of Biotechnology and*
 358 *Bioengineering*, 1(1), 37–42.

359 Goupeyou-Youmsi, J., Rakotondranaivo, T., Puchot, N., Peterson, I., Girod, R., Vigan-Womas, I.,
 360 Paul, R., Ndiath, M. O., & Bourgouin, C. (2020). Differential contribution of *Anopheles*
 361 *coustani* and *Anopheles arabiensis* to the transmission of *Plasmodium falciparum* and
 362 *Plasmodium vivax* in two neighbouring villages of Madagascar. *Parasites and Vectors*, 13(1),
 363 1–16. <https://doi.org/10.1186/s13071-020-04282-0>

364 Joseph M Mwangangi, Ephantus J Muturi, Simon M Muriu, Joseph Nzovu, Janet T Midega, &
 365 Charles Mbogo. (2013). The role of *Anopheles arabiensis* and *Anopheles coustani* in indoor
 366 and outdoor malaria transmission in Taveta District, Kenya. *Parasites and Vectors*, 6(114),
 367 1–9.

368 Lafferty, K. D., & Mordecai, E. A. (2016). The rise and fall of infectious disease in a warmer world.
 369 In *F1000Research* (Vol. 5). Faculty of 1000 Ltd.
 370 <https://doi.org/10.12688/F1000RESEARCH.8766.1>

371 Lobo, N. F., St. Laurent, B., Sikaala, C. H., Hamainza, B., Chanda, J., Chinula, D., Krishnankutty,
 372 S. M., Mueller, J. D., Deason, N. A., Hoang, Q. T., Boldt, H. L., Thumlop, J., Stevenson, J.,
 373 Seyoum, A., & Collins, F. H. (2015). Unexpected diversity of *Anopheles* species in Eastern
 374 Zambia: Implications for evaluating vector behavior and interventions using molecular tools.
 375 *Scientific Reports*, 5(December), 1–10. <https://doi.org/10.1038/srep17952>

376 National Malaria Control Programme. (2020). National Malaria Strategic Plan, 2021 – 2025. In
 377 *National Malaria Strategic Plan, 2021 – 2025*.

378 National Malaria Elimination Programme, & Federal Ministry of Health Nigeria. (2014). *Nigeria*
 379 *National Malaria Strategic Plan (2014-2020)* (pp. 1–132).

380 Nelly Armanda Kala-Chouakeu, Edmond Kopya, Vasileia Balabanidou, Borel Tchamen Djiappi,
 381 Kyriaki Maria Papapostolou, Timoléon Tchuinkam, & Christophe Antonio-Nkondjio. (2022).
 382 DDT Resistance in *Anopheles pharoensis* from Northern Cameroon Associated with High

383 Cuticular Hydrocarbon Production. *Genes*, 13, 1–8. [https://doi.org/doi:](https://doi.org/doi:10.3390/genes13101723)
384 10.3390/genes13101723

385 Nkya, T. E., Fillinger, U., Sangoro, O. P., Marubu, R., Chanda, E., & Mutero, C. M. (2022). Six
386 decades of malaria vector control in southern Africa: a review of the entomological evidence-
387 base. *Malaria Journal*, 21(1), 1–16. <https://doi.org/10.1186/s12936-022-04292-6>

388 Olatunbosun-Oduola, A., Abba, E., Adelaja, O., Taiwo-Ande, A., Poloma-Yoriyo, K., & Samson-
389 Awolola, T. (2019). Widespread report of multiple insecticide resistance in *Anopheles*
390 *gambiae* s.l. mosquitoes in eight communities in southern Gombe, north-eastern Nigeria.
391 *Journal of Arthropod-Borne Diseases*, 13(1), 50–61. <https://doi.org/10.18502/jad.v13i1.932>

392 Oyewole, I. O., Awolola, T. S., Ibidapo, C. A., Oduola, A. O., Okwa, O. O., & Obansa, J. A. (2007).
393 Behaviour and population dynamics of the major anopheline vectors in a malaria endemic
394 area in southern Nigeria. *Journal of Vector Borne Diseases*, 44(1), 56–64.

395 Oyewole, I. O., Ibidapo, C. A., Okwa, O. O., Oduola, A. O., Adeoye, G. O., Okoh, H. I., & Awolola,
396 T. S. (2010). Species Composition and Role of *Anopheles* Mosquitoes in Malaria
397 Transmission Along Badagry Axis of Lagos Lagoon, Lagos, Nigeria. *International Journal*
398 *of Insect Science*, 2, IJIS.S4698. <https://doi.org/10.4137/ijis.s4698>

399 PMI. (2021). *The PMI VectorLink Nigeria 2019-2020 Annual Entomology Report. September*.
400 [https://d1u4sg1s9ptc4z.cloudfront.net/uploads/2021/08/Entomological-Monitoring-Report-](https://d1u4sg1s9ptc4z.cloudfront.net/uploads/2021/08/Entomological-Monitoring-Report-Nigeria-2019-2020.pdf)
401 [Nigeria-2019-2020.pdf](https://d1u4sg1s9ptc4z.cloudfront.net/uploads/2021/08/Entomological-Monitoring-Report-Nigeria-2019-2020.pdf)

402 Saili, K., de Jager, C., Sangoro, O. P., Nkya, T. E., Masaninga, F., Mwenya, M., Sinyolo, A.,
403 Hamainza, B., Chanda, E., Fillinger, U., & Mutero, C. M. (2023). *Anopheles rufipes*
404 implicated in malaria transmission both indoors and outdoors alongside *Anopheles funestus*
405 and *Anopheles arabiensis* in rural south-east Zambia. *Malaria Journal*, 22(1), 1–12.
406 <https://doi.org/10.1186/s12936-023-04489-3>

407 Sinka, M. E. (2013). Global distribution of the dominant vector species of malaria,
408 *Anopheles* mosquitoes. In *New insights into malaria vectors* (pp. 109–143).

409 Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P.,
410 Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Van Boeckel, T., Godfray,
411 H. C. J., Harbach, R. E., & Hay, S. I. (2010). The dominant *Anopheles* vectors of human
412 malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and
413 bionomic précis. *Parasites and Vectors*, 3(1). <https://doi.org/10.1186/1756-3305-3-117>

414 Suh, P. F., Elanga-Ndille, E., Tchouakui, M., Sandeu, M. M., Tagne, D., Wondji, C., & Ndo, C.
415 (2023). Impact of insecticide resistance on malaria vector competence: a literature review. In
416 *Malaria Journal* (Vol. 22, Issue 1). BioMed Central Ltd. [https://doi.org/10.1186/s12936-023-](https://doi.org/10.1186/s12936-023-04444-2)
417 [04444-2](https://doi.org/10.1186/s12936-023-04444-2)

418 Tabue, R. N., Awono-Ambene, P., Etang, J., Atangana, J., Antonio-Nkondjio, C., Toto, J. C.,
419 Patchoke, S., Leke, R. G. F., Fondjo, E., Mnzava, A. P., Knox, T. B., Tougordi, A., Donnelly,

- M. J., & Bigoga, J. D. (2017). Role of *Anopheles (Cellia) rufipes* (Gough, 1910) and other local anophelines in human malaria transmission in the northern savannah of Cameroon: a cross-sectional survey. *Parasites and Vectors*, 10(1), 1–11. <https://doi.org/10.1186/s13071-016-1933-3>
- Wahedi, J. A., Ande, A. T., Oduola, A. O., & Obembe, A. (2021). Bendiocarb resistance and associated deltamethrin and DDT resistance in *Anopheles gambiae* s.l. populations from North Eastern Adamawa State, Nigeria. *Ceylon Journal of Science*, 50(1), 63. <https://doi.org/10.4038/cjs.v50i1.7848>
- Wahedi, J. A., Ande, A. T., Oduola, A. O., Obembe, A., Tola, M., Oyeniyi, T. A., & Awolola, T. S. (2020). Dynamics of malaria vector indices in two vegetation zones within North Eastern Adamawa State, Nigeria. *Tropical Biomedicine*, 37(3), 637–649. <https://doi.org/10.47665/tb.37.3.637>
- WHO. (2023). *World malaria report 2023*. World Health Organization. <https://www.wipo.int/amc/en/mediation/>
- Wilkins, E. E., Howell, P. I., & Benedict, M. Q. (2006). IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis*. *Malaria Journal*, 5, 1–7. <https://doi.org/10.1186/1475-2875-5-125>
- World Health Organization. (2016). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes Second edition* (2nd ed.). World Health Organization.
- Yoriyo, K. P., Alo, E. B., Naphtali, R. S., Samdi, L. M., Oduola A. O. & Awolola T. S. (2014). *Anopheles* species abundance and vectorial competence in four local government areas of Gombe state, Northeastern Nigeria. *International Journal of Advanced Studies in Engineering and Scientific Inventions*, 2(1), 19–25.