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Species Composition, Feeding Behaviour and Plasmodium falciparum infectivity of Malaria Vectors along an altitudinal gradient in parts of Plateau State, Nigeria

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Abstract

Background

Malaria still persists as a major cause of mosquito-borne diseases, particularly in the tropics. Among several risk factors, the feeding behaviour of the vectors is critical. This study, conducted in 2023 investigated the species composition, feeding patterns and sources of blood meals of *Anopheles* mosquitoes across four Local Government Areas (LGAs) in Plateau State, Nigeria.

Results

Overall, 798 mosquitoes were sampled and initially identified morphologically and thereafter by polymerase chain reaction (PCR). Samples were collected using Centers for Disease Control mosquito traps (CDC), (35.2%) and pyrethrum spray catch (PSC) (64.8%). *An. gambiae* sensu lato (86.5%) was the dominant species, while *An. rufipes* (0.4%) ($p < 0.05$) was the least abundant. *Anopheline* species diversity and abundance was more in the study sites in Shendam (81.0%) followed by Mangu (17.5%); these 2 LGAs accounted for most of the collection, with higher collections made in October (57.6%) and April (25.3%) ($p < 0.05$). *An. gambiae* and *An. funestus* were the only species occurring indoors, while *An. funestus* exhibited a random feeding pattern; *An. gambiae* fed throughout the night. Most (83.2%) collections with PSC were fed ($p > 0.05$). PCR identification showed that 331 (46.83%) of the 798 anophelines collected were *An. coluzzii*, 157 (47.43%) *An. gambiae s.s*, 4 (1.21%) *An. gambiae s.l*, while 15 (4.53%) were unamplified ($p < 0.005$). The blood fed mosquitoes were 239 (72.21%), with 97.67% feeding on humans, and 2.33% feeding on dogs. Mangu (1.19%) and Shendam (3.13%) were the only locations where feeding on dogs was recorded ($p > 0.05$). Overall, *P. falciparum* sporozoite was detected in 18.75% of the collection, with *An. gambiae* ss accounting for most (21.88%) of the infection ($p > 0.05$).

Conclusions

This study reinforces the need for continuous vector surveillance and highlights the role of behavioral plasticity in mosquito feeding, particularly in regions where outdoor sleeping habits may expose populations to increased risk of mosquito bites.

Background

Emerging infectious diseases have taken the center stage of global public health watch since the outbreak of the COVID-19 pandemic reported in 2019, particularly infectious diseases transmitted by vectors through the infectious bites of blood-sucking arthropods (Siriyasatien et al., 2023). Notably, across the globe, mosquito-borne infections continue to pose significant public health concern causing deaths in humans, wildlife, livestock and severe economic losses (Moyer, 2009).

Apart from the nuisance they cause, mosquitoes are known to cause significant public health diseases with about 100 of the known 3,500 species able to transmit infectious pathogens to humans including the deadly malaria parasite. Other infections transmitted by mosquitoes include dengue fever, chikungunya fever, yellow fever, lymphatic filariasis, rift valley fever, Japanese encephalitis, and other viral encephalitis (Kalaivani et al., 2015).

Malaria remains foremost in terms of morbidity and mortality caused by mosquito-borne pathogen. *Anopheles gambiae* s.l. (Ekedo & Ukpai, 2018) and other members of the genus *Anopheles* are responsible for transmitting malaria infection. Although, control efforts have span across decades of interventions, malaria continues to be a major public health challenge, especially in sub-Saharan Africa, including Nigeria. Reports have indicated that in 2023 alone, malaria infections were estimated at 263 million with about 597,000 deaths attributed to the infection. This represents a rise in malaria infection by about 11 million compared to 2022, even though there was not much difference in the deaths recorded between the two years. However, about 95% of the global deaths due to malaria is accounted for by the WHO African Region, with majority of the burden suffered by children under five years old, of which nearly 40% of the recorded deaths in this vulnerable population was recorded in Nigeria World Health Organization (WHO, 2024).

The female *Anopheles* mosquito feeds on blood, an important factor associated with the epidemiology of malaria infection (Garrett-Jones et al., 1980). This behavior of the female mosquito influences its ability to transmit the *Plasmodium* parasite (Koutsos et al., 2007), and for an infection to be established, it depends on the vertebrate hosts from which the vector feeds and this determines to what extent the disease is distributed spatially (Richards et al., 2006). The major malaria vectors have been known to feed primarily on humans, although they may feed secondarily on cattle and other domestic animals. However, the choice of these hosts for blood feeding depends on their availability (Garrett-Jones et al., 1980). The choice and subsequent feeding success of a vector (Becker et al., 2012; Main et al., 2016) together with other factors such as host size and accessibility, density, defense mechanisms, and proximity to mosquito habitats (Lyimo & Ferguson 2009; Okwa, 2012); flight behavior, environmental factors, and feeding periodicity of the mosquitoes are also key factors to be considered (Molaei et al., 2008). The abdominal State of female *Anopheles* mosquitoes, specifically whether they are unfed, freshly fed, or gravid, is directly related to malaria sporozoite rates, with infection often found in both fed and unfed individuals. The physiological state of the mosquito influences its likelihood of carrying *Plasmodium* sporozoites in their salivary glands, as well as their foraging behavior (Aschale et al., 2020; Machani et al., 2020).

The transmission of mosquito borne pathogens happens due to the feeding habits of mosquitoes. Most Anopheles species exhibit varied blood-feeding patterns and sometimes overlapping host preferences (Becker et al., 2012). The correct identification of the preferred host for malaria vectors fosters a better understanding of how local malaria transmission is maintained within a population (Altahir et al., 2022). An in depth understanding of how mosquitoes interact with the hosts and disease pathogens, as well as how the community shapes the dynamics of their behavior can help to give insight into region-specific

disease transmission dynamics, and diseases spread and help in designing effective vector control tools (Sriwichai et al., 2016).

This study therefore focused on identifying the malaria vectors in Plateau State and understanding their feeding behaviour in an attempt to create more insight and seek out better measures aimed at vector control and the prevention of disease transmission.

Methods

Study area

This study was conducted in Plateau State, which is a state in north-central Nigeria. Plateau State is the twelfth largest State in Nigeria by land mass. According to population statistics provided on the website of the National Population Commission (National Population Commission (NPC) [Nigeria], & National Bureau of Statistics (NBS), 2017), more than four million people reside in the State, majority of whom are engaged in agrarian, mining or pastoral livelihood activities. Administratively, seventeen local government areas constitute the State of which eight lie in the geographic highlands (>800-1,500m above sea level) and the remaining LGAs in lowland areas of the State.

The study was conducted in four LGAs of Plateau State (Jos North, Jos South, Mangu and Shendam). The State is located in the Northern Guinea Savanna vegetation belt and has one of the two plateaux in Nigeria. The highland geographical landscape predominates in the northern part of the State including Jos, the state capital. The Plateau highland elevation averages 1280 metres having its highest peak rising above 1529 m of the famous Shere hills and is estimated to cover an area of about 8600 km^2. The lowland area averages 400m above sea level. The geographical coordinates lie between latitude 9.8965ºN and longitude 8.8583ºE. The climate is semi-temperate, and temperature can range between 18°C (64.4 ºF) to 25°C (77.0 ºF). Annual rainfall in Jos can reach up to 1400 mm (55.1 in). The Plateau State serves as a major hydrological source for several rivers in northern Nigeria, many of which are characterized by rapid flow. In the dry months, water levels are low resulting in isolated stagnant pools that provide suitable breeding habitats for mosquitoes. (Goselle et al., 2017). It is reported that there are over 1,000 mining leases that have left thousands of mining ponds, some small others medium to large and very deep ponds created by tin mining rigs.

Study design

This was a longitudinal study that lasted for three (3) sampling days in each quarter over 12 consecutive months in 2023. Collection of adult mosquito samples occurred in January, April, July and October, with houses selected randomly for the study. From the four LGAs: two communities, one urban and the other rural, were picked in Mangu and Shendam respectively, with a community each in Jos North and Jos South. Collection of adult mosquito samples was done using CDC light traps deployed in two (2) households while the PSC technique was used in ten (10) households in each community; no household

had both CDC and PSC deployed to collect the samples. At each outing, mosquito collections were done for three (3) consecutive days.

Sample collection

Two trapping methods were employed for mosquito collections. The CDC light trapping method was employed for hourly collection of mosquitoes from both indoors and outdoors locations while the Pyrethrum Spray Catch method served for the purpose of collecting indoor mosquitoes that stayed back after a blood meal.

CDC collection method

Both indoors and outdoors mosquito samples were collected using the CDC light traps (CDC-LT). The traps were deployed by suspending at an approximate distance of 1.5 m from the ground (near the foot of the bed). This is the effective flight zone for host-seeking mosquitoes. A human bait slept under an untreated net where the trapping was done. The trap ran through the night from 6:00 pm to 6:00 am, and hourly mosquito catches were transferred from the trap into labeled paper cups (Swai et al., 2023) and thereafter identified.

PSC collection method

Mosquitoes that stayed back in the room after a blood meal were collected indoors through the Pyrethrum Spray Catch method. The collection was done between 6:00 am and 9:00 am. In selected rooms where people slept, white linen was used to cover the floor and other flat surfaces after removing all uncovered food items. A pyrethrum-based insecticide spray was then sprayed in the room after shutting everywhere, and 10 minutes afterwards, the knocked down mosquitoes were collected into labeled petri dishes for identification (Ndiath et al., 2011).

Morphological identification of mosquito species

All adult mosquito samples collected were taken to the lab, and anophelines were sorted from non-anopheline samples; male mosquitoes were also sorted from female samples. Morphological identification of the sorted female samples was done using dissecting microscope and the guide to mosquito species identification by Coetzee (2004) and Kent (2006). The identified samples were preserved using silica gel in Eppendorf tubes.

Molecular identification of mosquito species

DNA extraction

The appendages (wings and legs) of the collected samples (n=331) were dissected out from each mosquito and placed in separate tubes and genomic DNA samples were extracted using the kit manufactured by the Nigeria Institute of Medical Research (NIMR), Lagos State, (BIOTECH). After adding 500µl lysis buffer, the samples were homogenized mechanically using mortar and pestle, and the

mixture vortexed and incubated for 10 minutes at 56℃. This was followed by centrifugation at 10,000 rpm for 1 minute. Thereafter, 200 µL of absolute ethanol was added, and the mixture transferred to a spin column and spun for 30 seconds at 10,000rpm. The supernatant was discarded, and the tube blotted on tissue paper, after which 500 µL of wash buffer 1 was added and spun again for 30 seconds at 10,000 rpm. The flow-through was discarded and the collection tube blotted on a tissue paper. The spin column was then centrifuged at 12,000rpm for 3 minutes to remove the remaining ethanol and thereafter transferred to a fresh microcentrifuge tube. 50 µL of elution buffer was then added and incubated at room temperature for to 2 minutes. The mix was finally centrifuged at 10,000rpm for 1 minute to elute the DNA which was then stored at -20 °C until required.

DNA amplification and electrophoresis

The species-specific primers for *Anopheles sensu stricto* (S form; 5-CCAGACCAAGATGGTTCGCTG-3), *An. gambiae s.s* (5-GCT TAC TGG TTT GGT CGG CATTG-3), *An. colluzzi*, 5-TAGCCAGCTCTTGTCCACTAGTTTT-3), *Anopheles merus* (5 CAACCCACTCCCTTGACGATG -3), *An. arabiensis* (5-GTGTTAAGTGTCCTTCTCCGTC -3), and An. *quadriannulatus* (5-GCATGTCCAAGATGGTTCGCTG -3) were used for PCR reaction. Each reaction mix contained 2.5µl master mix, 0.5 µl of IMP-UN, IMP-S1 and IMP-M1 specific primers, 5.5µl ddH_2O, 1µl of DNA, AR-3T, QD-3T GA-3T, and ME-3T. The thermal conditions were an initial denaturation at 95℃ for 5min (1 cycle), denaturation at 95℃ for 30 seconds and annealing at 59.2℃ for 30 seconds, followed by extension at 72℃ for 30 sec (30 cycles), a final extension at 72℃ for 5min (1 cycle), and a final hold at 4℃. The PCR product was run on a 1.5% agarose gel at 100v with 150mA for 45min and visualized under UV transilluminator (Wilkins et al., 2005)

Detection of mosquito bloodmeal sources

Abdomens of the anopheline specimens tested for blood meal sources were teased out singly. DNA was extracted using the NIMR extraction kit as described above. The procedure described by Kent & Norris (2006) was adopted for PCR amplification and detection of blood meal sources. In brief, the cytochrome b-based PCR blood meal identification assay included

Pig 573F-5'CCTCGCAGCCGTACATCTC3', Human 741F-5'GGCTTACTTCTTCATTCTCTC CT 3', Goat 894F-5'CCTAATCTTAGTACTGTACCCTTCCTC3', Dog 368F 5'GGAATTGTACTATTATTCGCAACCAT3', Cow 121F 5'CATCGGCACAAATTTAGTCG3', UNREV 1025 5'GGTTGTCCTCCAATTCATGTTA 3'. The cycling conditions were an initial denaturation at 95℃ for 15min (1 cycle), denaturation for 1 min at 95℃, annealing at 58℃ for 30 sec, followed by extension at 72℃ for 1min (35 cycles), a final extension at 72℃ for 5min (1 cycle), and a final hold at 4℃. The PCR product was run on a 1.5% gel at 100 V with 150 mA for 45 min and visualized under a UV transilluminator.

Detection of sporozoites in the *Anopheles* mosquitoes

The head and salivary glands of 64 collected samples were teased out from each mosquito, and the DNA samples extracted as explained above. The detection of the sporozoite utilized a nested PCR

technique. The first amplification used genus-specific primers rPLU5 (CCTGTTGTTGCCTTAAACTTC) and rPLU6 (TTAAAATTGTTGCAGTTAAAACG), following the cycling conditions of: initial denaturation for 5 min at 95°C, and a second denaturation for 1 min at 94°C. Annealing and extension were both for 2 min at 58°C and 72°C each and final extension at 72°C for 5 min (24 cycles). This was followed by a second amplification using species-specific primers: rFAL(TTAAACTGGTTTGGGAAAACCAAATATATT and fFAL (ACACAATGAACTCAATCATGACTACCCGTC) and rVIV(CGCTTCTAGCTTAATCCACATAACTGATAC and fVIV(ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA) targeting *P. falciparium* and *P. vivax* (as secondary) respectively. The initial denaturation was for 5 min at 95°C, followed by a further denaturation at 94°C for 1min. Annealing and extension both lasted for 2 min each at 58°C and 72°C respectively and final extension at 72°C for min (30 cycles). The PCR product was run on a 1.5% agarose gel at 100 V with 150 mA for 45min and visualized under a UV transilluminator (Snounou et al., 1993).

Data analysis

The data on mosquito abundance and feeding behaviour collected as well as weather variable data were first cleaned in Microsoft Excel and subsequently imported into R version 4.5.1 (2025-06-13 ucrt) for statistical analysis. Given that some expected counts were less than 5, Fisher's Exact test was used to assess whether there were significant differences in anopheline species abundance across trapping methods, collection locations, and sampling months. Additionally, the same test was used to determine whether the abdominal State of the species and their blood meal sources differed significantly. Results were considered statistically significant at a threshold of $p < 0.05$.

Results

Overall abundance of anopheline species collected

Table 1 contains a summary of all anopheline species collected using the two trapping methods (CDC and PSC). A total of 798 anopheline mosquitoes identified morphologically yielded four species. CDC trapped anophelines accounted for 35.2% of the collection while PSC accounted for the remaining 64.8%. Among the collections made, *An. gambiae* was the most abundant of the anopheline species (86.5%) followed by *An. funestus* (12.5%) with the least being *An. rufipes* accounting for only 0.4%. Fisher’s Exact statistical test revealed that mosquito abundance differed significantly between the two trapping methods ($p<0.05$).

Abundance of anopheline species collected across locations

Across locations (Table 2), significantly higher numbers of anophelines were collected in Shendam, 646 (81.0%) compared to the other locations. Mangu had the second most abundant collection (17.5%). Overall, higher number of anophelines were collected in October (57.6%) and April (25.3%) with the least catches in January (3.6%). Fisher’s Exact statistical test showed a significant difference in mosquito species abundance across months ($p<0.05$) (Table 3).

TABLE 1 Species composition of anophelines collected by two methods

Species	Method of collection		Total
	CDC	PSC	
An. funestus	14(14.0.0)	86(86)	100(12.5)
An. coustani	5(100)	0(0.0)	5(0.6)
An. gambiae	259(37.5)	431(62.5)	690(86.5)
An. rufipes	3(100)	0(0.0)	3(0.4)
Total	281(35.2)	517(64.8)	798(100)

Fisher's Exact Test: p-value = $4.985e^{-09}$

TABLE 2 Distribution of anopheline species from sampling sites across the four Local Government Areas Location

Species	Location				Total
	Jos north	Jos south	Mangu	Shendam	
An. funestus	2(2.0)	6(6.0)	17(17.0)	75(75.0)	100(12.5)
An. coustani	0(0.0)	0(0.0)	0(0.0)	5(100)	5(0.6)
An. gambiae	0(0.0)	3(0.4)	122(17.7)	565(81.9)	690(86.5)
An. rufipes	1(33.3)	0(0.0)	1(33.3)	1(33.3)	3(0.4)
Total	3(0.4)	9(1.1)	140(17.5)	646(81.0)	798(100)

Fisher's Exact Test: p-value = $7.836e^{-06}$

TABLE 3 Monthly distribution of anopheline species.

Species	Month				Total
	January	April	July	October	
An. funestus	12(12.0)	38(38.0)	31(31.0)	19(19.0)	100(12.5)
An. coustani	0(0.0)	5(100)	0(0.0)	0(0.0)	5(0.6)
An. gambiae	15(2.2)	158(22.9)	76(11.0)	441(63.9)	690(86.5)
An. rufipes	2(66.7)	1(33.3)	0(0.0)	0(0.0)	3(0.4)
Total	29(3.6)	202(25.3)	107(13.4)	460(57.6)	798(100)

Fisher's Exact Test: p-value = $2.2e^{-16}$

Abundance of anophelines from indoors and outdoors location

Figure 2 illustrates the distribution of the species collected indoors relative to feeding time. Two *Anopheles* species were encountered indoor: *An. gambiae* and *An. funestus*. *An. funestus* exhibited a very random feeding pattern, peaking at 21:00-22:00, while *An. gambiae* fed throughout the night with four feeding peaks recorded, with the highest being at 01:00-02:00. A larger number of Anopheles species were encountered outdoors (Figure 3). However, Only *An. gambiae* was encountered almost throughout the night, with peak feeding time observed at 22:00-23:00 and 24:00-01:00 (Figure 3).

Molecular identification and abdominal status of anophelines collected

In Figure 4, the abdominal status of the anophelines collected using the PSC method is shown in which 83.2% had had blood meal, 7.7% were gravid, and 5.6% half gravid. Only 3.5% were unfed. Statistical tests however indicated no significant difference in the physiological State of the collected samples ($p>0.05$).

Of the 798 mosquitoes collected, 331 were molecularly identified using PCR (Figure 5, Plate 1). Of these, 155(46.83%) were identified as *An. coluzzi*, 157 (47.43%) as *An. gambiae s.s,* 4 (1.21%) as *An. gambiae s.l*, and the remaining 15 (4.53%) were unamplified ($p<0.005$).

Identification of blood meal hosts of collected anophelines

Among all specimens identified, 239 (72.21%) were blood fed. Further analysis revealed that most of the fed mosquitoes fed on humans (97.67%), only 2.33% fed on dogs. Feeding on dogs among *An. coluzzi* (2.59%) and *An. gambiae s.s* (2.04%). However, both species also feed on humans (97.41% and 97.96% respectively) ($p>0.05$) (Table 4). Across locations, Mangu (1.19%) and Shendam (3.13%) were the only locations where feeding on dogs was recorded ($p>0.05$). While feeding on humans was recorded across all three locations (Table 5).

Detection of *Plasmodium* sporozoite in collected anophelines

Of the 64 samples tested for *P. falciparum* sporozoites (Table 6), 12 were found positive, giving a total prevalence of 18.75% ($p < 0.001$). Out of the 12 positive samples, 4 (14.29%) were *An. colluzzi,* 7 (21.88%) *An. gambiae* ss while the remaining 1 (25.00%) was, identified simply as *An. gambiae* s.l. as it failed to identify to the species level. However, statistical test indicated no significant difference ($p > 0.05$) in the sporozoite rates observed across the different *Anopheles* species.

Sources of bloodmeals in the anophelines showed that *An. gambiae* s.s. and *An. coluzzi* which occurred in near equal proportions, were co-dominant hosts and fed almost exclusively on humans (Plate 2). Both species also feed in near equal proportions on dog blood meal (Table 4).

TABLE 4 Blood meal hosts of anopheline species

Species	Source of blood meal		Total
	Dog	Human	
An. coluzzi	3 (2.59)	113 (97.41)	116 (53.95)
An. gambiae s.s.	2 (2.04)	96 (97.96)	98 (45.58)
An. gambiae s.l.	0 (0.00)	1 (100)	1 (0.47)
Total	5 (2.33)	210 (97.67)	215 (100)

Fisher's Exact Test: p = 1.000

TABLE 5 Source of mosquito bloodmeals encountered across locations

Location	Blood meal source		Total
	Dog	Human	
Jos North	0 (0.00)	3 (100)	3 (1.40)
Mangu	1 (1.19)	83 (98.81)	84 (39.07)
Shendam	4 (3.13)	124 (96.88)	128 (59.53)
Total	5 (2.33)	210(97.67)	215 (100)

Fisher's Exact Test = 0.674

TABLE 6 Detection of sporozoites in collected mosquito samples

Species	No. examined	No. infected	% Infected
An. colluzzi	28	4	14.29
An. gambiae ss	32	7	21.88
Unamplified	4	1	25.00
Total	64	12	18.75

Overall: Fisher's Exact Test = 0.594

Across species: Chi square =25.000, df=1, p-value < 0.001

Discussion

Mosquitoes constitute a significant public health threat to vulnerable populations in malaria-endemic countries, with Nigeria being the chief. Haematophagous *Anopheles gambiae* s.l. in Nigeria is incriminated with malaria transmission and other infectious pathogens. This study was conducted to identify and determine the anopheline species responsible for maintaining *P. falciparum* transmission in selected urban and rural communities of Plateau State in the Middle Belt of Nigeria.

Analysis of anopheline samples collected from sites in the four LGAs in 2023 using two different trapping methods yielded798 *Anopheles*. Pyrethrum Spray Catch collections were the most abundant in contrast to published accounts by Adunga et al. (2021), Mayagaya et al. (2015), and Getachew et al. (2019) who observed higher numbers of mosquitoes collected using traps. This is thought to be impacted by the anophelines active bloodmeal search as against when resting. Specifically, Adunga et al. (2021) recorded higher numbers of unfed mosquitoes caught by the same sampling techniques and attributed this to the higher number of catches made using the trap. Results presented here show that most of the mosquito samples collected were blood fed, confirming the known behavior of the anophelines after a blood meal as they rest to initiate digestion before taking a flight.

Four mosquito species were identified morphologically in the collection (*Anopheles coustani, Anopheles gambiae, Anopheles funestus*, and *Anopheles rufipes*), with *An. gambiae* as the dominant species (86.5%), followed by *An*. *funestus* (12.5%). These two have been identified as the primary malaria vectors particularly *An. gambiae* s.s in the middle belt and Plateau State as well as other parts of Nigeria (Abba et al., 2025; Yina et al., 2023). Other reports by Oyewole et al. (2007) stated that *An. gambiae* s.s. was the predominant species in studies conducted in parts of Southern Nigeria and suggested that they could as well be largely responsible for malaria transmission in the coastal area. Other studies (Egwu et al., 2018; Lapang et al., 2019; Adeogun et al., 2023) also reported the dominance of *An. gambiae* larvae in Shendam in Plateau and Ohaffia in the southeast of Nigeria.

In this study, Shendam LGA in the lowland Plateau (average 400m asl) accounted for the highest abundance of anophelines encountered, followed by Mangu LGA in the hotter part of the highland Plateau and at an elevation of approximately 1,127 m asl. These locations have weather conditions that favour vector breeding and can also favour malaria transmission compared to the other two locations in Jos North and Jos South, all above 1000m asl. This is consistent with findings from highland sites in Plateau (Nanvyat et al., 2018) and Lagos (Bello et al., 2025) States in Nigeria where temperature, rainfall, and humidity were identified as key climatic determinants of malaria vector abundance and distribution and disease transmission.

Higher abundance of *Anopheles* spp. was encountered in October and April an indication that breeding habitats favoured mosquito abundance. The findings in this study clearly reveal that transmission of malaria and other *Anopheles* borne pathogens could favour seasonal transmission in both lowland and highland communities of Plateau State, with the greater impact being in the lowlands (Awolola et al., 2002; Oyewole et al., 2007), owing to the increase in the population density of the vectors at a given time. This is similar to the report by Oyewole et al. (2007) who found that *Anopheles* mosquitoes abound more

between May and June, a period corresponding to increased rainfall and also found higher sporozoite rates and EIR compared to the other months. Compared to our findings, these months are usually the onset of rains in Plateau State in which more breeding places become available for the breeding of the malaria vectors. However, the number of mosquitoes encountered was less compared to October, which corresponds to the late rainy season in the State.

Only *An. gambiae* and *An. funestus*, were encountered indoors. The peak biting activity for *An. gambiae* was observed between 23:00–24:00 and 01:00–02:00, which is the period when individuals are most likely to be in deep sleep. Notably, biting activity commenced around 21:00, coinciding with the time individuals typically retire to bed. A similar biting pattern was noted for *An. funestus* which occurred in very low density and not unexpectedly exhibited a lower biting density. These observations highlight the nocturnal and opportunistic feeding behavior of these vectors, which tend to optimize feeding times when human activity is minimal, and host defenses are reduced (Oyewole et al., 2007; Kabbale et al., 2013).

The increase in biting during the early hours of the morning may represent a compensatory strategy by mosquitoes to secure blood meals prior to sunrise, especially if earlier feeding attempts were unsuccessful. This behavior reflects an evolutionary adaptation aimed at enhancing feeding success while minimizing host detection and disruption (Guglielmone & Robbins, 2018). The observed feeding behaviour is important particularly for malaria, highlighting the need for sustained protective measures (e.g., use of insecticide-treated nets) throughout the night and into the early morning hours.

Outdoor mosquito collections revealed four morphologically identified species: *Anopheles gambiae* s.l., *An. coustani, An. funestus*, and *An. rufipes*. Among these, *An. gambiae* s.l. demonstrated a near-continuous nocturnal biting pattern, with notable peaks between 22:00–23:00, 24:00–01:00, and 03:00–04:00. This pattern closely resembles the indoor activity observed, reinforcing the role of *An. gambiae* as both an endophagic and exophagic mosquito (Tirados et al., 2006). However, it exhibits a greater tendency for endophagic behavior.

The presence of biting activity at 03:00–04:00 suggests an adaptive strategy by the vector to maximize feeding opportunities before dawn, particularly as outdoor human activity decreases with falling early morning temperatures. In communities in Shendam, where nighttime temperatures average approximately 21–28°C, individuals initially sleep outdoors, favoring the vectors early feeding behavior. However, as cooler morning conditions drive people back indoors, mosquitoes intensify their feeding activity during this window to secure bloodmeal (Bugoro et al., 2011).

The low feeding density observed for *An. coustani, An. funestus*, and *An. Rufipes*, with their activity concentrated between 21:00 and 02:00, may reflect secondary vector status or limited population density. Nonetheless, their presence outdoors during biting hours is epidemiologically significant, especially in areas with inconsistent or partial use of indoor vector control interventions.

The predominance of blood-fed and gravid female *Anopheles* in the indoor collections suggests endophilic resting behaviour following blood feeding. In Dutse LGA, Northwest Nigeria, Sadiq et al. (Abubakar et al., 2023) reported that a majority of indoor *Anopheles gambiae* complex were freshly fed, with gravid and half-gravid stages also present, reflecting similar post-feeding resting patterns. Similarly, (Ezihe et al., 2017) reported that most *Anopheles* were fed or partially fed during indoor collection using the PSC method, consistent with high human–vector contact indoors. Although there was a significant difference in the abdominal stages of the mosquitoes, the result however suggests an active indoor feeding and resting behaviour that is epidemiologically important for malaria transmission.

Contrary to the species identified morphologically in this study, PCR analysis revealed the presence of only *An. colluzzi* and *An. gambiae* (*An. gambiae ss* and *An. gambiae sl*). This discovery underscores the need for proper mosquito identification particularly the use of molecular based techniques which are more specific and less prone to errors as identification of the vector species is critical to their control. Study has shown that in the *An. gambiae* species complex, there are at least eight morphologically indistinguishable species, which are primarily zoophilic (Takken et al., 2024). Among these, *An. gambiae* and *An. colluzzi* are the most closely related species and the primary malaria vectors due to their tendency to feed on humans, their long lifespan, their indoor resting behaviour, and their relatively high abundance (Coetzee 2004; Niang et al., 2019). *An. gambiae* is widespread across much of sub-Saharan Africa, whereas *An. colluzzi* has a more restricted distribution, occurring mainly in West Africa (Sinka et al., 2016). However, their status as important vectors of malaria make them important in this study.

Furthermore, blood meal analysis reveals that both vector species fed humans and dogs. Even though it was observed that, all species have a higher preference for human blood compared to dogs, only *An. colluzzi* and *An. gambiae ss* fed on dogs, with feeding on dogs noted in Mangu and Shendam Local Government Areas. It has been stated that pathogen, vector, and host factors are important in the transmission of vector-borne diseases (Onyango et al., 2020), and host feeding patterns potent possible risks for disease transmission. Other findings have revealed that mosquitoes feed on humans, dogs, and goats (Getachew et al., 2019). Similar to our findings, Jeyaprakasam et al. (2022) reported a preference for *An. gambiae* for humans, followed by dogs. This indicates that the mosquitoes are more attracted to humans and therefore, more prone to malaria and other diseases transmitted by mosquitoes. A recent study in Plateau state (Ogbu et al., 2020), highlighted that most homes visited are found with one or two dogs cohabiting with the occupants. Such social leaving and proximity to these animals could offer the vectors an alternate host when the human host is not readily available. This study further agrees with earlier works that reported that malaria vectors have shifted little from feeding on humans to feeding on animals (Adugna et al., 2021). Although, the fact that these vectors are seen feeding on dogs, there is the likelihood of transmitting zoonotic infection from these animals to humans, further study is needed in this area.

Overall, the sporozoite rate recorded in this study was 18.75%, with higher infectivity in *An. gambiae* ss (21.88%) compared to *An. coluzi* (14.29%). Compared to this study, other studies, such as that by Khatib et al. (2025), have reported a very low sporozoite rate of about 0.3% out of 2,731 *An. arabiensis* and *An.*

gambiae s.s. sampled in Zanzibar. Similarly, Abba et al. (2025) also found a sporozoite rate of 0.08% in anophelines in their studies conducted in Plateau State, Nigeria. However, the high rate of sporozoite detection in the current study agrees with the earlier report by Mafuyai et al. (2022) that there is a high incidence of malaria, particularly in Southern Plateau State. Thus, this result suggests that active malaria transmission is occurring in the area, likely driven by *An. gambiae* s.s., judging from the recorded sporozoite rate.

Conclusions

This study has revealed the abundance of the malaria vector in Plateau State Nigeria, particularly, *An. gambiae.* The vector species abound particularly in the late and early periods of rain where there is an abundance of breeding habitats with majority of the vectors being endophagic with a high preference for human blood even though some feed on dogs. Feeding activity is highest in the late hours of the night and early hours of the morning when human activity is minimal to avoid disruption to their feeding. The study indicated a high infectivity rate particularly in *An. gambiae* ss suggesting active malaria transmission in the area. The result of the PCR performed in this study highlights the need for proper vector identification particularly in infectious disease surveillance as this will enable appropriate and targeted vector control.

Declarations

Ethics Approval and Consent to Participate in the study

This study protocol was approved by the University of Jos Central Health Research Ethics Committee (UJCHREC/26/062). Verbal informed consent was also obtained from all heads of household where mosquitoes were collected.

Consent for publication

Not applicable

Availability of Data and Materials

The corresponding author will upon reasonable request make available without undue reservations the raw data supporting the conclusions of this article.

Competing interests

Authors declare no conflict of interest.

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Clinical trial number

Not applicable

Author contributions

HBM, TI, GSM, NN, and MAA: conceptualization. EOO, LPM, MJM, IL, NMJ, AO, and RLS: field data collection. IL, LPM and ZOI: Sample processing and analysis: AO, NG, DEO and EOO: data analysis and manuscript drafting. HBM, NN, MAA, and GSM: draft review. All authors reviewed the manuscript and approved the submitted version.

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Plate

Plate 1 and 2 are available in the Supplementary Files section.

Figures

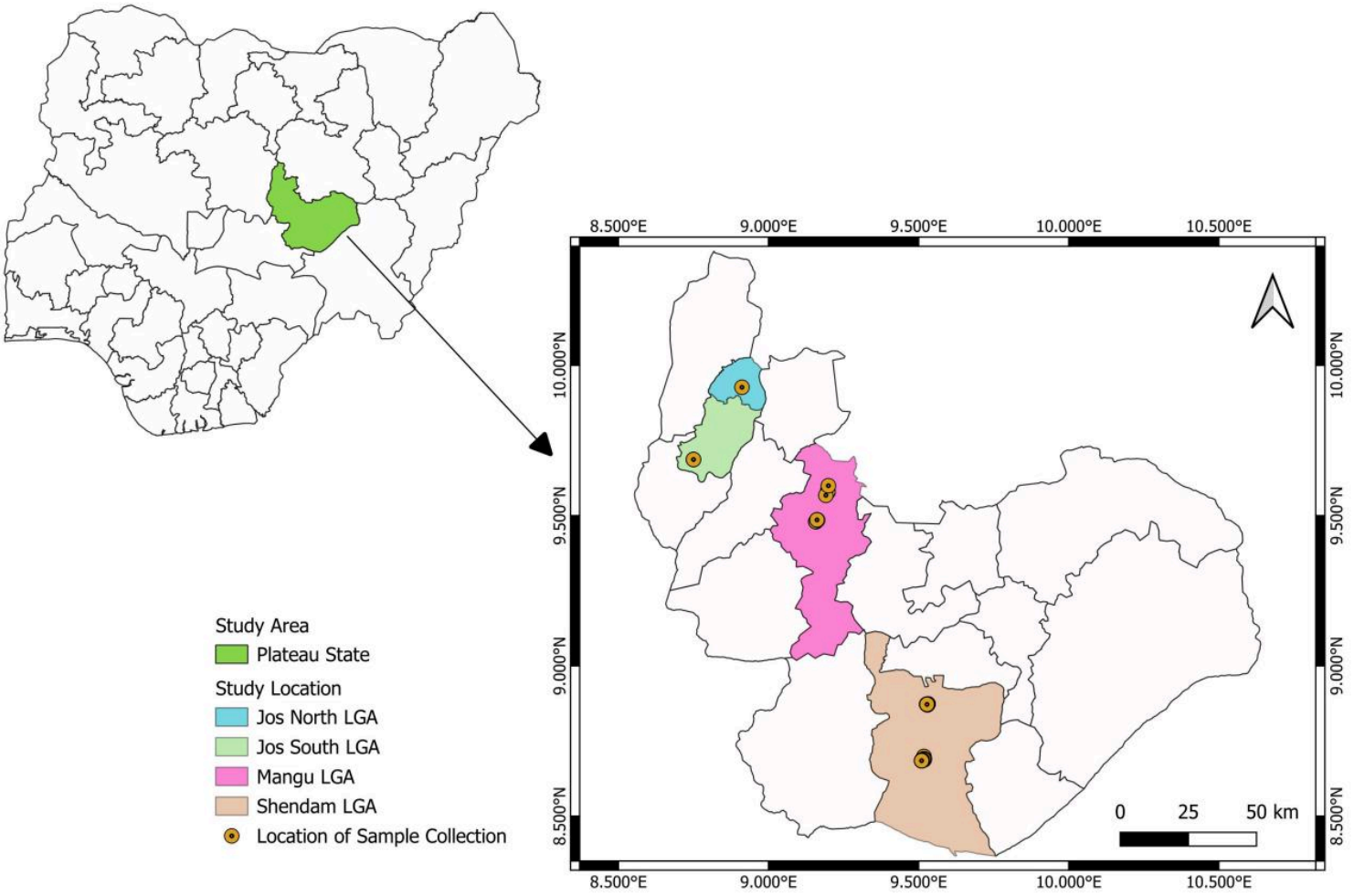


Figure 1

Map of Nigeria showing the four states sampled in Plateau State (Generated using QGIS version 3.8.4)

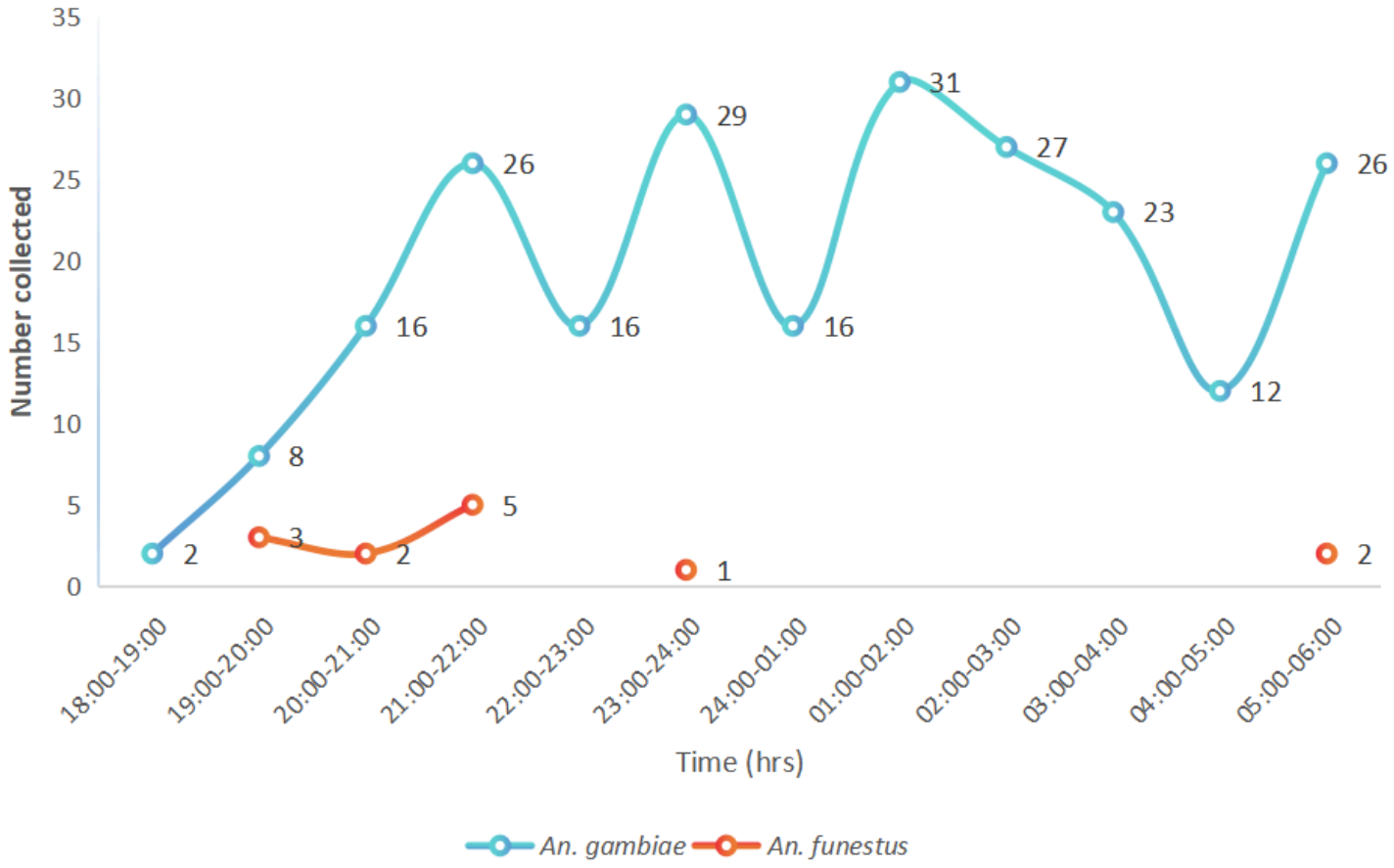


Figure 2

Indoor feeding behaviour of *An. gambiae* s.l. and *An. funestus*

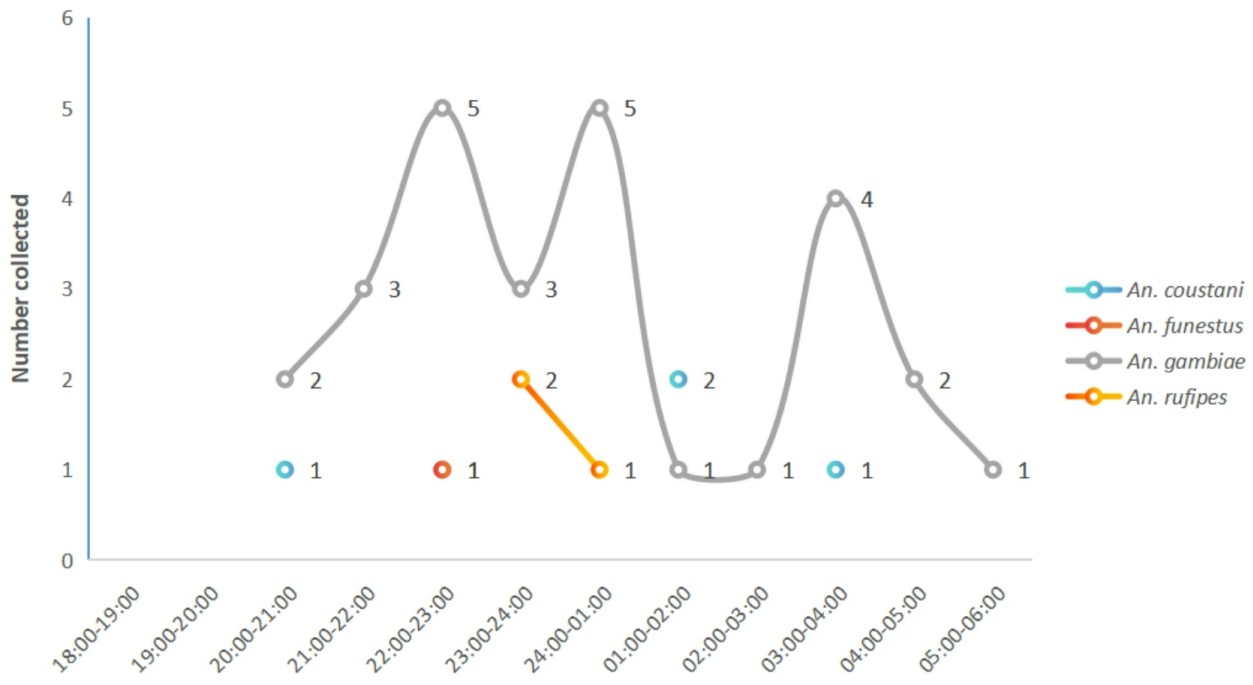


Figure 3

Outdoor feeding behaviour of four anopheline species

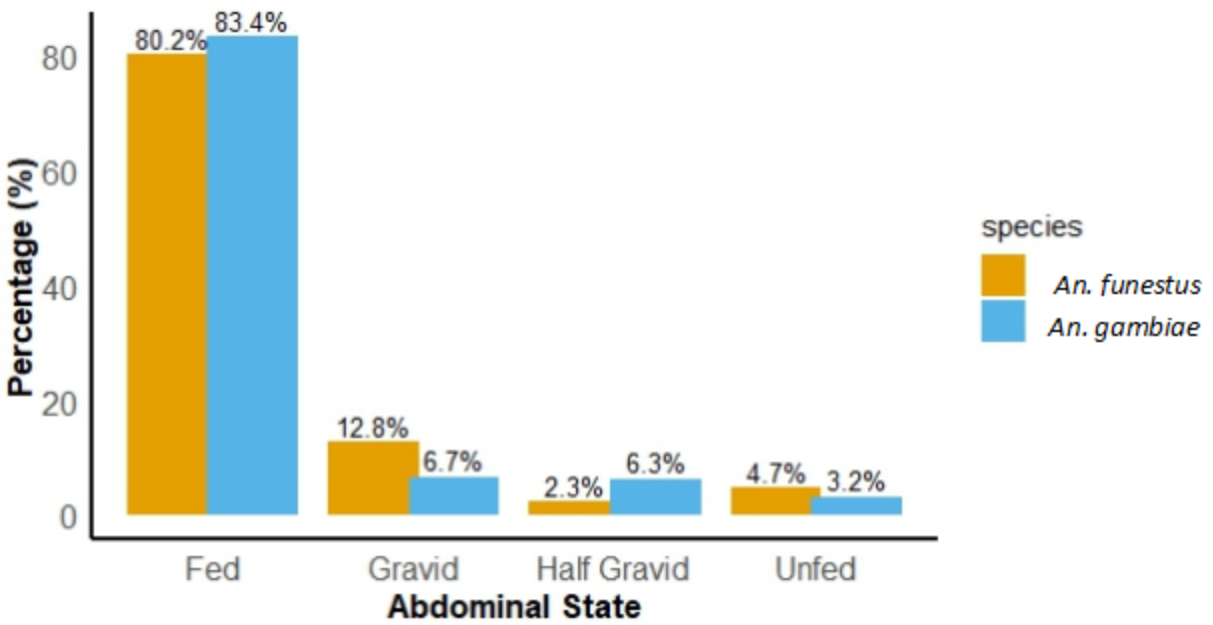


Figure 4

Abdominal Condition of *An. gambiae*s.l. and *An. funestus* Collected Using the PSC Method Across Location and Season

Fisher's Exact Test: p-value = 0.1125

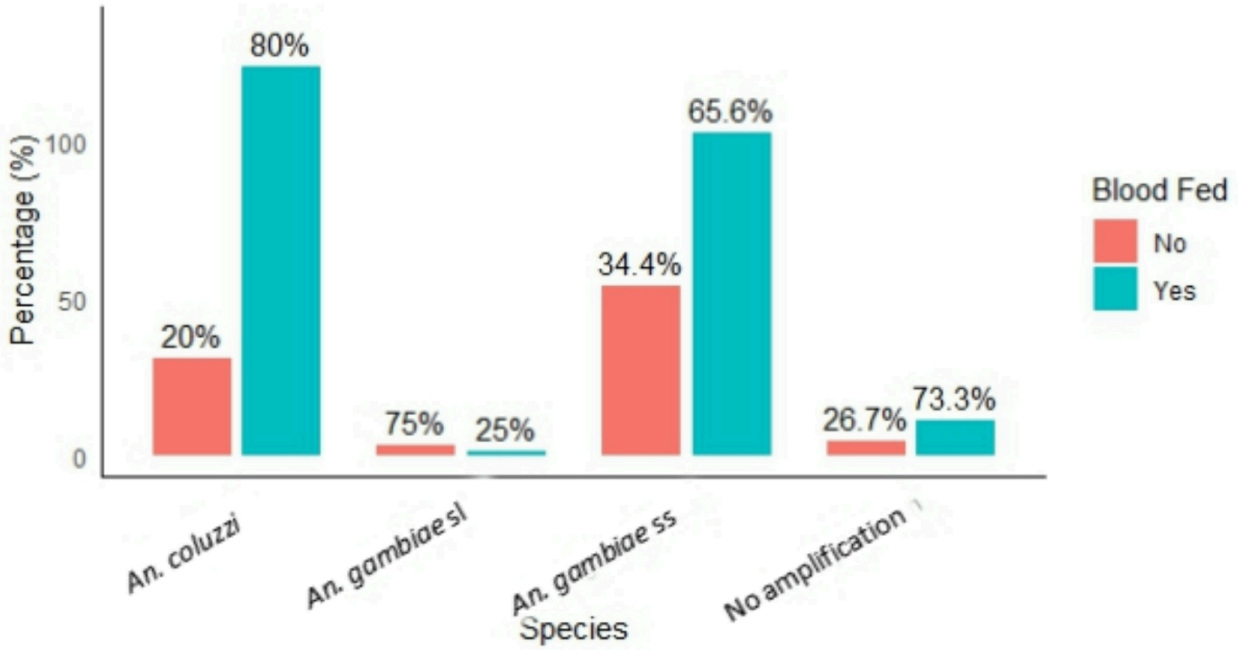


Figure 5

All anopheline species identified by PCR.

Fisher's Exact Test: p = 0.004

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage6.png
- floatimage7.png