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THE PMI VECTORLINK PROJECT CAMEROON

ANNUAL ENTOMOLOGY REPORT

OCTOBER 2018–SEPTEMBER 2019

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ACRONYMS

BTC	Biotechnology Center
CDC	U.S. Centers for Disease Control and Prevention
CRID	Center for Research in Infectious Diseases
ELISA	Enzyme-Linked Immuno-Sorbent Assay
EIR	Entomological Inoculation Rate
HBI	Human Blood Index
HBR	Human Biting Rate
HLC	Human Landing Catch
ITN	Insecticide-treated Net
IRS	Indoor residual spraying
Kdr	Knock Down Resistance
LT	Light trap
NMCP	National Malaria Control Program
OCEAC	Organization for the Coordination of Endemic Diseases in Central Africa
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

From October 2018 to September 2019, the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted malaria vector surveillance in five sentinel sites in Cameroon. Monthly entomological monitoring was done in Simatou and Gounougou in the North, while bimonthly collections occurred in Mangoum, Nyabessang, and Bonabéri in the South. Human landing catches (HLCs), pyrethrum spray catches (PSCs), and U.S. Centers for Disease Control and Prevention Light Traps (CDC LTs) were used to collect adult mosquitoes in households and assess vector composition, human biting rate (HBR), endophagic index, indoor resting density, parity rate, human blood index (HBI), infection rate, and entomological inoculation rate (EIR). In addition, insecticide susceptibility, intensity of resistance, and synergist assays with piperonyl butoxide (PBO) were conducted.

Species composition results across sites showed a high diversity of *Anopheles* species, nine of which transmit malaria: *An. gambiae* s.l., *An. funestus* s.l., *An. nili*, *An. moucheti*, *An. demeillonni*, *An. pharoensis*, *An. ziemanni*, *An. multicinctus*, and *An. marshalli*. Analysis of the *An. gambiae* complex and *An. funestus* group using polymerase chain reaction (PCR) revealed the presence of *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* in all five sites, with an overall proportion of 32.2%, 59.0%, and 8.2%, respectively. Hybrids of *An. gambiae*/*An. coluzzii* (0.5%) were also found in Simatou, Mangoum, and Nyabessang. Two subspecies of the *An. funestus* group were identified in Simatou and Gounougou: *An. funestus* s.s. (74.7%) and *An. leesoni* (25.3%).

The mean HBR of *Anopheles* mosquitos collected ranged from 23 bites/person/night (b/p/n) in Mangoum to 93 b/p/n in Simatou. Changes in the biting behavior of *An. gambiae* s.l. and *An. moucheti* were observed in Mangoum, Bonabéri, and Nyabessang, where both species continued to bite until 7:00 or 8:00 a.m. The average indoor resting density of *Anopheles* mosquitos across all sites during the collection period was 19.6 females/room/night; the highest was observed in Simatou (33.5 females/room/night) and the lowest in Bonabéri (0.03 female/room/night). The overall parity rate varied from 57.1% in Nyabessang to 76.4% in Gounougou, and the HBI ranged from 21.7% in Simatou to 74.2% in Mangoum. The endophagic index for *An. gambiae* s.l. was 0.50 in Simatou, 0.53 in Mangoum, 0.47 in Gounougou, 0.49 in Nyabessang, and 0.3 in Bonabéri, indicating more outdoor biting in the three latter sites. The monthly estimated EIRs recorded were 41.5 infective bites/person/month (ib/p/m) in Gounougou, 80.5 ib/p/m in Simatou, 53.6 ib/p/m in Mangoum, 34.8 ib/p/m in Nyabessang, and 27.6 ib/p/m in Bonabéri.

An. gambiae s.l. were resistant to pyrethroids (permethrin, deltamethrin, and alpha-cypermethrin) in all sites in 2018 and 2019. Additionally, high resistance intensity was observed in Gounougou, Simatou, and Mangoum for deltamethrin, permethrin, and alpha-cypermethrin; moderate resistance was found in Nyabessang and Bonabéri for permethrin and alpha-cypermethrin. Furthermore, exposure to PBO before testing with pyrethroids did not fully restore susceptibility of *An. gambiae* s.l.

In 2018, *An. gambiae* s.l. were resistant to pirimiphos-methyl in three of the four sites tested (Simatou, Mangoum, and Nyabessang) but were susceptible in all five sites in 2019. Susceptibility to clothianidin (13.2 mg/paper) and chlorfenapyr (100 µg/bottle and 200 µg/bottle) was observed at all sites except Mangoum.

Target site resistance mechanisms–*Kdr*-w (L1014F), *Kdr*-e (L1014S), and N1575Y for pyrethroids, and Ace-1 for organophosphates and carbamates–and metabolic resistance mechanisms were observed in *An. gambiae* s.l. tested in the different sites. Additional resistance markers (CYP6P9A, CYP6P5, CY6M7, and GSTe2) were found within *An. funestus* s.l. from Gounougou. CYP6M2, CYP6P3, and GSTe2 were also observed in *An. gambiae* s.l. collected from Gounougou.

These results will guide Cameroon's National Malaria Control Program in the selection of the appropriate insecticides and tools for malaria vector control across the country.

1. INTRODUCTION

Malaria is a leading public health challenge in Cameroon. According to the 2018 National Malaria Control Program (NMCP) Report, malaria was responsible for 25.8% of health facility consultations and 14.3% of deaths. Children under 5 years of age and pregnant women are disproportionately vulnerable; in 2018, hospital morbidity due to malaria was 31.5% among children under 5 and 22.3% among pregnant women. Given the scale of the problem, the Ministry of Public Health and its partners are implementing high-impact interventions to reduce malaria morbidity and mortality. These include i) the free distribution of insecticide-treated nets (ITNs) through mass campaigns and antenatal consultations for pregnant women, ii) seasonal chemo-prophylaxis of malaria for children ages 3 to 59 months, and iii) free treatment of uncomplicated and severe malaria for children under 5.

Entomological surveillance is a key component of malaria vector control as it provides information on vector species, including the spatial and temporal distribution, density, ecology, biting, feeding, and resting behavior, as well as the rate of infectivity, transmission, and susceptibility to the insecticides used in vector control interventions.

In October 2018, the U.S. President's Malaria Initiative (PMI) VectorLink Project began conducting entomological surveillance in Cameroon in five sentinel sites located in various regions representing different ecologies present in the country. VectorLink works in close collaboration with the NMCP and three research institutions—the Biotechnology Center (BTC), and the Center for Research in Infectious Diseases (CRID), and the Organization for the Coordination of Endemic Diseases Control in Central Africa (OCEAC).

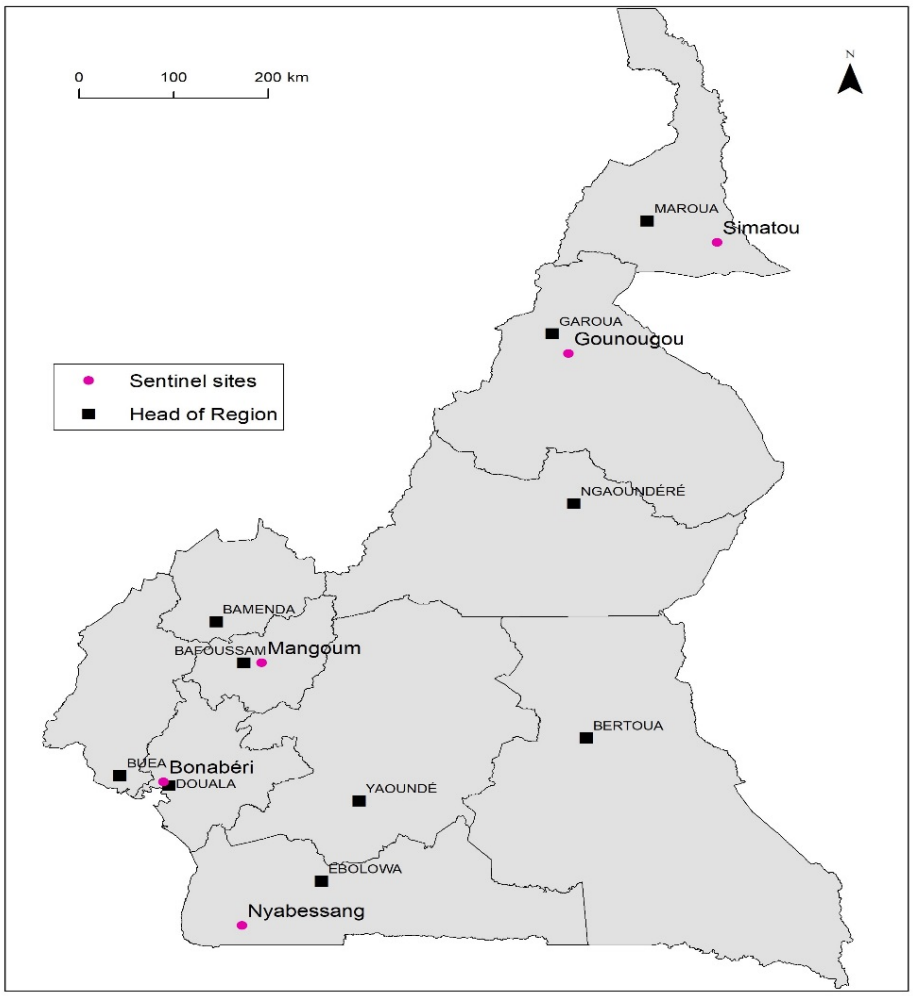
The data collected guides the selection and distribution of malaria vector control tools. In Cameroon, these data will also guide the NMCP and stakeholders in conducting a feasibility study before planning for potential indoor residual spraying (IRS).

2. MATERIAL AND METHODS

2.1 STUDY SITES

From October 2018 to September 2019, VectorLink Cameroon conducted entomological surveillance in five sentinel sites (Figure 1). Adult mosquito collections were done monthly in Gounougou and Simatou, and every other month in Nyabessang and Mangoum and insecticide resistance monitoring was conducted twice per site during the reporting period. In November 2018, security concerns led the team to replace the preselected site of Tiko with one in Bonabéri, and starting in December 2018, the team conducted collections every other month there. Gounougou and Simatou are located in the dry savannah and Sahelian zones of the North and Extreme North, Mangoum is in the wet, savannah zone of the West, Nyabessang is in the forest area of the South, and Bonabéri is located in the coastal zone of the Littoral region.

Figure 1: Geographical Locations of PMI Entomological Sentinel Sites in Cameroon



2.2 LONGITUDINAL MONITORING OF MALARIA VECTORS

VectorLink Cameroon collected adult mosquitoes using human landing catches (HLCs), pyrethrum spray catches (PSCs), and CDC light traps (CDC LTs) in all sentinel sites. The same houses were used each month for collections and 12 collections were completed in Gounougou and Simatou while six collections were done in Bonabéri, Mangoum, and Nyabessang. All mosquitoes were identified morphologically using identification keys (Gillies, M.T. & Coetzee, M. 1987). All mosquitoes were preserved on silica gel in Eppendorf tubes for further laboratory processing to identify sibling species, resistance mechanisms, infection status, and source of blood meal using polymerase chain reaction (PCR) and Enzyme-Linked Immunosorbent Assays (ELISAs). Table 1 provides additional information on mosquito collection methods used.

Table 1: Adult Mosquito Collection Methods for Vector Surveillance

Collection Method	Time	Collection Location	Frequency	Sample
HLCs	6:00 p.m.–6:00 a.m. from Oct–Dec 2018 and 6:00 p.m.–8:00 a.m. from Jan–Sept 2019	Indoors and outdoors	Two nights per site	Three houses per site (same houses every month)
PSCs	6:00 a.m.–8:00 a.m.	Indoors	Two days per site	Twenty houses per site (the same houses most of the time)
CDC LTs	6:00 p.m.–6:00 a.m.	Indoors and outdoors	Two nights per site	Four houses per site (same houses every month)

2.2.1 HUMAN LANDING CATCHES

HLCs were performed indoors and outdoors in three houses for two consecutive nights, to collect adult mosquitoes landing on human baits. From October to December 2018, the collections occurred from 6:00 p.m. to 6:00 a.m. Based on the high number of mosquitos collected during the early morning hours in these first three months, the collection time was extended to 8 a.m. in January 2019 in order to study the behavior change of the vectors. With legs exposed to attract host-seeking mosquitoes, two human baits, situated about 1.5-2 meters apart, were seated indoors and another two were seated outdoors and served as mosquito collectors. The two teams of 12 collectors each worked in two shifts—6:00 p.m. to 12:00 a.m. and 12:00 a.m. to 8:00 a.m. The collectors swapped positions (indoor and outdoor) every hour following the VectorLink SOP 02/01. The doors of the houses were kept closed when collections were underway. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before the mosquitoes could bite. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made bags for a total of 12 or 14 hours. Mosquitoes collected all night and at hourly intervals were identified and the ovaries of subsamples (about 20% of the total) of unfed *Anopheles* were dissected to determine parity (VectorLink SOP 10/01). All *Anopheles* and the carcasses of the dissected *Anopheles* were individually stored in labeled Eppendorf tubes containing silica gel.

2.2.2 PYRETHRUM SPRAY CATCHES

The PSCs were carried out during morning hours, between 6:00 a.m. and 8:00 a.m. for two consecutive days in 20 sleeping rooms. White cloth/sheets were placed on the floor from wall to wall in sampled rooms. After closing the windows and doors and covering or removing drinking water and food items, the rooms were sprayed with the commercial pyrethroid + piperonyl butoxide (PBO) insecticide. For houses with open eaves, collectors sprayed from outside through the eaves before entering and spraying indoors. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected from the white sheets. The

mosquitoes were kept in Petri dishes and then sorted by species using an identification key. The abdominal status of all female anophelines was determined, and individuals were sorted into four categories: unfed, blood-fed, half-gravid, and gravid. To determine blood meal status, female *Anopheles* mosquitoes were classified according to their abdomen status and were kept individually in labeled Eppendorf tubes containing silica gel for further laboratory analysis.

2.2.3 CDC Light Traps

CDC light traps were installed for two consecutive nights, one indoors and one outdoors, of four houses (8 traps per night) in each site at each collection period between 6:00 p.m. and 6:00 a.m. The traps were suspended 1.5 meters above the ground. Indoors, the trap was installed in a bedroom used for sleeping by at least one household member and containing at least one treated mosquito net (typically received from the different ITN distribution campaigns) and in use, and near the feet of the sleeper. Outdoors, the trap is set un-baited near the house of collection. Two volunteers were recruited to check on the traps during collection nights. The next morning, *Anopheles* collected were identified and the ovaries of subsamples of unfed *Anopheles* that were still alive were dissected. All *Anopheles* mosquitoes including the carcasses of the subsamples dissected were individually stored in labeled Eppendorf tubes containing silica gel.

Table 2 summarizes the indicators calculated based on the number of mosquitoes captured through each collection method.

Table 2: Vector Surveillance Indicators by Collection Method

Collection Method	Indicator	Definition
HLC	Human Biting Rate	Mean number of bites per person per night
	Peak biting time	Hour of highest human biting rate
	Parity Rate	Percentage of parous mosquitoes/total dissected
	Exophagic Rate	Proportion of mosquitoes biting outside
	Endophagic Rate	Proportion of mosquitoes biting inside
PSC	Indoor Resting Density	Mean number of mosquitoes per house per day
	% of fed females	Number of fed mosquitoes / total collected by PSC
CDC LT	Indoor/Outdoor Density	Mean number of mosquitoes collected indoors or outdoors per trap per night

2.3 Insecticide Resistance Monitoring

In 2018 and 2019, the team completed insecticide resistance monitoring in five sites (Gounougou, Simatou, Mangoum, Nyabessang, and Bonabéri). *An. gambiae* s.l. larvae and pupae were collected per site from different larval habitats, pooled, and reared to adulthood in the field laboratory`. Insecticide susceptibility tests were conducted on 2-5 day old adult females using WHO tube tests. CDC bottle assays were used to test the susceptibility to chlorfenapyr. For each WHO susceptibility test and CDC bottle assay, two control groups of 20-25 female *An. gambiae* s.l. were used and tested similarly.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%) were tested in all sites. Resistance was defined following the WHO criteria, with less than 90% mortality indicating confirmed resistance, between 90-97% mortality indicating possible resistance, and greater than 98% indicating susceptibility. When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at five- and ten-times the diagnostic concentration of permethrin, deltamethrin, alpha-cypermethrin, and pirimiphos-methyl.

Synergist assays with PBO were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of P450s in pyrethroid resistance. A high percentage mortality and/or reversal of susceptibility using PBO indicated probable involvement of enzyme activities such as P450s in this insecticide resistance mechanism.

Clothianidin-impregnated papers were treated locally at the dose of 13.2 mg/paper using a protocol designed by VectorLink and the susceptibility testing was done as described above with a seven-day delay mortality recording. CDC bottles were treated with chlorfenapyr at two selected doses of 100 µg/bottle and 200 µg/bottle following a modified protocol designed by Brogdon et al, 1998. The mosquitoes were exposed for one hour and the mortality was recorded up to three days (VectorLink SOP 04/01).

In Gounougou and Simatou, where *An. funestus* s.l. and *An. gambiae* s.l. were found in households in the morning, fed females of these two species were collected, reared at the CRID insectary, and the F1 aged two to five days were tested for metabolic resistance using PCR methods (Riveron et al., 2013, Riveron et al., 2014, Kwiatkowska et al., 2013).

3. RESULTS AND DISCUSSION

From October 2018 to September 2019, mosquitos were collected in five sentinel sites to assess vector species composition, density, behavior, and transmission.

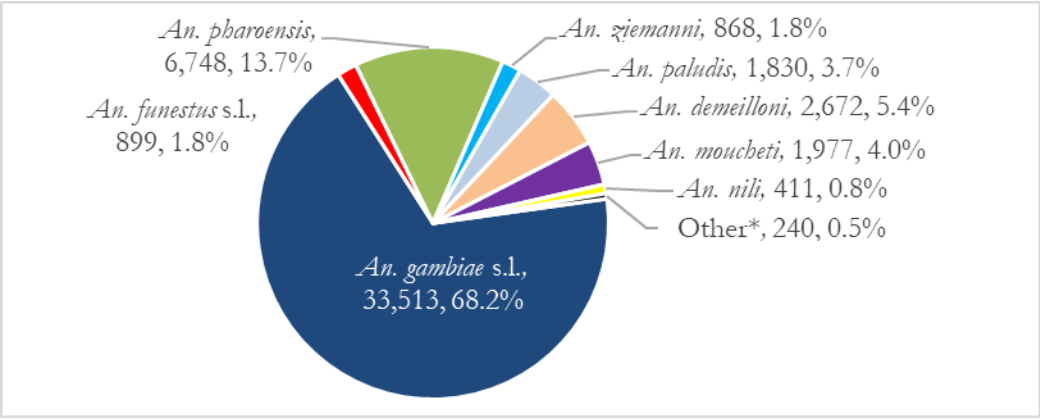
3.1 SPECIES COMPOSITION OF MOSQUITOS COLLECTED BY HLC, PSC, AND CDC LIGHT TRAP ACROSS ALL SITES

From October 2018 to September 2019, VectorLink Cameroon collected 49,158 *Anopheles* mosquitos by HLCs across the five sentinel sites. The predominant species represented were *An. gambiae* s.l. (68.2%), *An. pharoensis* (13.7%), *An. demeilloni* (5.4%), *An. moucheti* (4.0%), and *An. paludis* (3.7%) (Figure 2 and Annex A). *An. gambiae* s.l. were collected at all five sentinel sites. *An. moucheti* and *An. nili* were only found at Nyabessang. This site is surrounded by large rivers that offer suitable breeding sites for these two species.

A total of 18,725 *Anopheles* mosquitos, representing 14 different species, were collected using CDC LTs from October 2018 to September 2019 in four sites (Gounougou, Mangoum, Nyabessang, and Simatou). Bonabéri did not yield any *Anopheles* mosquitos using CDC LTs. *An. ziemanni* (42.8%), *An. gambiae* s.l. (31.3%), and *An. pharoensis* (16.1%) were the most abundant. *An. gambiae* s.l. was the only species present at all four sites. A total of 7,598 *An. ziemanni* were collected in Simatou (94.8% of total) and 351 were collected in Gounougou (5.2% of total) (Figure 3 and Annex B).

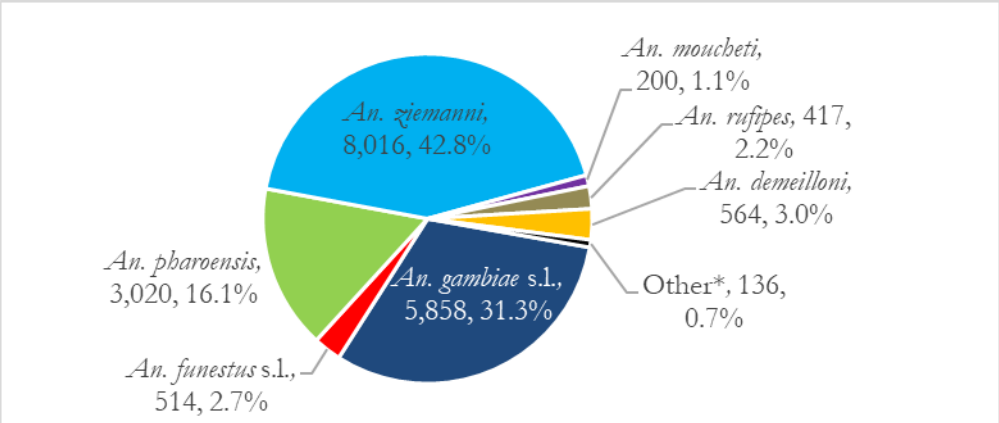
A total of 16,127 *Anopheles* mosquitos were caught using PSCs. *An. gambiae* s.l. (82.4%), *An. funestus* s.l. (6.7%), *An. demeilloni* (5.6%), and *An. rufipes* (4.8%) were the most abundant (Figure 4).

Figure 2: Species Composition of *Anopheles* Collected across All Sites Using HLCs (October 2018–September 2019)



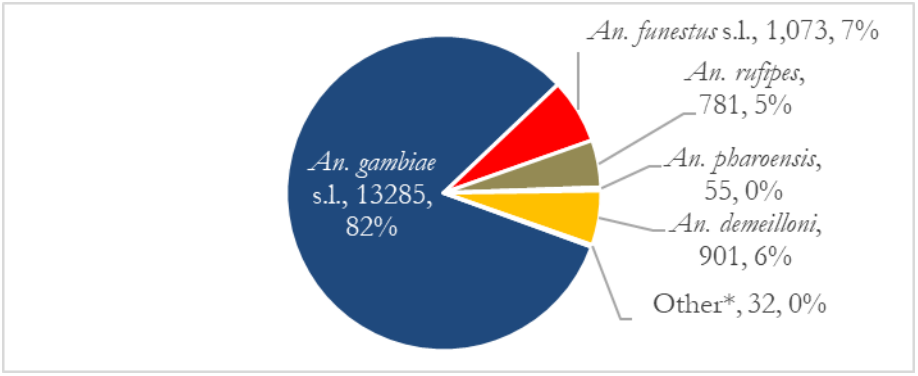
*Other species include: *An. rufipes* (n=45), *An. marshalli* (n=56), *An. christyi* (n=1), *An. multicinctus* (n=113), *An. coustani* (n=4), *An. welcomei* (n=7), *An. tenebrosus* (n=9), and *An. smithii* (n=5).

Figure 3: Species Composition of *Anopheles* Collected across All Sites Using CDC LTs (October 2018–September 2019)



*Other species include *An. paludis* (n=53), *An. hancocki* (n=1), *An. marshalli* (n=3), *An. christyi* (n=1), *An. cinerus* (n=3), *An. multicinctus* (n=63), and *An. nili* (n=12).

Figure 4: Species Composition of *Anopheles* Collected across All Sites Using PSCs (October 2018–September 2019)



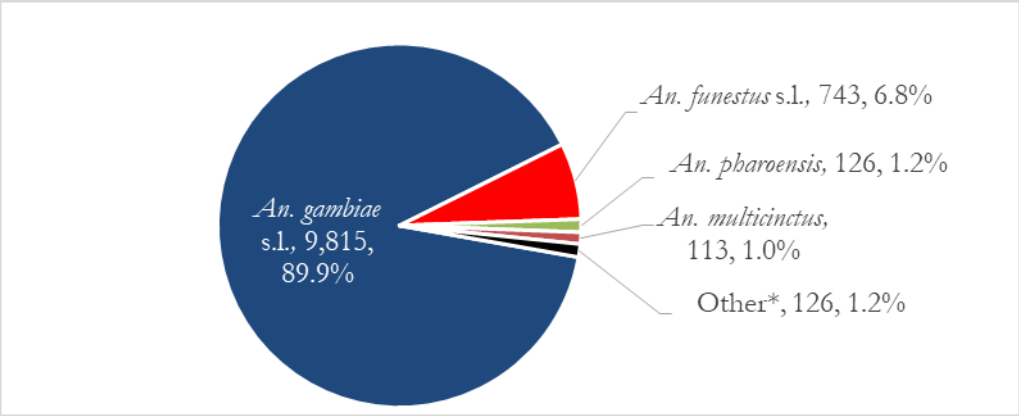
*Other species: *An. ziemanni* (n=12), *An. moucheti* (n=7), *An. nili* (n=1), *An. hancocki* (n=11), and *An. multicinctus* (n=1)

3.2 Species Composition of Mosquitos Collected by HLC, PSC, and CDC Light Trap by Site

3.2.1 Gounougou

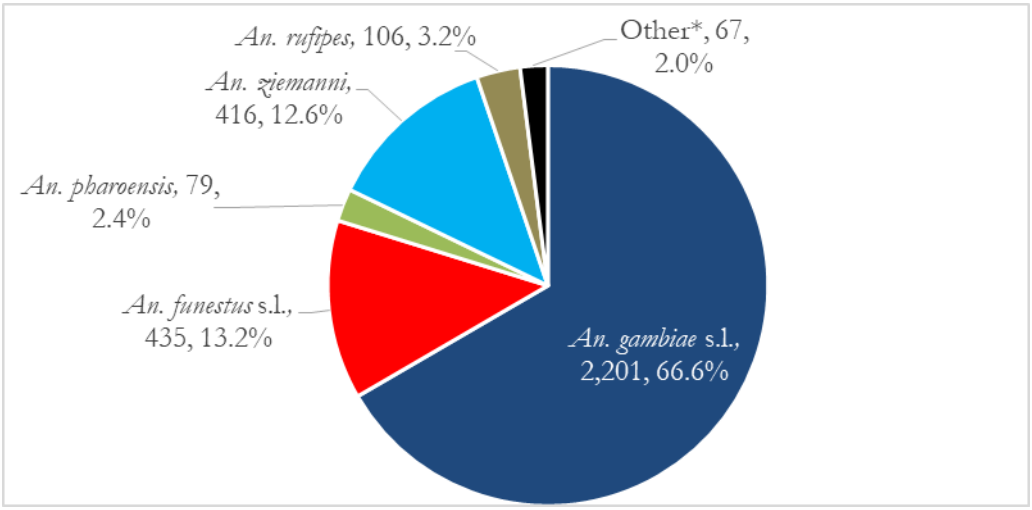
An. gambiae s.l. represented 90% (9,815) of the 10,923 total *Anopheles* species collected by HLCs from October 2018 to September 2019. Small numbers of other vectors including *An. funestus* (6.8%, 743), *An. pharoensis* (1%, 126) and *An. multicinctus* (1.0%, 113) were also collected (Figure 5). A total of 3,304 and 7,640 *Anopheles* mosquitos were collected in Gounougou through CDC LTs and PSCs, respectively. For both methods, *An. gambiae* s.l. was the main vector collected, representing 66.6% (2,201) of those collected by CDC-LTs and 84% of those collected by PSCs (Figure 6 and 7).

Figure 5: Species Composition of Anopheles Collected from Gounougou Using HLCs (October 2018–September 2019)



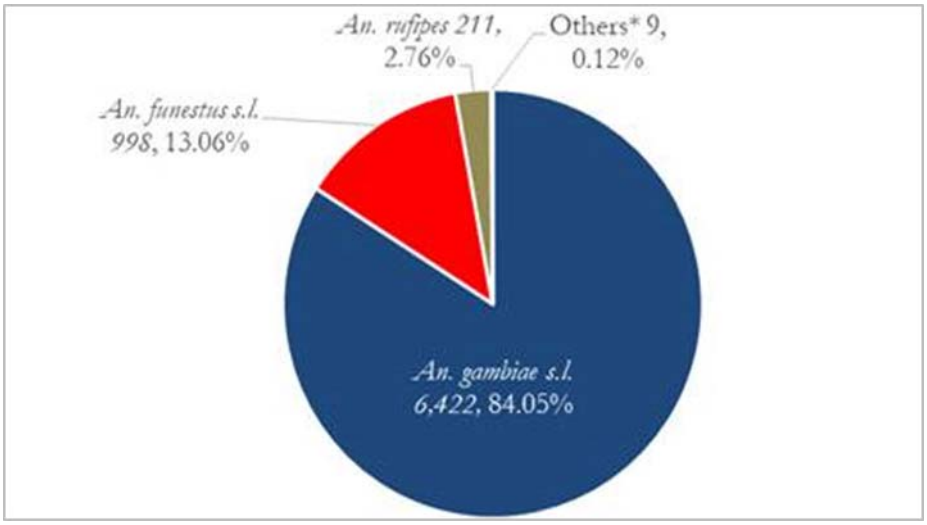
*Other species: *An. ziemanni* (n=99), *An. rufipes* (n=11), *An. tenebrosus* (n=9), *An. smithii* (n=5), *An. christyi* (n=1), and *An. coustani* (n=1).

Figure 6: Species Composition of *Anopheles* Collected from Gounougou Using CDC LTs (October 2018–September 2019)



*Other species include: *An. multicinctus* (n=63), *An. christyi* (n=1), and *An. cinerus* (n=3).

Figure 7: Species Composition of *Anopheles* Collected from Gounougou Using PSCs (October 2018–September 2019)



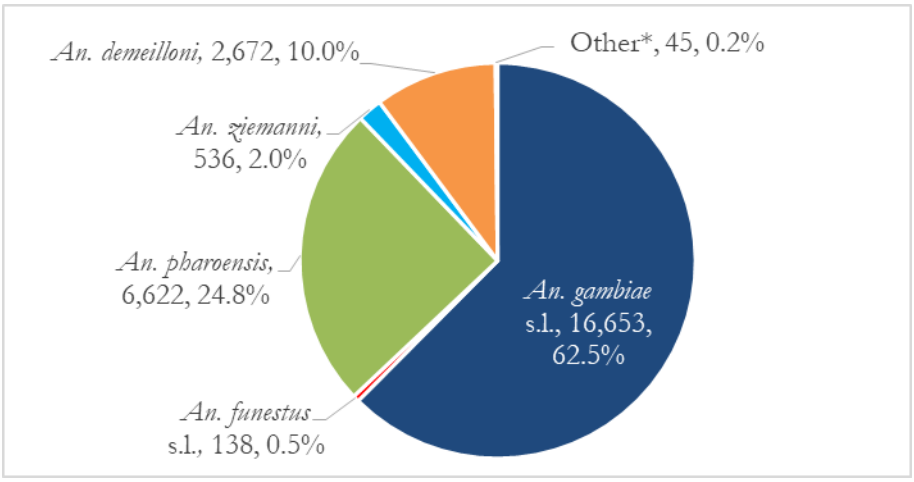
*Other species include: *An. ziemanni* (n=5), *An. pharoensis* (n=3), and *An. multicinctus* (n=1).

3.2.2 SIMATOU

In Simatou, 26,666 Anopheles mosquitos were collected using HLC representing more than 54% of the total Anopheles collected at all site through HLC. Simatou was particularly and highly productive compared to the other for sites. *Anopheles gambiae* s.l. represented 62.5% (16,653) of the Anopheles collected and high proportion of *An. pharoensis* (24.8%, 6,622) and *An. demeilloni* (10%, 2,672) were recorded in this site (Figure 8).

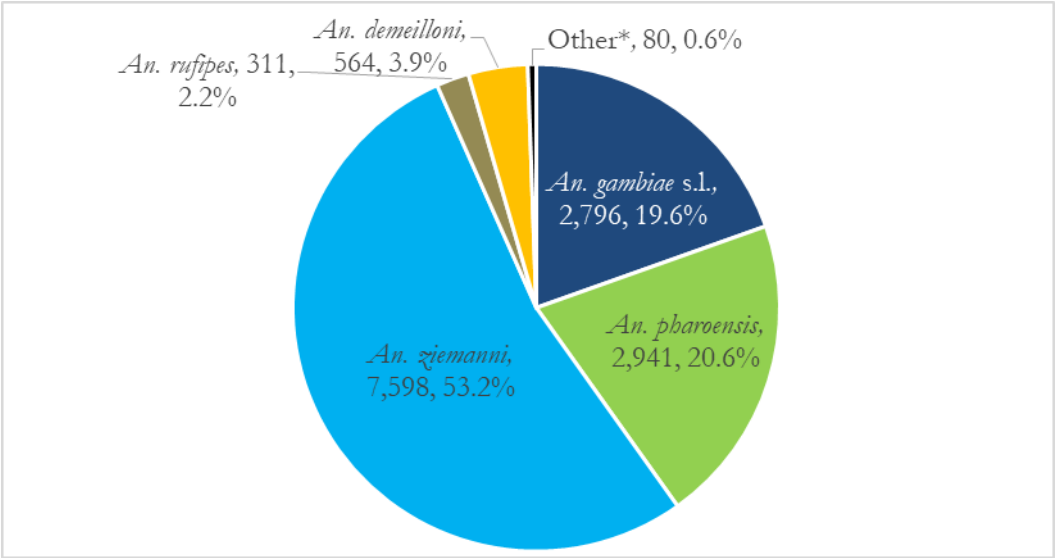
The total number of *Anopheles* collected by CDC LTs in Simatou was 14,290. In contrast to the HLC collection, the majority of mosquitos caught in CDC LTs were *An. ziemanni* (53.2%, 7,598) and *An. pharoensis* (20.6%; 2,941). *An. gambiae* s.l. (19.6%, 2,793) represented the third most abundant vector collected through the method. However, among the 8,065 A*nopheles* mosquitos collected through PSC method, the proportion of *An. gambiae* s.l. (79.9%, 6440) collected was higher than the other species (Figures 9 and 10).

Figure 8: Species Composition of *Anopheles* Collected from Simatou Using HLCs (October 2018–September 2019)



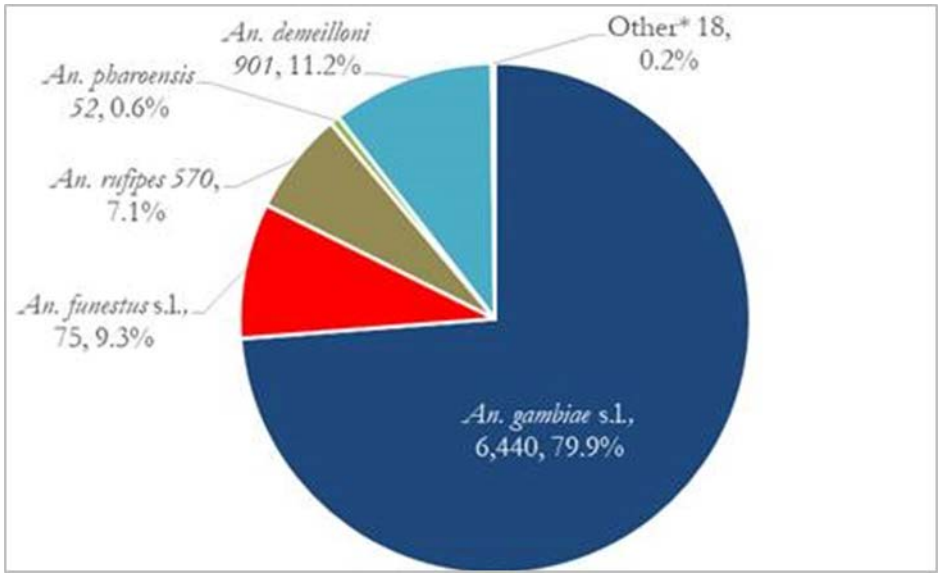
*Other species: *An. rufipes* (n=34), *An. coustani* (n=3), *An. paludis* (n=1), and *An. welcomei* (n=7).

Figure 9: Species Composition of *Anopheles* Collected from Simatou Using CDC LTs (October 2018–September 2019)



*Other species include: *An. funestus* s.l. (n=79) and *An. hancocki* (n=1).

Figure 10: Species Composition of *Anopheles* Collected from Simatou Using PSCs (October 2018–September 2019)



*Other species include: *An. ziemanni* (n=7) and *An. hancocki* (n=11).

3.2.3 MANGOUM

Very limited *Anopheles* vector species were recorded in Mangoum. The population was mostly composed of *An. gambiae* s.l. Only three additional species were collected using HLCs, i.e. *An. funestus* (0.5%, 18), *An. ziemanni* (1.1%, 36), and *An. paludis* (0.1%, 2) (Figure 11). Both CDC LTs and PSCs recorded 100% *An. gambiae* s.l. with 704 and 308 mosquitos collected respectively (Figures 12 and 13).

Figure 11: Species Composition of *Anopheles* Collected from Mangoum Using HLCs (October 2018–September 2019)

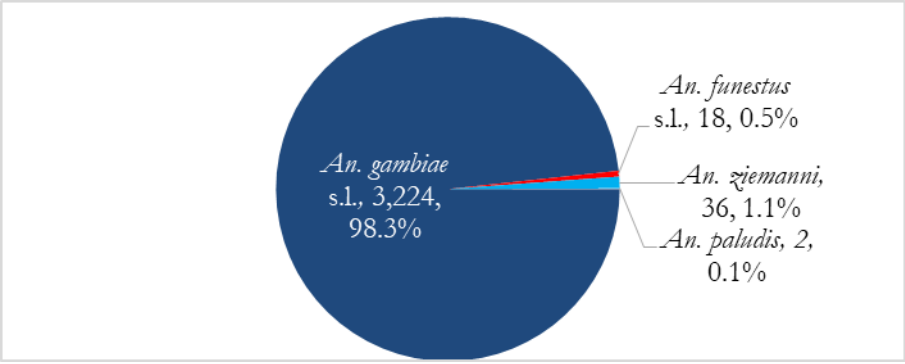


Figure 12: Species Composition of *Anopheles* Collected from Mangoum Using CDC LTs (October 2018–September 2019)

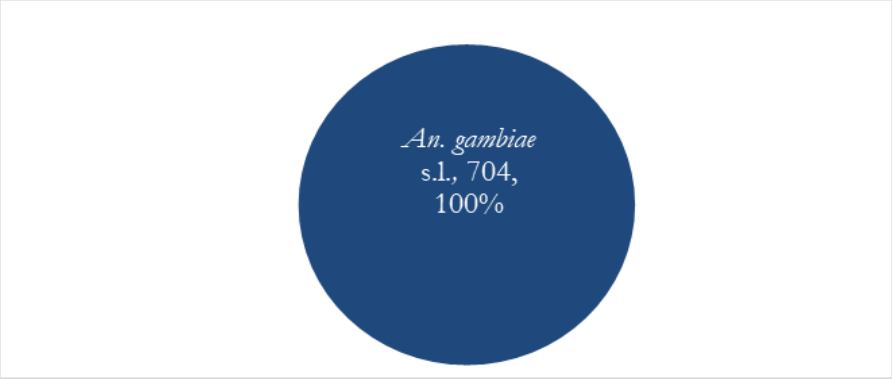
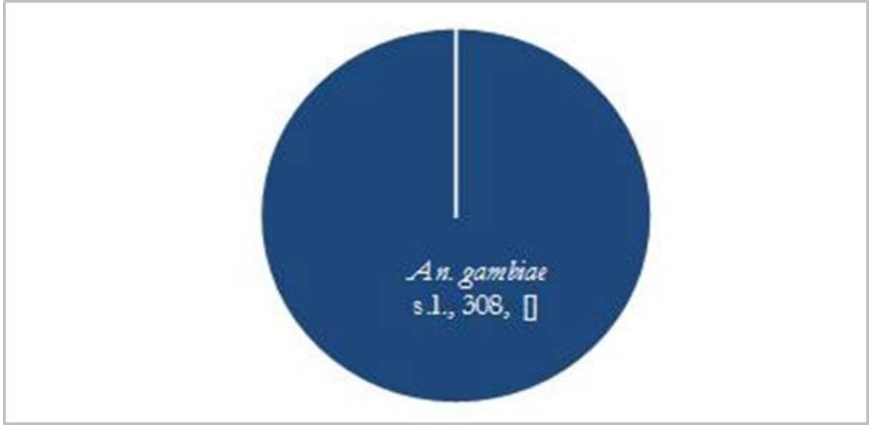


Figure 13: Species Composition of *Anopheles* Collected from Mangoum Using PSCs (October 2018–September 2019)



3.2.4 NYABESSANG

An. moucheti represented the main vector in Nyabessang and the only site where the species was collected from. The population of *An. moucheti* (36.7%; 1,977) and *An. paludis* (33.9%; 1,827) constituted more than 70% of the total *Anopheles* collected using HLC (4,467) (Figure 14) and similarly, *An. moucheti* represented 46.8% (200) of the *Anopheles* collected through CDC LTs, followed by *An. gambiae* s.l. (36.8%; 157) (Figure 15). Only the PSC method yielded higher percentage of *An. gambiae* s.l. compared to *An. moucheti* and *An. nili*, the two additional species collected (Figure 16).

Figure 14: Species Composition of *Anopheles* Collected from Nyabessang Using HLCs (October 2018–September 2019)

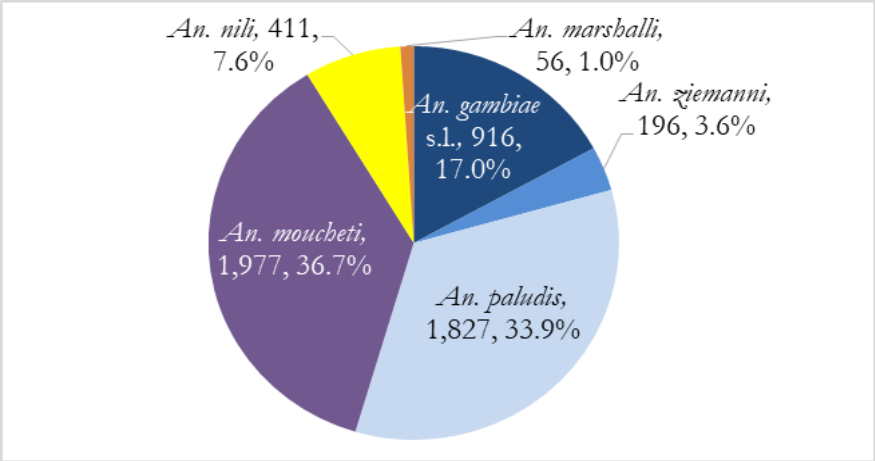


Figure 15: Species Composition of *Anopheles* Collected from Nyabessang Using CDC LTs (October 2018–September 2019)

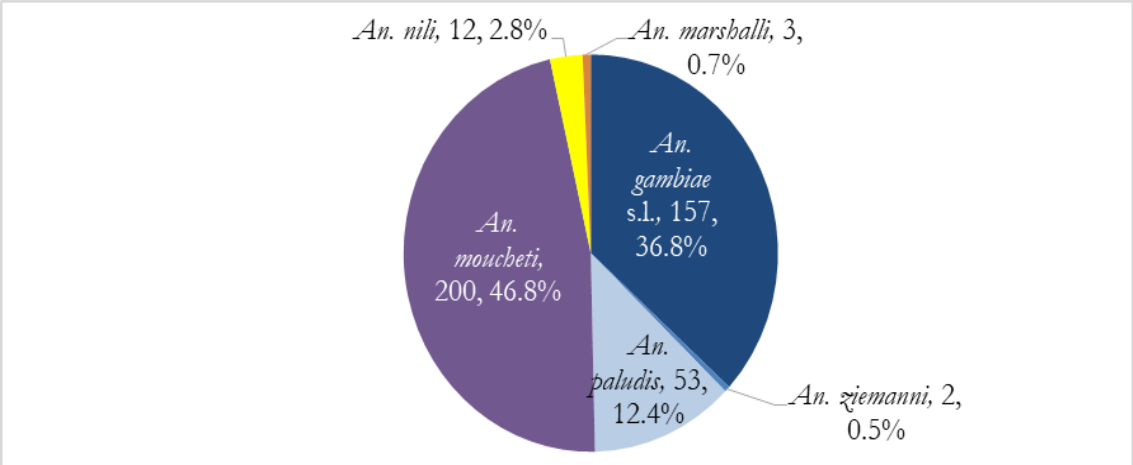
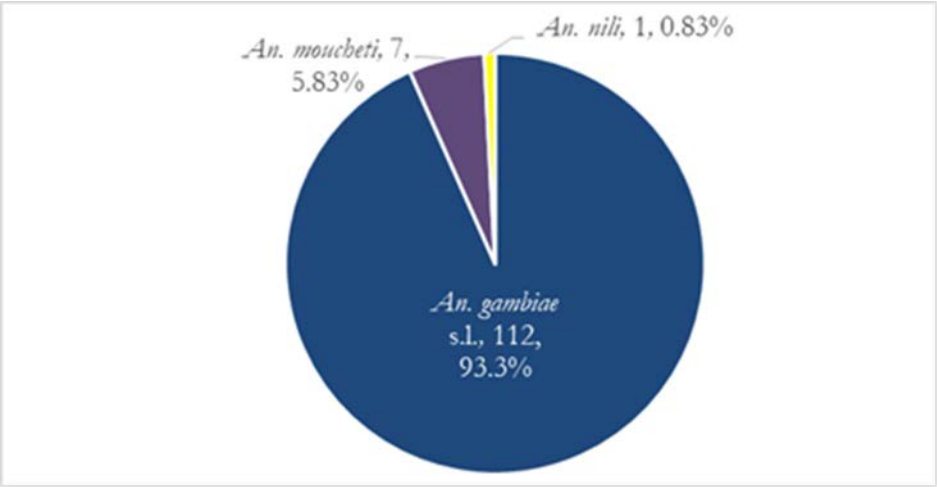


Figure 16: Species Composition of *Anopheles* Collected from Nyabessang Using PSCs (October 2018–September 2019)



3.2.5 BONABÉRI

Bonabéri recorded the least number of mosquitoes among the five sites (2,905 for HLC and 3 for PSC). Only HLC and PSC yielded *Anopheles* with the majority being *An. gambiae* s.l. for both methods. A single *An. ziemanni* was collected using HLC (Figures 17-18). No *Anopheles* was collected using CDC-LT collection method.

Figure 17: Species Composition of *Anopheles* Collected from Bonabéri Using HLCs (December 2018–September 2019)

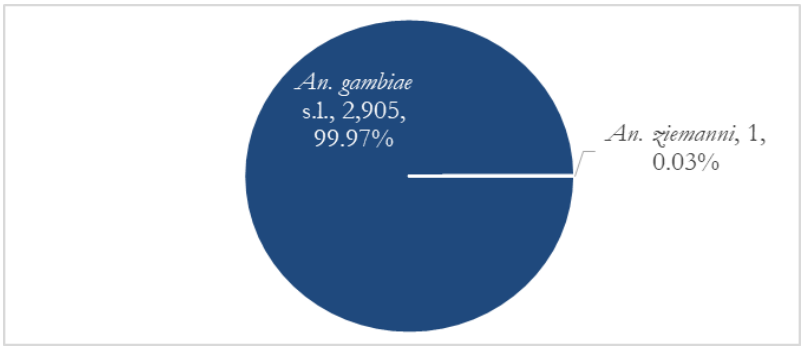
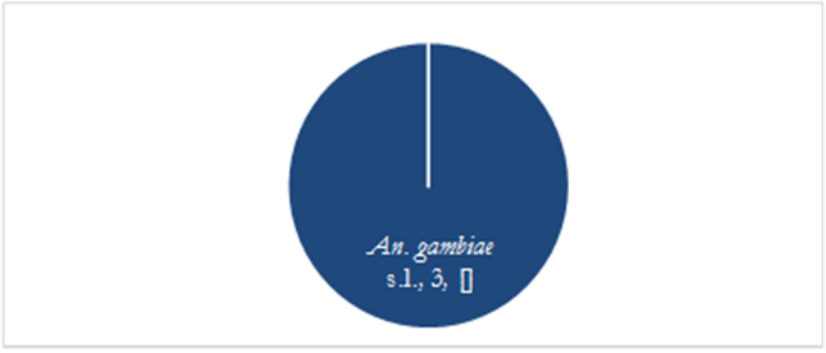


Figure 18: Species Composition of *Anopheles* Collected from Bonabéri Using PSCs (December 2018–September 2019)



3.3 SPECIES COMPOSITION OF *AN. GAMBIAE* COMPLEX AND *AN. FUNESTUS* GROUP

A total of 2,536 *An. gambiae* s.l. and 486 *An. funestus* s.l. were tested by PCR for molecular identification of the sub-species of each complex following the protocol described by Santolamazza et al, 2008 for *An. gambiae* s.l. and Koekemoer et al, 2002 for *An. funestus* s.l. (Table 4).

Three species from the *An. gambiae* complex were identified: *An. gambiae* (32.2%), *An. coluzzii* (59.0%), and *An. arabiensis* (8.2%). Hybrids of *An. gambiae*/*An. coluzzii* (0.5%) were also found. The species composition varied depending on the site location and climate (Figures 19-23). *An. arabiensis* were found in Gounougou and Simatou in the Northern, drier areas, and *An. coluzzii* were collected in humid areas in the South (Bonabéri, Nyabessang) and in rice cultivation zones (Simatou, Gounougou). Two species of the *An. funestus* group were found in Simatou and Gounougou: *An. funestus* s.s. (74.7%) and *An. leesoni* (25.3%) (Figure 24-25 & Annex C).

Figure 19: Species Composition of An. gambiae Complex in Gounougou

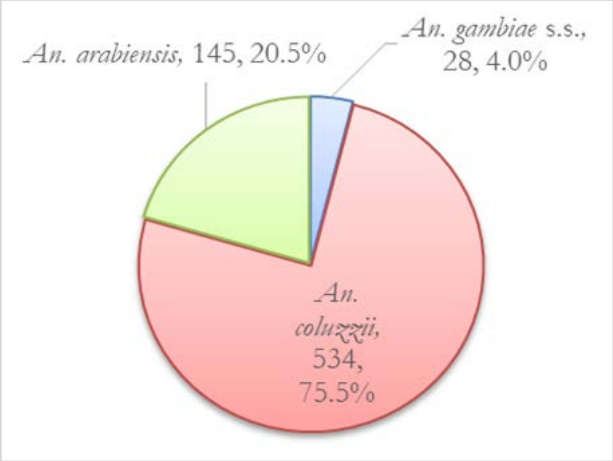


Figure 20: Species Composition of An. gambiae Complex in Simatou

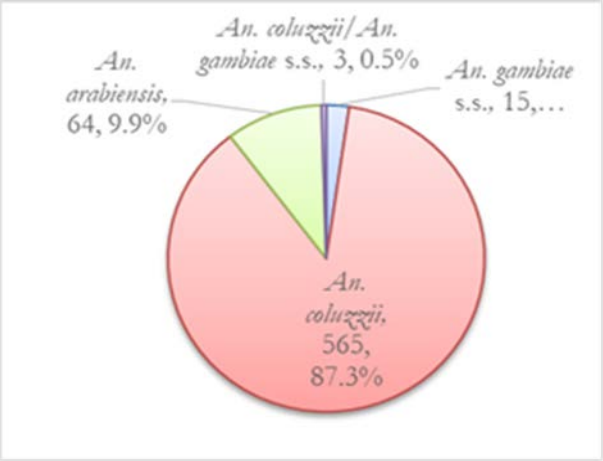


Figure 21: Species Composition of *An. gambiae* Complex in Nyabessang

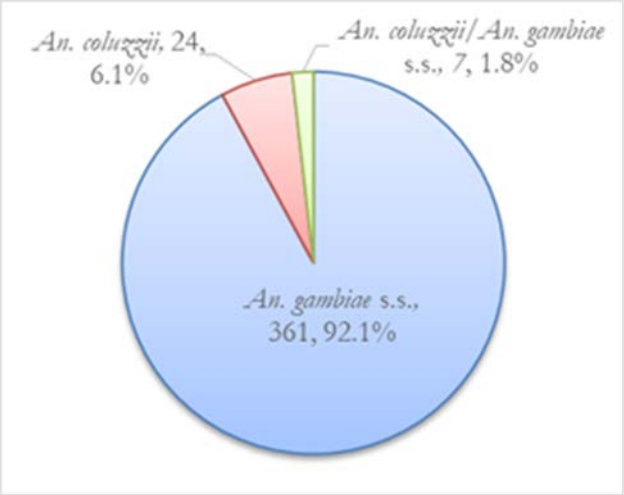


Figure 22: Species Composition of *An. gambiae* Complex in Mangoum

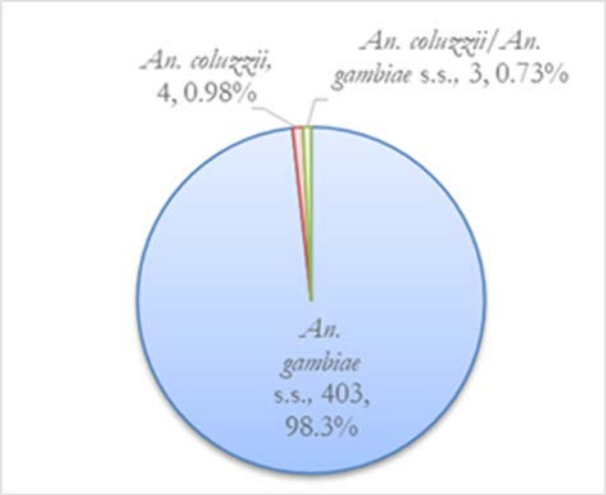


Figure 23: Species Composition of *An. gambiae* Complex in Bonabéri

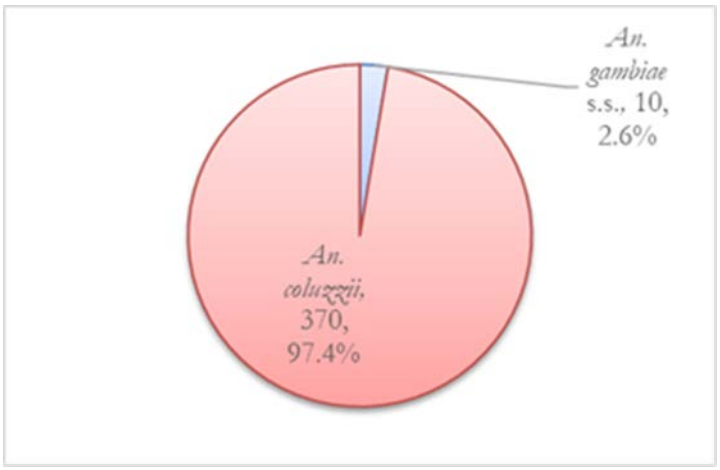


Figure 24: Species Composition of *An. funestus* Group in Simatou

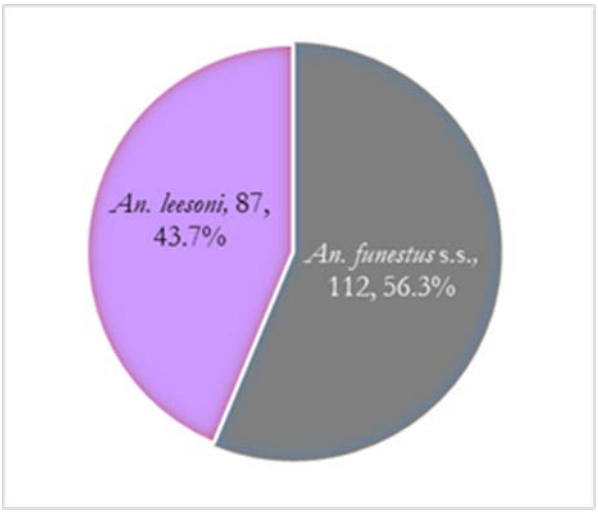
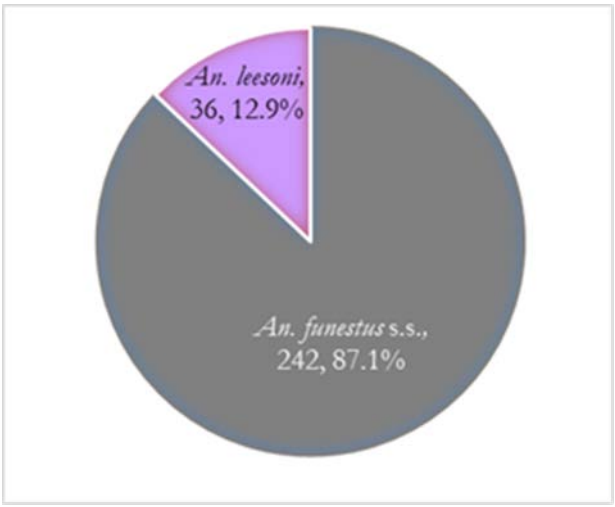


Figure 25: Species Composition of *An. funestus* Group in Gounougou



3.4 HUMAN BITING RATE AND SEASONAL VARIATION

3.4.1 GOUNOUGOU

The average total HBR for *An. gambiae* s.l. was 34.1 bites per person per night (b/p/n) with 32.1 b/p/n indoors and 36.0 b/p/n outdoors, while *An. funestus* averaged 2.6 b/p/n (2.9 b/p/n indoors and 2.2 b/p/n outdoors) as detailed in Annex D. The HBR varied monthly and peaked for *An. gambiae* s.l. in August 2019 (Figure 26). The peak biting time of *An. gambiae* s.l. collected in Gounougou was observed between 1:00–2:00 a.m. (Figure 27). *An. gambiae* s.l. continued biting after 6:00 a.m. The endophagic index of the *An. gambiae* s.l. (0.47) was lower than the one of *An. funestus* s.l. (0.57), indicating *An. gambia*e s.l. in this area likely bite more outdoors than indoors (see Annex E).

Figure 26: Human Biting Rate of *Anopheles* in Gounougou (October 2018–September 2019)

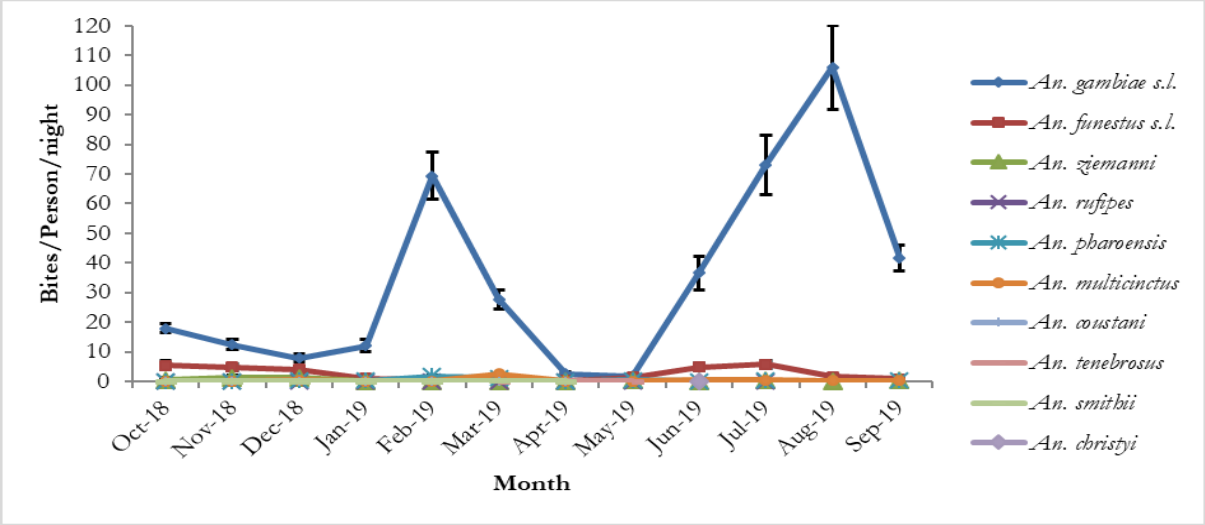
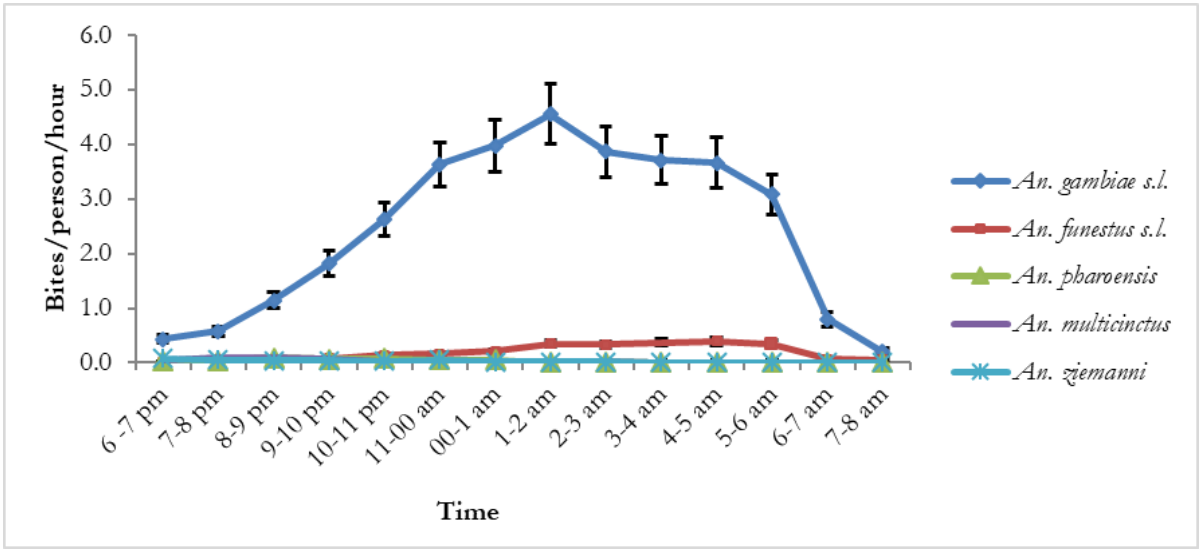


Figure 27: Hourly Biting Rate of *Anopheles* in Gounougou (October 2018–September 2019)



3.4.2 SIMATOU

In Simatou, the average total HBR was 57.8 b/p/n for *An. gambiae* s.l., with 58.3 b/p/n indoors and 57.3 b/p/n outdoors, while *An. funestus* s.l. averaged 0.48 b/p/n (0.5 b/p/n indoors and 0.4 b/p/n outdoors). For *An. pharoensis,* the total average *HBR* was 22.9 b/p/n and 9.2 b/p/n for *An. demeilloni* (see detail in Annex D). Seasonal variation was also observed in this site (Figure 28) with the highest HBR observed in July (281.2 b/p/n) during the rainy season. *An. gambiae* s.l. biting peaked between 1:00–2:00 a.m. and 3:00–4:00 a.m. while the peak time of *An. pharoensis* was 8:00–9:00 p.m. *An. gambiae* s.l. *An. pharoensis*, and *An.* demeilloni were still biting until 8:00 a.m. (Figure 29). The endophagic indices of the major *Anopheles* species found in Simatou were 0.50 for *An. gambiae* s.l. and *An. pharoensis*, 0.53 for *An. funestus,* 0.56 for *An. rufipes* and 0.43 for *An. ziemanni.* Outdoor biting was higher than indoors for *An. ziemanni* (see Annex E).

Figure 28: Human Biting Rate of *Anopheles* in Simatou (October 2018–September 2019)

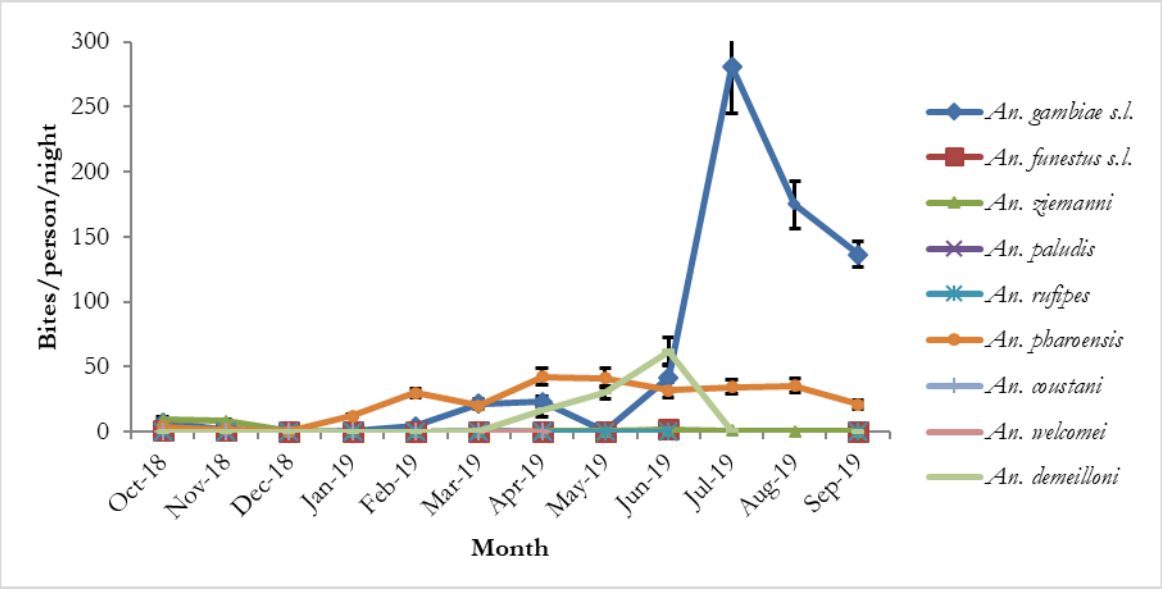
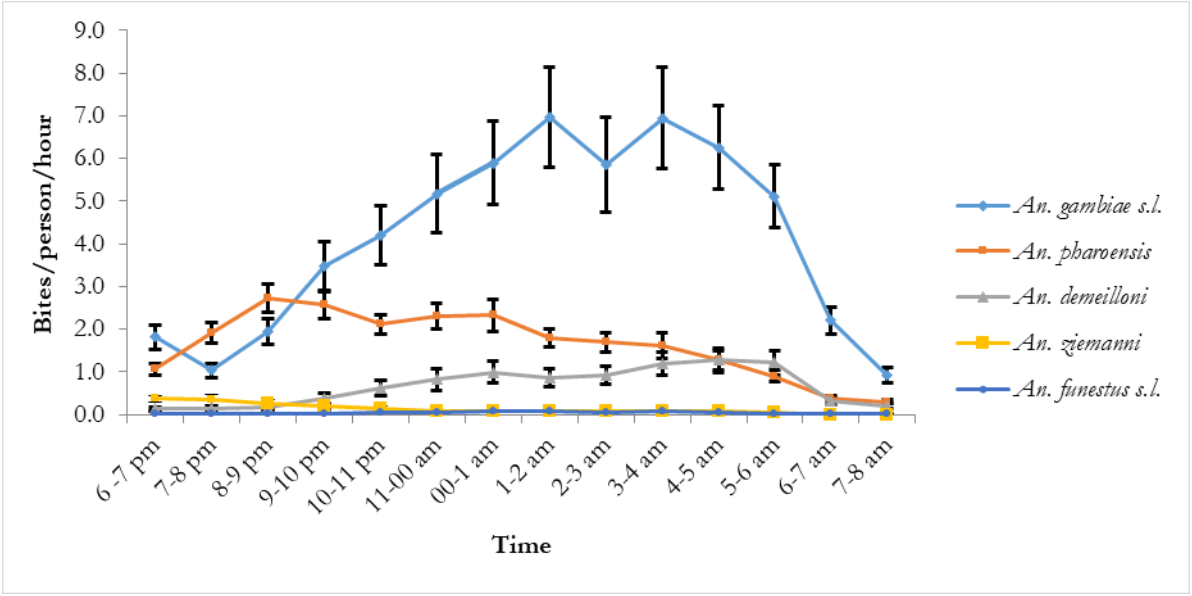


Figure 29: Hourly Biting Rate of *Anopheles* in Simatou (October 2018–September 2019)



3.4.3 MANGOUM

In Mangoum, the average total HBR of *An. gambiae* s.l. was 22.4 b/p/n, with 23.9 b/p/n indoors and 20.8 b/p/n outdoors, while *An. funestus* s.l. averaged 0.13 b/p/n (0.15 b/p/n indoors and 0.10 b/p/n outdoors). For *An. ziemanni*, the average total HBR was 0.25 b/p/n (Figure 30 & Annex C).

An. gambiae s.l. biting peaked between 5:00–6:00 a.m. and continued until 8:00 a.m. (Figure 31). The endophagic indexes of *An. gambiae* s.l., *An. funestus*, and *An. ziemanni* were above 50%, indicating that these species bite more indoors than outdoors (see Annex E).

Figure 30: Human Biting Rate of *Anopheles* in Mangoum (October 2018–September 2019)

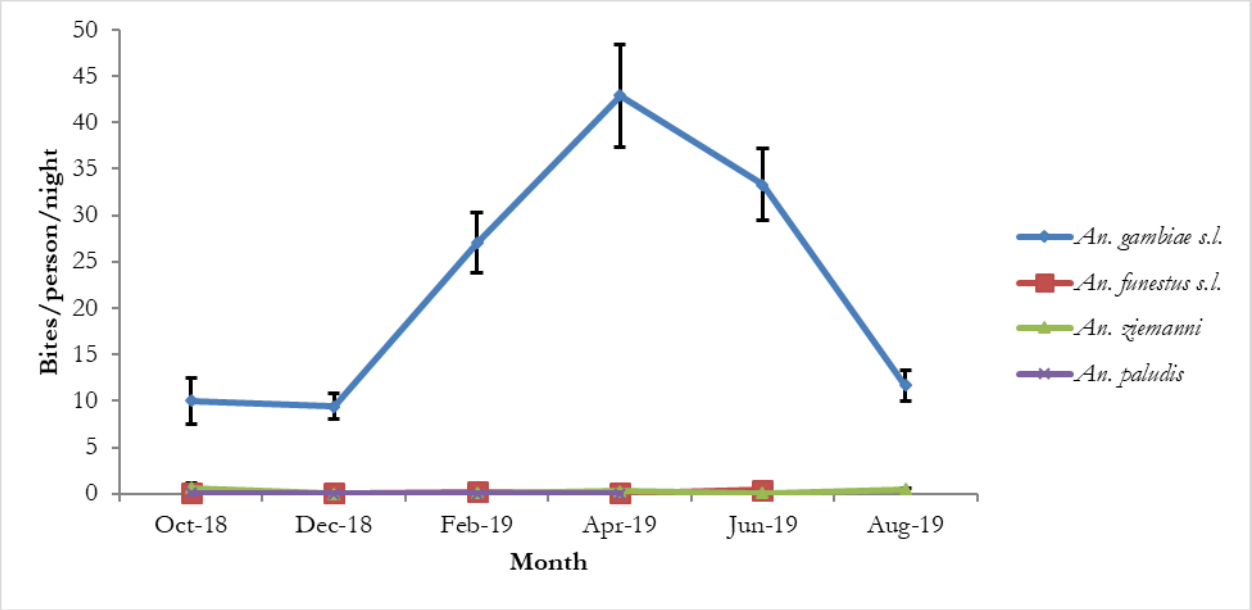
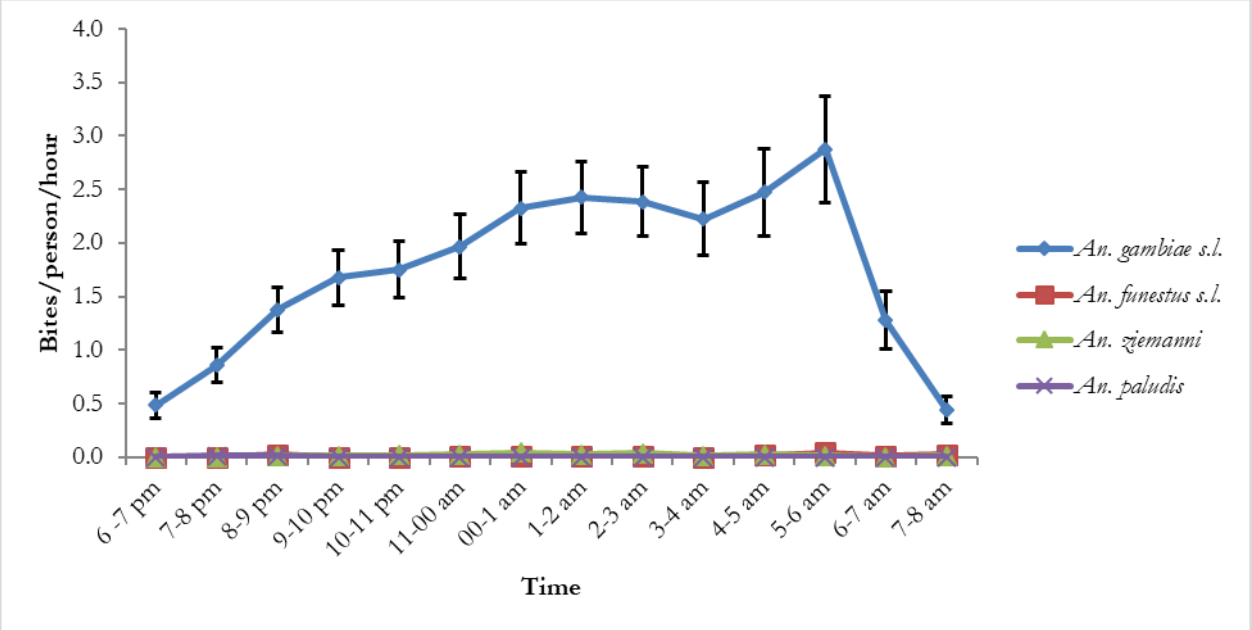


Figure 31: Hourly Biting Rate of *Anopheles* in Mangoum (October 2018–September 2019)



3.4.4 NYABESSANG

Four species were predominant in Nyabessang: *An. moucheti*, *An. paludis, An. gambiae* s.l., and *An. nili*. The average total HBR was 13.7 b/p/n for *An. moucheti* with 14.1 b/p/n indoors and 13.3 b/p/n outdoors, while *An. gambiae* s.l. averaged 6.4 b/p/n (6.2 b/p/n indoors and 6.5 b/p/n outdoors).

For *An. paludis*, the average total HBR was 12.69 b/p/n, and 2.9 b/p/n for *An. nili* (Figure 32 & Annex C). Peak biting of *An. moucheti* occurred between 11:00 p.m.–12:00 a.m. and from 5:00–6:00 a.m. for *An. gambiae* s.l. *An. moucheti* and *An. gambiae* s.l. continued biting until 8:00 a.m. (Figure 33). *An. moucheti* was found to be endophagic because more biting occurred indoors than outdoors (see Annex E).

Figure 32: Human Biting Rate of *Anopheles* in Nyabessang (October 2018–September 2019)

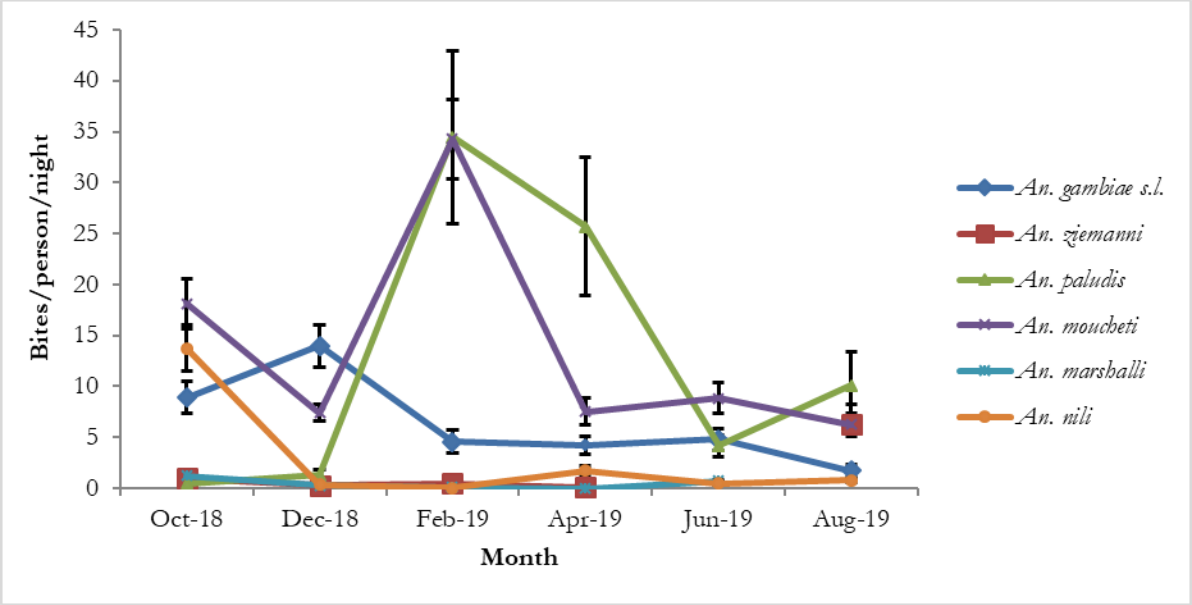
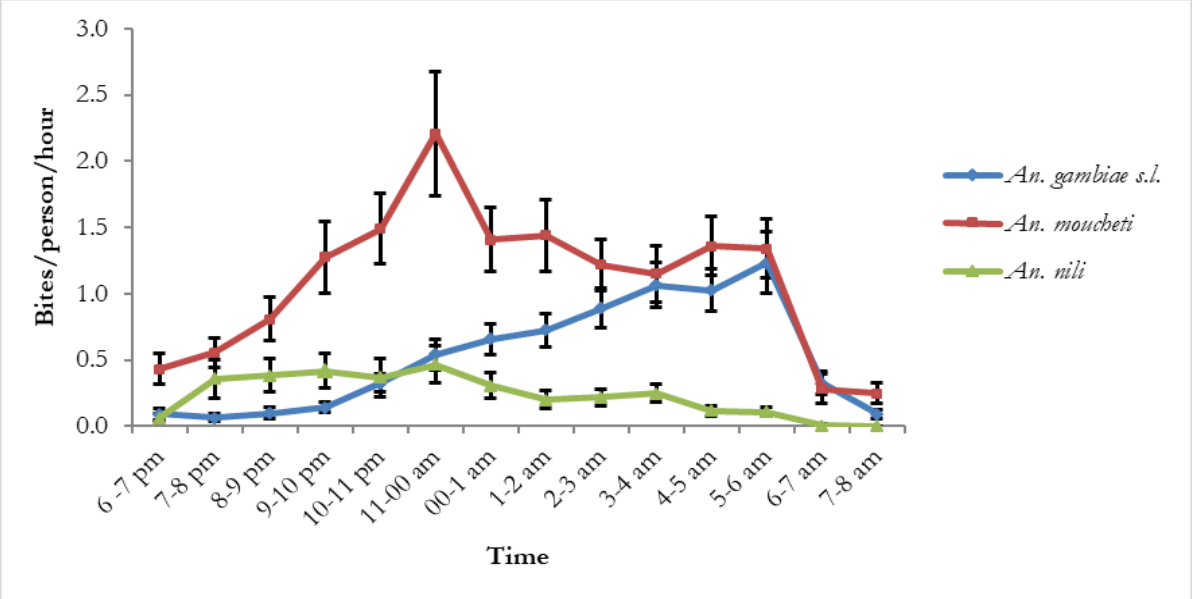


Figure 33: Hourly Biting Rate of *Anopheles* in Nyabessang (October 2018–September 2019)



3.4.5 BONABÉRI

An. gambiae s.l. was the major *Anopheles* found in Bonabéri, and the average total HBR was 24.2 b/p/n with 13.3 b/p/n indoors and 35.1 b/p/n outdoors.

The highest biting rates were recorded in February (32.7 b/p/n) and August 2019 (39 b/p/n) (Figure 34; see also Annex C). The peak biting time of *An. gambiae* s.l. was observed between 2:00–3:00 a.m. (Figure 35). Biting occurred until 8:00 a.m. at this site, and the vector was found to be exophagic (Annex E).

Figure 34: Human Biting Rate of *An. gambiae* s.l. in Bonabéri (December 2018–September 2019)

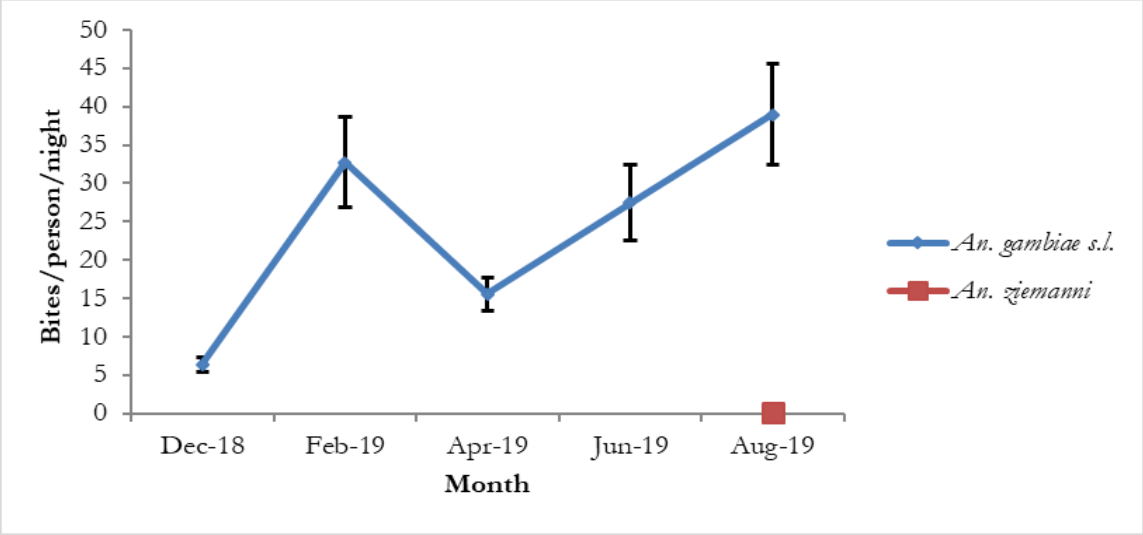
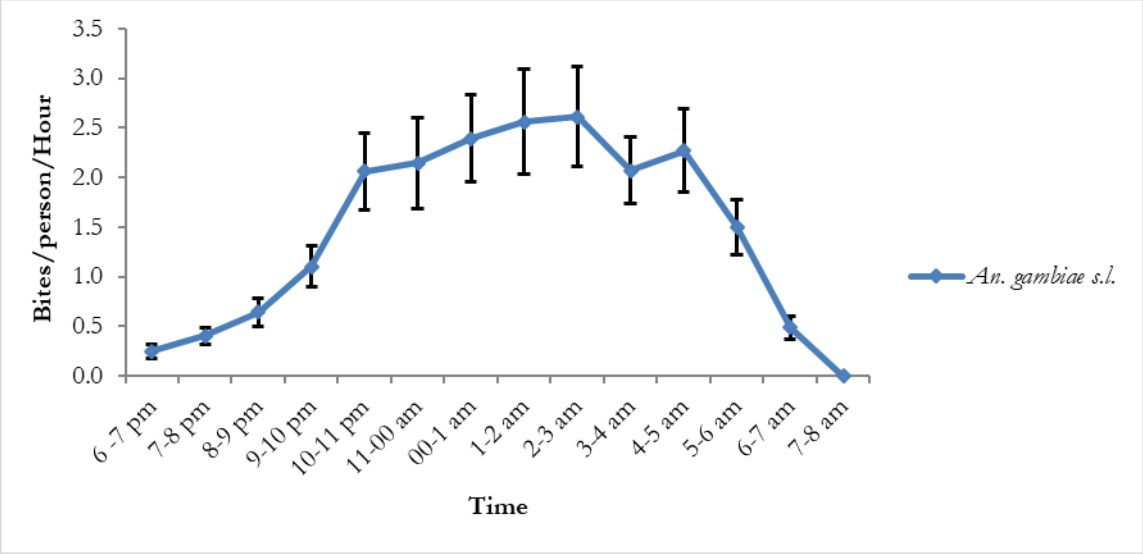


Figure 35: Hourly Biting Rate of *An. gambiae* s.l. in Bonabéri (December 2018–September 2019)



3.5 INDOOR RESTING DENSITY

The average *Anopheles* indoor resting density per room was 19.6 females/room/night (16,127 total females/ 820 rooms). Figures 36 to 40 illustrate the trend in each site from October 2018 to September 2019. Bonabéri yielded *Anopheles* mosquitos in December 2018 and August 2019; no *Anopheles* were collected in any other month at this site.

Figure 36: Indoor Resting Density of *Anopheles* in Gounougou (October 2018–September 2019)

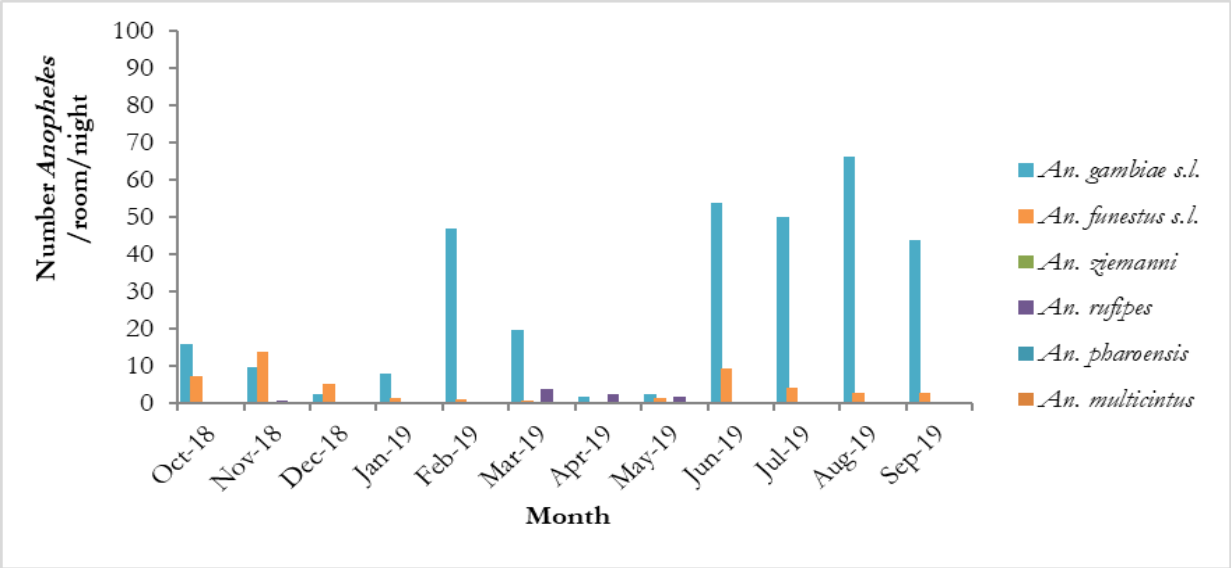


Figure 37: Indoor Resting Density of *Anopheles* in Simatou (October 2018–September 2019)

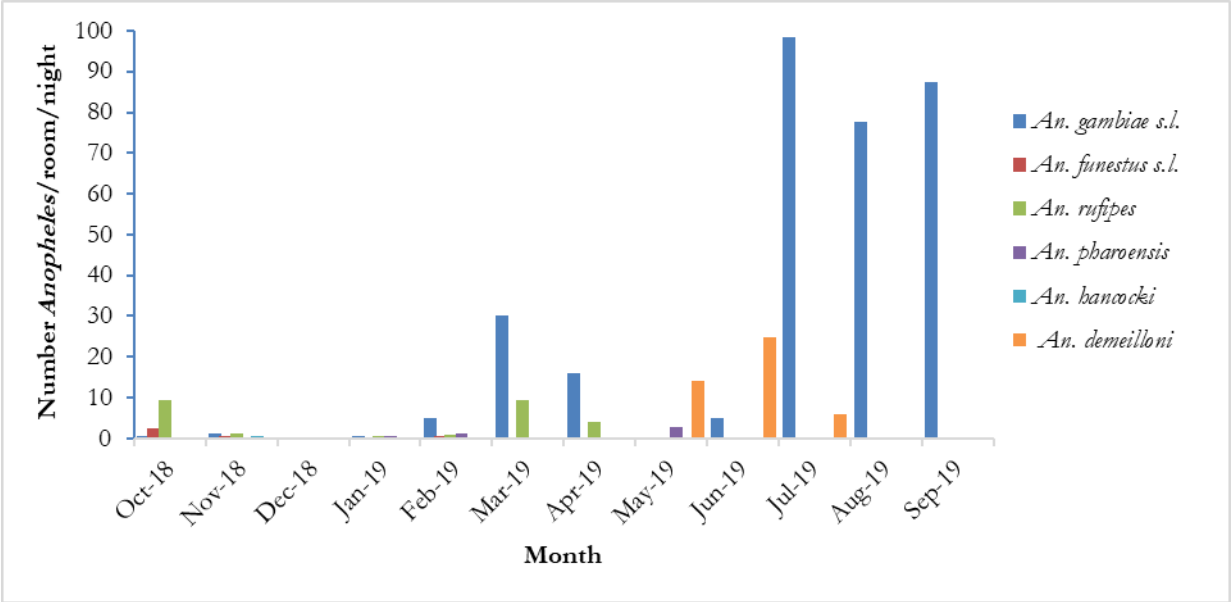


Figure 38: Indoor Resting Density of *Anopheles* in Mangoum (October 2018–September 2019)

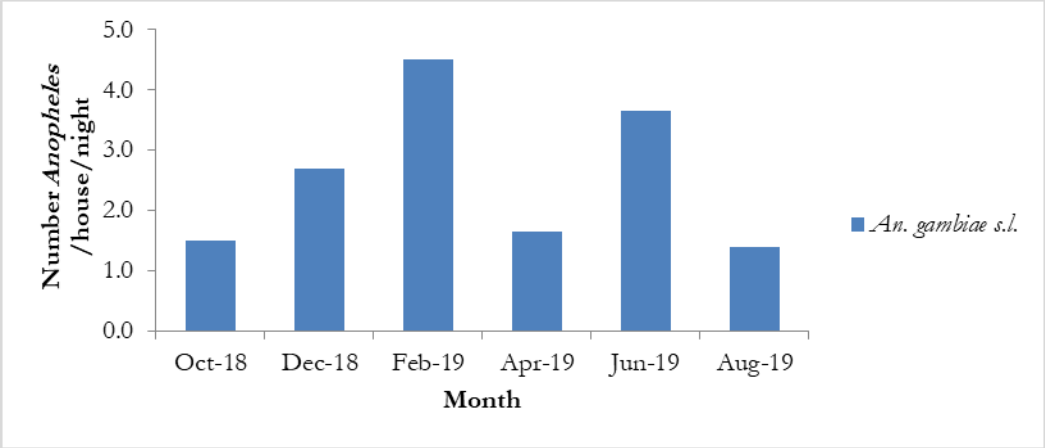


Figure 39: Indoor Resting Density of *Anopheles* in Nyabessang (October 2018–September 2019)

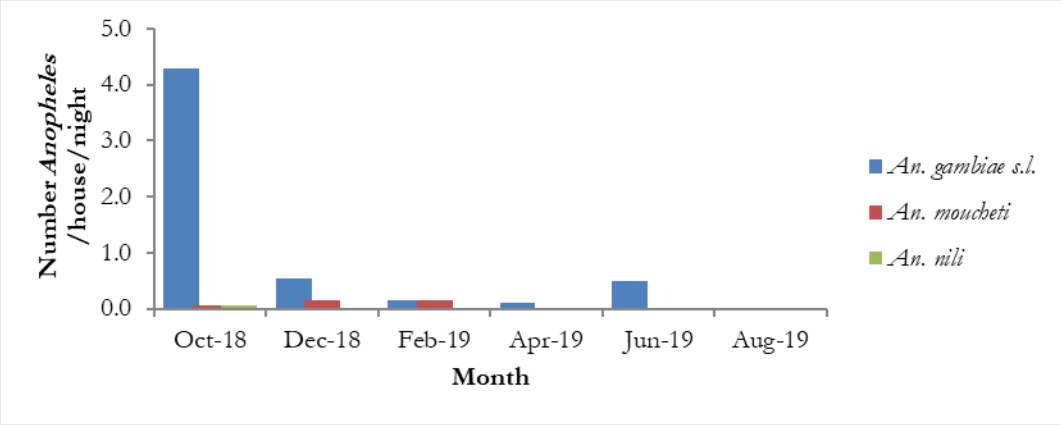
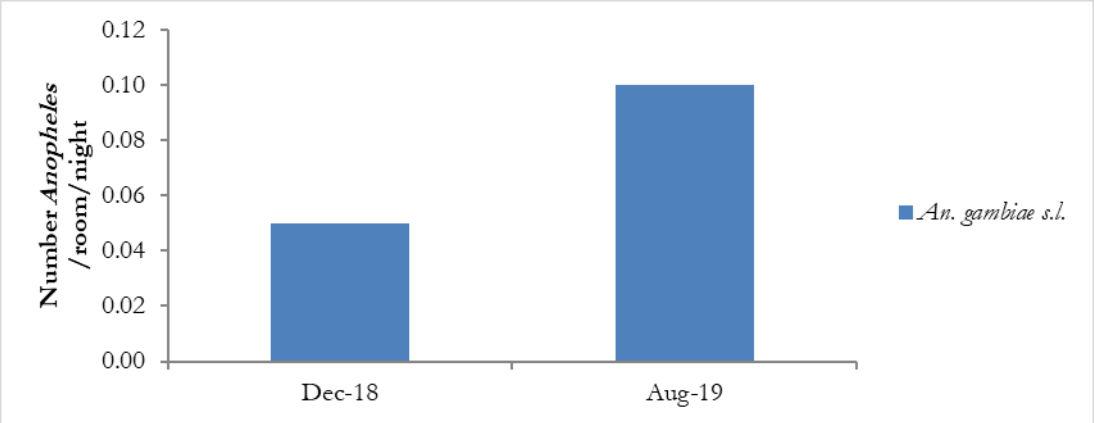


Figure 40: Indoor Resting Density of *Anopheles* in Bonabéri (October 2018–September 2019)



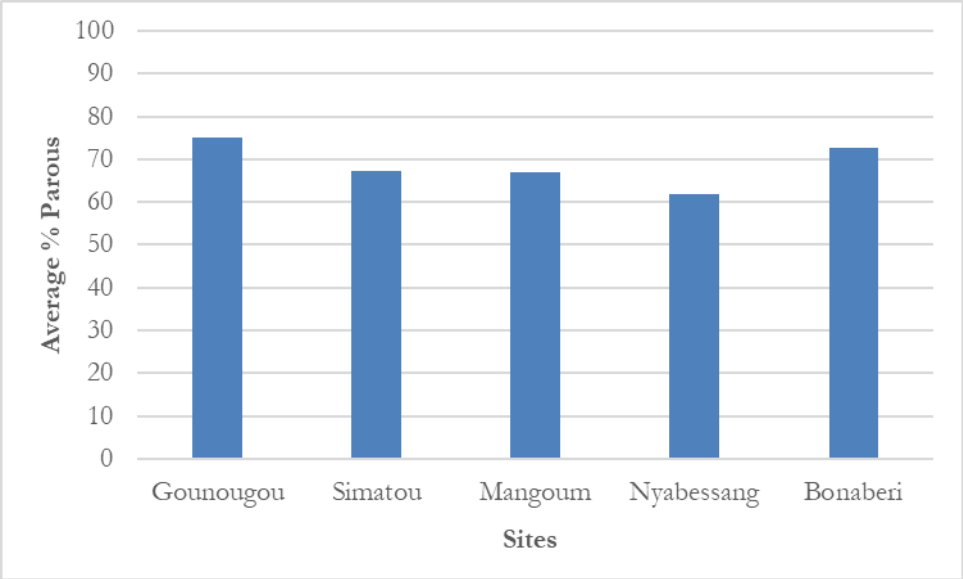
3.6 Host Preference

A total of 1,344 blood-fed *Anopheles* mosquitoes were analyzed using ELISAs, of which 432 were found to have fed on humans. The HBI was 32.1% (Annex F), varying from 21.7% in Simatou to 74.2% in Mangoum.

3.7 Parity

The ovaries of 11,051 *Anopheles* were dissected. The average parity rate across the five sites was 68.9%, with the lowest average rate observed in Nyabessang (57.1%) and the highest (76.4%) observed in Gounougou (see Figure 41 below and Annex G).

Figure 41: Average Parity Rate of *An. gambiae* s.l. across Sites (October 2018–September 2019)



3.8 Entomological Inoculation Rates per Site Using HLC

Out of 9,778 *Anopheles* mosquitos tested by ELISAs, 393 had the circumsporozoite antigen of *Plasmodium*. The total average infection rate was 4.0% (Annex H). Nine *Anopheles* species were found to be positive: *An. gambiae* s.l., *An. funestus* s.l., *An. nili*, *An. moucheti*, *An. demeillonni*, *An. pharoensis*, *An. ziemanni*, *An. multicinctus,* and *An. marshalli*. The infection rates were 3.6% (Gounougou), 3% (Simatou), 7.9% (Mangoum), 3.1% (Nyabessang), and 3.8% (Bonabéri). The EIR varied from 27.6 infected bites/person/month in Bonabéri to 80.5 infected bites/person/month in Simatou (Table 3).

Table 3: Entomological Inoculation Rate of Mosquitos Collected by HLCs (October 2018–September 2019)

Sentinel Site	Species	HBR	Infection Rate	EIR (infected bites/person/night)	Estimated Monthly EIR (infected bites/person/month)
Gounougou	*An. gambiae* s.l.	34.1	0.04	1.26	37.85
	An. funestus s.l.	2.6	0.03	0.08	2.42
	An. ziemanni	0.3	0.01	0.00	0.12

Sentinel Site	Species	HBR	Infection Rate	EIR (infected bites/person/ night)	Estimated Monthly EIR (infected bites/ person/month)
	An. multicinctus	0.4	0.09	0.04	1.06
Total EIR		**37.4**	**0.04**	**1.38**	**41.51**
Simatou	*An. gambiae* s.l.	57.8	0.05	2.89	86.70
	An. funestus s.l.	0.48	0.02	0.01	0.23
	An. ziemanni	1.86	0.00	0.01	0.22
	An. demeilloni	9.28	0.03	0.28	8.35
	An. rufipes	0.12	0.03	0.00	0.12
	An. pharoensis	22.9	0.01	0.32	9.62
Total EIR		**92.55**	**0.029**	**2.68**	**80.52**
Mangoum	*An. gambiae* s.l.	22.4	0.08	1.79	53.76
	An. ziemanni	0.25	0.03	0.0075	0.225
Total EIR		**22.63**	**0.08**	**1.79**	**53.63**
Nyabessang	*An. gambiae* s.l.	6.36	0.05	0.32	9.54
	An. moucheti	13.73	0.018	0.25	7.41
	An. nili	2.85	0.017	0.05	1.45
	An. ziemanni	1.36	0.05	0.07	2.04
	An. paludis	12.69	0.02	0.25	7.61
	An. marshalli	0.39	0.11	0.04	1.29
Total EIR		37.38	0.031	1.16	34.76
Bonabéri	*An. gambiae* s.l.	24.21	0.038	0.92	27.60
Total EIR		**24.21**	**0.038**	**0.92**	**27.60**

3.9 Insecticide Resistance Monitoring

3.9.1 Susceptibility Status of *An. gambiae* s.l.

Tables 4 and 5 below show the resistance status of *An. gambiae* s.l. to the different insecticides. Samples from four sites (Gounougou, Simatou, Nyabessang, and Mangoum) were tested in 2018 and extended to five in 2019 with the addition of Bonabéri. Resistance to the diagnostic dose of all pyrethroids was observed in all sites in 2018 and 2019. Resistance to pirimiphos-methyl was also observed in three sites (Simatou, Mangoum, and Nyabessang) in 2018, and susceptibility was recorded in all five sites in 2019. *An. gambiae* s.l. from

Simatou, Gounougou, and Bonabéri were susceptible to bendiocarb in 2018 and 2019; resistance was observed in those from Mangoum in 2019. Resistance is suspected in Nyabessang (95% mortality) in 2018.

High intensity pyrethroid resistance (less than 98% mortality at 10x the diagnostic dose) to deltamethrin, permethrin, and alpha-cypermethrin was observed in Gounougou, Simatou, and Mangoum. Moderate resistance (below 98% mortality at 5x or greater than 98% at 10x the diagnostic dose) was observed at Nyabessang and Bonabéri for permethrin and alpha-cypermethrin.

Pre-exposure of mosquitoes to PBO before deltamethrin, permethrin, or alpha-cypermethrin yielded a partial increase in mortality but did not lead to full restoration of susceptibility in all sites surveyed (Tables 15 and 16). Partial restoration of susceptibility was more significant in Simatou, Gounougou, and Nyabessang in 2018 and in Nyabessang and Bonabéri in 2019.

Susceptibility to clothianidin was recorded after 24 hours in Nyabessang, 48 hours in Bonabéri, and after 72 hours in Simatou and Gounougou in 2019. Resistance to clothianidin was seen after 72 hours in Mangoum (Figure 42). Susceptibility to chlorfenapyr (100 μg/bottle) was recorded at 24 hours after exposure in Nyabessang and after 72 hours in Simatou and Bonabéri (Figure 44). Susceptibility to chlorfenapyr (200 μg/bottle) was seen in Gounougou and Nyabessang after 24 hours and after 48 hours in Simatou and Bonabéri (Figure 43). Resistance to chlorfenapyr (both 100 and 200 μg/bottle) was recorded in Mangoum after 72 hours.

Table 4: WHO Susceptibility Test Results across Sites in 2018

Insecticides	Simatou		Gounougou		Mangoum		Nyabessang	
	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality
Pirimiphos methyl 1x	86	69 R	100	100 S	83	0 R	80	54 R
Pirimiphos methyl 5x	94	100 S	NA*	-	82	100 S	100	100 S
Permethrin 1x	88	16 R	NC*	-	83	0 R	100	8 R
Permethrin 5x	91	75 R	NC	-	84	36 R	100	99 S
Permethrin 10x	94	74 R	NC	-	83	54 R	NA	-
Permethrin + PBO	89	48 R	NC	-	85	2 R	100	14 R
Deltamethrin 1x	91	51 R	100	39 R	83	0 R	100	23 R
Deltamethrin 5x	92	65 R	100	73 R	86	8 R	100	27 R
Deltamethrin 10x	84	76 R	100	92 PR	82	35 R	100	100 S
Deltamethrin + PBO	94	91 PR	100	89 PR	83	25 R	100	86 R
Alpha-cypermethrin 1x	80	12 R	100	78 R	82	1 R	100	7 R
Alpha-cypermethrin 5x	89	46 R	100	85 R	84	5 R	100	35 R
Alpha-cypermethrin 10x	89	65 R	100	77 R	86	37 R	100	98 S
Alpha-cypermethrin + PBO	88	95 PR	100	94 PR	83	78 R	100	95 PR
Bendiocarb 1x	92	100 S	NC	-	NC	-	100	95 PR

R Resistant | PR Possibly Resistant | S Susceptible | NA= Not applicable / NC= Not completed

Table 5: WHO Susceptibility Test Results across Sites in 2019

Insecticides	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total exposed	% Mortality	Total Exposed	% Mortality
Pirimiphos methyl 1x	93	100 S	91	100 S	84	100 S	100	100 S	100	100 S
Permethrin 1x	89	11.3 R	80	1.3 R	80	0 R	80	6.3 R	89	8.7 R
Permethrin 5x	93	59.2 R	102	58 R	83	22 R	100	95 PR	95	90 PR
Permethrin 10x	88	88.5 R	87	68 R	82	90 PR	100	100 S	100	100 S
PBO + Permethrin	93	37.9 R	88	24 R	84	3.4 R	80	61 R	99	69 R
Deltamethrin 1x	85	21.1 R	88	8.0 R	83	0 R	80	40 R	83	59 R
Deltamethrin 5x	95	50.5 R	102	31.5 R	86	9.2 R	100	99 S	100	92 PR
Deltamethrin 10x	94	56.4 R	85	29.5 R	93	19 R	NA	-	96	92 PR
PBO + deltamethrin	87	68.7 R	81	12 R	80	38 R	80	97 PR	100	96 PR
Alpha-cypermethrin 1x	90	21.5 R	81	6.2 R	83	0 R	100	31 R	93	14 R
Alpha-cypermethrin 5x	82	40.3 R	94	17 R	89	3.5 R	100	97 PR	96	94 PR
Alpha-cypermethrin 10x	95	54.6 R	87	23 R	85	17 R	100	98 S	100	100 S
PBO + Alpha-cypermethrin	92	78.5 R	87	39 R	82	18 R	100	97 PR	95	57 R
Bendiocarb 1x	95	100 S	93	100 S	82	47 R	100	93 PR	100	99 S
Bendiocarb 5x	NA	-	NA	-	92	52.3 R	100	97 PR	NA	-

R Resistant PR Possibly Resistant S Susceptible NA= Not applicable / NC= Not completed

Figure 42: Susceptibility Test Results of *An. gambiae* s.l. to Clothianidin by Site in 2019

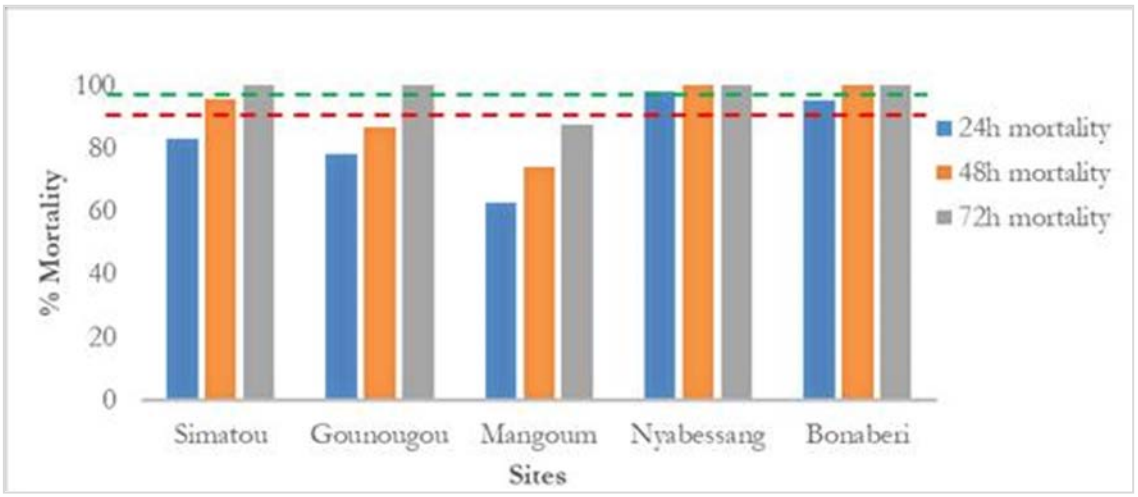


Figure 43: Susceptibility Test Results of *An. gambiae* s.l. to Chlorfenapyr (100 µg/bottle) by Site in 2019

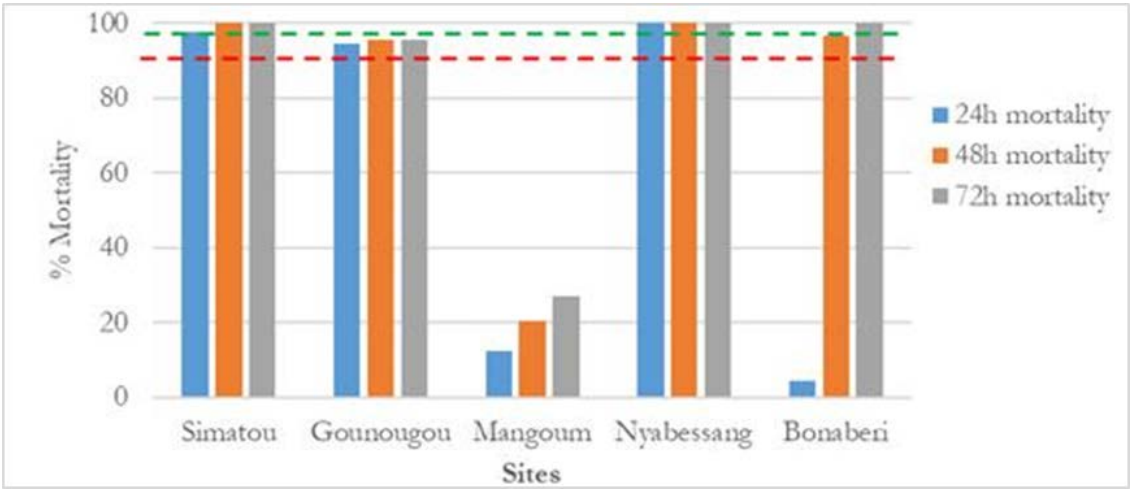
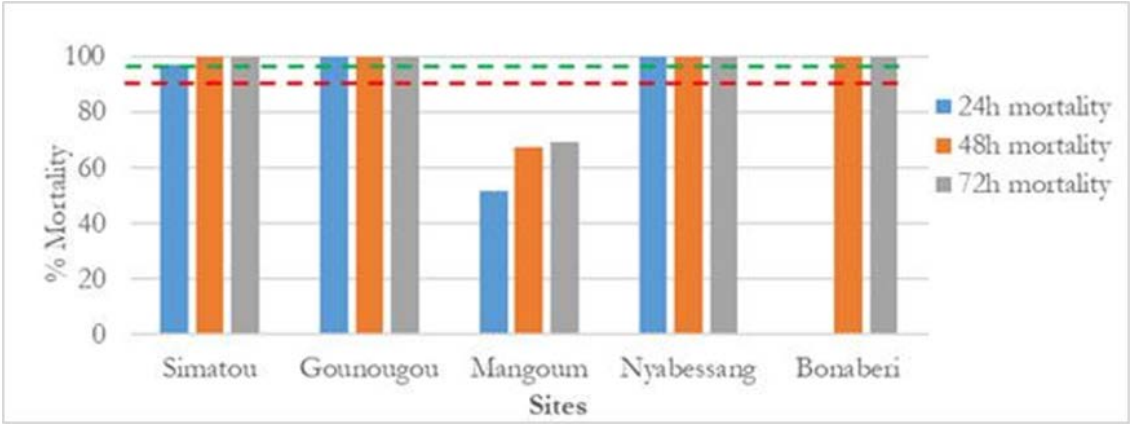


Figure 44: Susceptibility Test Results of *An. gambiae* s.l. to Chlorfenapyr (200 µg/bottle) by Site in 2019



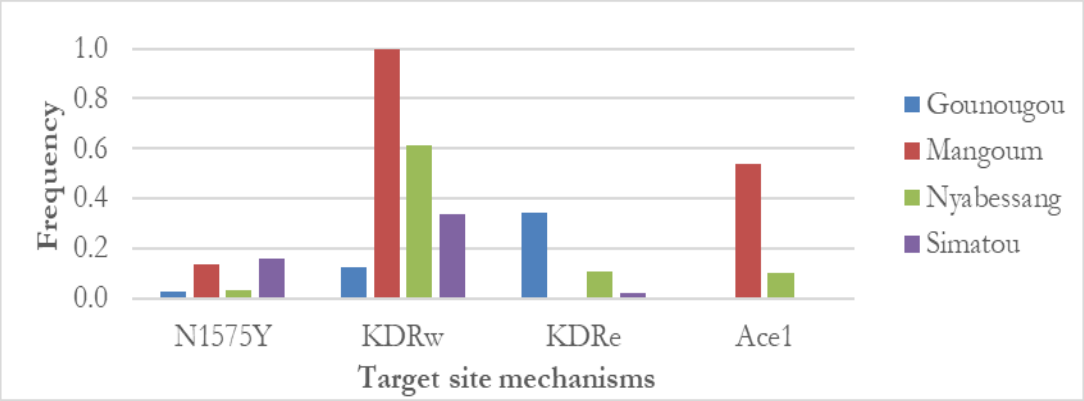
Note: the red and green lines in Figures 42-44 represent the resistance and susceptibility thresholds, respectively.

3.9.2 TARGET SITE RESISTANCE

Insecticide resistance in mosquitos can be related to target site mutations. Among them, resistance to pyrethroids and DDT is described as a substitution of amino acid leucine to either phenylalanine (L1014F, referred as *kdr*-West) or serine (L1014S, referred as *kdr*-East) at the position 1014 in the sodium channel gate. The N1575Y represents an additional mutation involved in the *kdr* mutation. For organophosphate and carbamate insecticides, the target site mechanism, known as *Ace-1,* is a substitution of an amino acid glycine to serine at position 119.

Figure 45 describes the frequency of each mechanism per collection site characterized within the samples tested in 2018 (Annex I). Four mutations were found: *kdr*-w (L1014F), *kdr*-e (L1014S), N1575Y for pyrethroids, and Ace-1 for organophosphates and carbamates. *Kdr*-w (L1014F) and N1575Y were present in all four sentinel sites. *Kdr*-w was only found in Mangoum. *Kdr*-e (L1014S) was found in three of the four sentinel sites (Gounougou, Simatou, and Nyabessang). Ace-1 (G296S) was found in Nyabessang and Mangoum.

Figure 45: Frequency of Target Site Mechanisms Involved in the Resistance of *An. gambiae* s.l.



Note: Insecticide testing was not carried out in Bonabéri in 2018.

3.9.3 METABOLIC RESISTANCE

Metabolic resistance represents the production of enzymes by the mosquitoes to decrease the insecticidal effect of the pyrethroid insecticides. Different enzymes are involved in the metabolic resistance and are referred to mono-oxygenase (the CY group), esterases, or Glutation-S-Transferases (GSTe2). The metabolic resistance is expressed as a number fold change of the mosquito population tested (Figure 46-47). CYP6P9A, CYP6P5, CY6M7, and GSTe2 were found in *An. funestus* s.l. from Gounougou, and CYP6M2, CYP6P3, and GSTe2 were found in *An. gambiae* s.l., also from Gounougou.

Figure 46: Frequency of *An. funestus* s.l. Fold-Changes

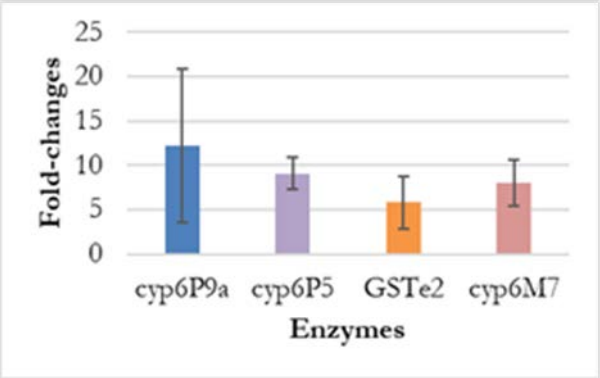
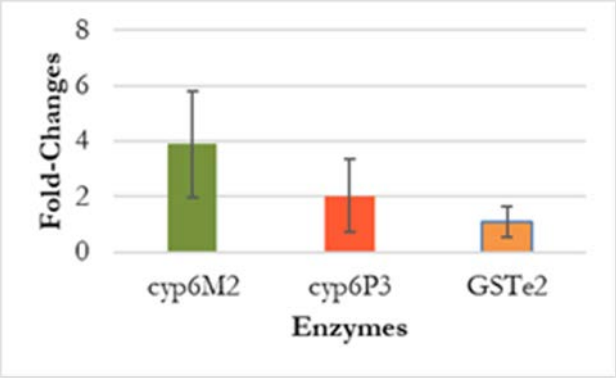


Figure 47: Frequency of *An. gambiae* s.l. Fold-Changes



4. Discussion and Conclusions

Bionomic surveys conducted in Cameroon from October 2018 to September 2019 in the five sentinel sites showed a large diversity of *Anopheles* species across sites. *An. gambiae* s.l., *An. ziemanni*, *An. pharoensis,* and *An. funestus* s.l. were the most abundant and were found in all sites (except Bonabéri) and collection methods (HLCs, PSCs, and CDC light traps). *An. moucheti* and *An. nili* were collected only in Nyabessang.

An. gambiae s.l. were collected at variable proportions depending on the collection method. In Bonabéri and Mangoum, *An. gambiae* s.l. was the only vector collected using HLCs and PSCs. *An. moucheti* and *An. nili* were also collected in Nyabessang using PSCs. Notably, the extended HLC collection time to 8:00 a.m. showed changes in biting time of *An. gambiae* s.l., *An. funestus* s.l., and *An. moucheti*. Biting by all three species occurred until 8:00 a.m. in Mangoum, Nyabessang, Simatou, and Bonabéri.

The longevity of the vectors represents an important parameter to consider for IRS implementation. All five sites recorded an average annual parity rate above 60% for *An. gambiae* s.l. Gounougou recorded the highest overall rate, with 75.1% parous *An. gambiae* s.l.

Within Africa, Cameroon has one of most diverse populations of malaria vectors (Nkondjio & al, 2019). Nine *Anopheles* species involved in malaria transmission were collected and confirmed to have sporozoites through ELISAs. The estimated monthly EIR varied from 27.6 infected bites per person per month (Bonabéri) to 80.5 infected bites per person per month (Simatou).

Three sub-species of *An. gambiae* complex (*An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis)* and hybrids of *An. gambiae*/*An. coluzzii* were identified. *An. arabiensis* were found in the drier areas (Gounougou and Simatou). Additionally, *An. leesoni* and *An. funestus* s.s. were identified in Gounougou and Simatou and were the only members of the *An. funestus* group from these sites.

Resistance to all three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) was observed in all sites in 2018 and 2019. The resistance intensity varied by site and insecticide. Higher resistance intensity was observed in Gounougou, Simatou, and Mangoum for deltamethrin, permethrin, and alpha-cypermethrin, while moderate resistance to permethrin and alpha-cypermethrin was seen in Nyabessang and Bonabéri. Furthermore, synergist assays with PBO showed partial restoration of susceptibility of all three pyrethroids at all sites.

Resistance of *An. gambiae* s.l. to pirimiphos-methyl was recorded in 2018 in three sites (Simatou, Mangoum, and Nyabessang). The tests conducted in 2019, however, showed full susceptibility to pirimiphos-methyl at all five sites. This could be explained by either the quality of the papers used in 2018 or the population of the mosquitos tested. The 2020 collection data will help confirm the results obtained during both years.

Susceptibility to bendiocarb was recorded in Simatou, Gounougou, and Bonabéri in 2018 and 2019, while the population of Mangoum was resistant and possible resistance was observed in Nyabessang. Moreover, susceptibility to clothianidin was observed at different periods: 24 hours for clothianidin (Nyabessang), 48 hours (Bonabéri), and 72 hours (Simatou and Gounougou). The results of CDC bottle assays using chlorfenapyr (100 µg/bottle) showed susceptibility of *An. gambiae* s.l. 24 hours after exposure at Nyabessang and 72 hours after exposure at Simatou and Bonabéri. Susceptibility to chlorfenapyr (200 µg/bottle) was also observed 24 hours after exposure in Gounougou, Nyabessang, Simatou, and Bonabéri.

The population of *An. gambiae* s.l. tested was characterized by two types of insecticide resistance mechanisms: target site mutations (*Kdr*, *Ace-1,* and N1575Y) and metabolic resistance.

Considering the change in biting behavior of *Anopheles* mosquitos and the high level of resistance to insecticides used for ITNs, additional vector control interventions such as IRS may be needed to reduce the malaria transmission and burden in Cameroon.

5. RECOMMENDATIONS

5.1 FOR NMCP

- Use the current data on insecticide resistance to draft or update the insecticide resistance management plan.
- Given the high resistance of *An. gambiae* s.l. to pyrethroids, the partial restoration of susceptibility using PBO, and the change in biting behavior of malaria vectors, consider introducing:
  - IRS in selected areas using non-pyrethroid insecticides (e.g. pirimiphos-methyl, bendiocarb)
  - Next-generation ITNs (PBO and Interceptor G2) in select areas.

5.2 FOR PMI

- Support the country to update its insecticide resistance management plan and scale up vector surveillance by increasing the number of sentinel sites across the country.

ANNEX A: SPECIES COMPOSITION OF *ANOPHELES* COLLECTED USING HLC (OCTOBER 2018–SEPTEMBER 2019)

Species	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	Total	Frequency (%)
An. gambiae s.l.	9,815	16,653	3,224	916	2,905	**33,513**	**68.2%**
An. funestus s.l.	743	138	18	0	0	**899**	**1.8%**
An. ziemanni	99	536	36	196	1	**868**	**1.8%**
An. paludis	0	1	2	1,827	0	**1,830**	**3.7%**
An. moucheti	0	0	0	1,977	0	**1,977**	**4.0%**
An. rufipes	11	34	0	0	0	**45**	**0.1%**
An. marshalli	0	0	0	56	0	**56**	**0.1%**
An. pharoensis	126	6,622	0	0	0	**6,748**	**13.7%**
An. christyi	1	0	0	0	0	**1**	**0.0%**
An. multicinctus	113	0	0	0	0	**113**	**0.2%**
An. nili	0	0	0	411	0	**411**	**0.8%**
An. coustani	1	3	0	0	0	**4**	**0.0%**
An. welcomei	0	7	0	0	0	**7**	**0.0%**
An. tenebrosus	9	0	0	0	0	**9**	**0.0%**
An. smithii	5	0	0	0	0	**5**	**0.0%**
An. demeilloni	0	2,672	0	0	0	**2,672**	**5.4%**
Total	10,923	26,666	3,280	5,383	2,906	**49,158**	**100.0%**

ANNEX B: SPECIES COMPOSITION OF *ANOPHELES* COLLECTED USING CDC LTS (OCTOBER 2018–SEPTEMBER 2019)

Species	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	Total	Frequency (%)
An. gambiae s.l.	2,201	2,796	704	157	0	5,858	31.3%
An. funestus s.l.	435	79	0	0	0	514	2.7%
An. ziemanni	416	7,598	0	2	0	8,016	42.8%
An. paludis	0	0	0	53	0	53	0.3%
An. moucheti	0	0	0	200	0	200	1.1%
An. hancocki	0	1	0	0	0	1	0.0%
An. rufipes	106	311	0	0	0	417	2.2%
An. marshalli	0	0	0	3	0	3	0.0%
An. pharoensis	79	2,941	0	0	0	3,020	16.1%
An. christyi	1	0	0	0	0	1	0.0%
An. cinerus	3	0	0	0	0	3	0.0%
An. multicinctus	63	0	0	0	0	63	0.3%
An. nili	0	0	0	12	0	12	0.1%
An. demeilloni	0	564	0	0	0	564	3.0%
Total	**3,304**	**14,290**	**704**	**427**	**0**	**18,725**	**100.0%**

ANNEX C: SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. COMPLEX AND *AN. FUNESTUS* S.L. GROUP

Sites	*An. gambiae* s.l.				Total *An. gambiae* s.l.	*An. funestus* s.l.		Total *An. funestus* s.l.	Grand Total
	An. gambiae (%)	*An. coluzzii* (%)	*An. arabiensis* (%)	*An. coluzzii/ An. gambiae* (%)		*An. funestus* s.s. (%)	*An. leesoni* (%)		
Simatou	15 (2.3)	565 (87.3)	64 (9.9)	3 (0.5)	**647**	112 (56.3)	87 (43.7)	**199**	**846**
Gounougou	28 (4.0)	534 (75.5)	145 (20.5)	0 (0.0)	**707**	242 (87.1)	36 (12.9)	**278**	**985**
Mangoum	403 (98.3)	4 (1.0)	0 (0.0)	3 (0.7)	**410**	9 (100.0)	0	**9**	**419**
Nyabessang	361 (92.1)	24 (6.1)	0 (0.0)	7 (1.8)	**392**	0	0	**0**	**392**
Bonabéri	10 (2.6)	370 (97.4)	0 (0.0)	0 (0.)	**380**	0	0	**0**	**380**
Total	**817**	**1,497**	**209**	**13**	**2,536**	**363**	**123**	**486**	**3,022**

ANNEX D: HUMAN BITING RATE OF *ANOPHELES* MOSQUITOES BY SITE

Table D.1: Human Biting Rate of *Anopheles* Mosquitos in Gounougou (October 2018–April 2019)

	October 2018		November 2018		December 2018		January 2019		February 2019		March 2019		April 2019	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	429	17.88	299	12.46	187	7.79	288	12.00	1,665	69.38	665	27.71	62	2.58
An. funestus s.l.	129	5.38	114	4.75	96	4.00	25	1.04	12	0.50	13	0.54	8	0.33
An. ziemanni	12	0.50	29	1.21	27	1.13	1	0.04	3	0.13	2	0.08	4	0.17
An. rufipes	3	0.13	1	0.04	1	0.04	1	0.04	1	0.04	0	0.00	2	0.08
An. pharoensis	2	0.08	3	0.13	8	0.33	8	0.33	41	1.71	29	1.21	3	0.13
An. multicinctus	1	0.04	3	0.13	9	0.38	1	0.04	2	0.08	61	2.54	1	0.04
An. coustani	0	0.00	1	0.04	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
An. tenebrosus	0	0	0	0	0	0	2	0.08	0	0.00	4	0.17	0	0.00
An. smithii	0	0	0	0	0	0	0	0.00	0	0.00	5	0.21	0	0.00
An. christyi	0	0	0	0	0	0	0	0.00	0	0.00	0	0.00	0	0.00

Table D.2: Human Biting Rate of *Anopheles* Mosquitos in Gounougou (May–September 2019)

Species	May 2019		June 2019		July 2019		August 2019		September 2019		Total	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	43	1.79	877	36.54	1,755	73.13	2,545	106.04	1,000	41.67	**9,815**	**34.1**
An. funestus s.l.	34	1.42	117	4.88	136	5.67	39	1.63	20	0.83	**743**	**2.6**
An. ziemanni	8	0.33	1	0.04	5	0.21	1	0.04	6	0.25	**99**	**0.3**
An. rufipes	1	0.04	0	0.00	0	0.00	0	0.00	0	0.00	**11**	**0.0**
An. pharoensis	12	0.50	4	0.17	5	0.21	0	0.00	11	0.46	**126**	**0.4**
An. multicintus	1	0.04	10	0.42	12	0.50	8	0.33	5	0.21	**113**	**0.4**
An. coustani	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**1**	**0.0**
An. tenebrosus	3	0.13	0	0.00	0	0.00	0	0.00	0	0.00	**9**	**0.0**
An. smithii	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**5**	**0.0**
An. christyi	0	0.00	1	0.04	0	0.00	0	0.00	0	0.00	**1**	**0.0**

Table D.3: Human Biting Rate of Anopheles Mosquitos in Simatou (October 2018–April 2019)

Species	October 2018		November 2018		December 2018		January 2019		February 2019		March 2019		April 2019	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	173	7.21	39	1.63	5	0.21	24	1.00	105	4.38	524	21.83	559	23.29
An. funestus s.l.	33	1.38	34	1.42	3	0.13	1	0.04	5	0.21	12	0.50	4	0.17
An. ziemanni	224	9.33	199	8.29	6	0.25	0	0.00	0	0.00	0	0.00	11	0.46
An. paludis	1	0.04	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
An. rufipes	5	0.21	9	0.38	2	0.08	5	0.21	1	0.04	6	0.25	1	0.04
An. pharoensis	92	3.83	49	2.04	15	0.63	297	12.38	722	30.08	479	19.96	1,017	42.38
An. coustani	3	0.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
An. welcomei	2	0.08	3	0.13	0	0.00	1	0.04	1	0.04	0	0.00	0	0.00
An. demeilloni	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	18	0.75	397	16.54

Table D.4: Human Biting Rate of *Anopheles* Mosquitos in Simatou (May–September 2019 and Total)

Species	May 2019		June 2019		July 2019		August 2019		September 2019		Total (Oct 2018–Sept 2019)	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	7	0.29	1,003	41.79	6,749	281.21	4,190	174.58	3,275	136.46	**16,653**	**57.82**
An. funestus s.l.	1	0.04	43	1.79	0	0.00	0	0.00	2	0.08	**138**	**0.48**
An. ziemanni	14	0.58	41	1.71	19	0.79	11	0.46	11	0.46	**536**	**1.86**
An. paludis	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**1**	**0.00**
An. rufipes	1	0.04	3	0.13	0	0.00	0	0.00	1	0.04	**34**	**0.12**
An. pharoensis	990	41.25	763	31.79	830	34.58	856	35.67	512	21.33	**6,622**	**22.99**
An. coustani	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**3**	**0.01**
An. welcomei	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**7**	**0.02**
An. demeilloni	729	30.38	1,489	62.04	22	0.92	0	0.00	17	0.71	**2,672**	**9.28**

Table D.5: Human Biting Rate of *Anopheles* Species in Mangoum (October 2018–August 2019)

	October 2018		December 2018		February 2019		April 2019		June 2019		August 2019		Total	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	240	10.00	226	9.42	649	27.04	1,029	42.88	800	33.33	280	11.67	**3,224**	**22.39**
An. funestus s.l.	1	0.04	1	0.04	5	0.21	2	0.08	9	0.38	0	0.00	**18**	**0.13**
An. ziemanni	14	0.58	0	0.00	2	0.08	7	0.29	2	0.08	11	0.46	**36**	**0.25**
An. paludis	2	0.08	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**2**	**0.01**

Table D.6: Human Biting Rate of *Anopheles* Species in Nyabessang (October 2018–August 2019)

Species	October 2018		December 2018		February 2019		April 2019		June 2019		August 2019		Total	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	214	8.92	335	13.96	109	4.54	101	4.21	115	4.79	42	1.75	**916**	**6.36**
An. ziemanni	24	1.00	7	0.29	11	0.46	3	0.13	0	0.00	151	6.29	**196**	**1.36**
An. paludis	11	0.46	31	1.29	827	34.46	616	25.67	100	4.17	242	10.08	**1,827**	**12.69**
An. moucheti	435	18.13	177	7.38	822	34.25	181	7.54	212	8.83	150	6.25	**1,977**	**13.73**
An. marshalli	30	1.25	7	0.29	2	0.08	0	0.00	17	0.71	0	0.00	**56**	**0.39**
An. nili	330	13.75	8	0.33	1	0.04	42	1.75	11	0.46	19	0.79	**411**	**2.85**

ANNEX E: HUMAN BITING RATE AND ENDOPHAGIC INDEX OF *ANOPHELES* ACROSS SITES

Table E.1: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Gounougou from October 2018–January 2019

Species	2018												2019			
	October				November				December				January			
	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index
An. gambiae s.l.	20.00	15.75	17.88	0.56	13.42	11.50	12.46	0.54	6.17	9.42	7.79	0.40	10.33	13.67	12.00	0.43
An. funestus s.l.	7.50	3.25	5.38	0.70	6.75	2.75	4.75	0.71	4.25	3.75	4.00	0.53	0.75	1.33	1.04	0.36
An. ziemanni	0.00	1.00	0.50	0.00	0.83	1.58	1.21	0.34	0.92	1.33	1.13	0.41	0.00	0.08	0.04	0.00
An. rufipes	0.00	0.25	0.13	0.00	0.08	0.00	0.04	1.00	0.08	0.00	0.04	1.00	0.00	0.08	0.04	0.00
An. pharoensis	0.00	0.17	0.08	0.00	0.00	0.25	0.13	0.00	0.17	0.50	0.33	0.25	0.00	0.67	0.33	0.00
An. multicinctus	0.00	0.08	0.04	0.00	0.08	0.17	0.13	0.33	0.08	0.67	0.38	0.11	0.00	0.08	0.04	0.00
An. coustani	0.00	0.00	0.00	0.00	0.00	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
An. tenebrosus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.08	0.00
An. smithii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
An. christyi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.2: Human Biting Rate and Endophagic Index of *Anopheles* Species in Gounougou from February–May 2019

Species	February				March				April				May			
	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index
An. gambiae s.l.	51.42	87.33	69.38	0.37	23.67	31.75	27.71	0.43	1.67	3.50	2.58	0.32	2.00	1.58	1.79	0.56
An. funestus s.l.	0.67	0.33	0.50	0.67	0.58	0.50	0.54	0.54	0.33	0.33	0.33	0.50	1.50	1.33	1.42	0.53
An. ziemanni	0.17	0.08	0.13	0.67	0.00	0.17	0.08	0.00	0.08	0.25	0.17	0.25	0.08	0.58	0.33	0.13
An. rufipes	0.08	0.00	0.04	1.00	0.00	0.00	0.00	0.00	0.17	0.00	0.08	1.00	0.00	0.08	0.04	0.00
An. pharoensis	1.75	1.67	1.71	0.51	1.00	1.42	1.21	0.41	0.17	0.08	0.13	0.67	0.42	0.58	0.50	0.42
An. multicinctus	0.08	0.08	0.08	0.50	0.92	4.17	2.54	0.18	0.00	0.08	0.04	0.00	0.00	0.08	0.04	0.00
An. coustani	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. tenebrosus	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.00	0.00	0.00	0.00	0.00	0.08	0.17	0.13	0.33
An. smithii	0.00	0.00	0.00	0.00	0.17	0.25	0.21	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. christyi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.3: Human Biting Rate and Endophagic Index* of Anopheles Species in Gounougou from June–August 2019

Species	June				July				August			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	34.92	38.17	36.54	0.48	73.58	72.67	73.13	0.50	99.42	112.67	106.04	0.47
An. funestus s.l.	5.25	4.50	4.88	0.54	5.17	6.17	5.67	0.46	2.00	1.25	1.63	0.62
An. ziemanni	0.08	0.00	0.04	1.00	0.17	0.25	0.21	0.40	0.00	0.08	0.04	0.00
An. rufipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. pharoensis	0.00	0.33	0.17	0.00	0.25	0.17	0.21	0.60	0.00	0.00	0.00	0.00
An. multicintus	0.17	0.67	0.42	0.20	0.17	0.83	0.50	0.18	0.33	0.33	0.33	0.50
An. coustani	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. tenebrosus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. smithii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. christyi	0.08	0.00	0.04	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.4: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Gounougou in September 2019 and Total from October 2018–September 2019

Species	September				Total (October 2018–September 2019)			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	49.33	34.00	41.67	0.59	32.16	36.00	34.08	0.47
An. funestus s.l.	0.83	0.83	0.83	0.50	2.97	2.19	2.58	0.57
An. ziemanni	0.25	0.25	0.25	0.50	0.22	0.47	0.34	0.31
An. rufipes	0.00	0.00	0.00	0.00	0.03	0.04	0.03	0.46
An. pharoensis	0.50	0.42	0.46	0.55	0.35	0.52	0.44	0.40
An. multicintus	0.08	0.33	0.21	0.20	0.16	0.63	0.39	0.20
An. coustani	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
An. tenebrosus	0.00	0.00	0.00	0.00	0.01	0.06	0.03	0.11
An. smithii	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.40
An. christyi	0.00	0.00	0.00	0.00	0.01	0.00	0.00	1.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.5: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Simatou from October 2018–January 2019

Species	2018												2019			
	October				November				December				January			
	HBR in	HBR out	Total HBR	Endo-phagic index	HBR in	HBR out	Total HBR	Endo-phagic index	HBR in	HBR out	Total HBR	Endo-phagic index	HBR in	HBR out	Total HBR	Endo-phagic index
An. gambiae s.l.	6.92	7.50	7.21	0.48	1.67	1.58	1.63	0.51	0.17	0.25	0.21	0.40	1.08	0.92	1.00	0.54
An. funestus s.l.	1.42	1.33	1.38	0.52	1.50	1.33	1.42	0.53	0.08	0.17	0.13	0.33	0.08	0.00	0.04	1.00
An. ziemanni	8.75	9.92	9.33	0.47	6.42	10.17	8.29	0.39	0.33	0.17	0.25	0.67	0.00	0.00	0.00	0.00
An. paludis	0.08	0.00	0.04	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. rufipes	0.42	0.00	0.21	1.00	0.08	0.67	0.38	0.11	0.08	0.08	0.08	0.50	0.25	0.17	0.21	0.60
An. pharoensis	3.58	4.08	3.83	0.47	1.83	2.25	2.04	0.45	0.50	0.75	0.63	0.40	12.83	11.92	12.38	0.52
An. coustani	0.08	0.17	0.13	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. welcomei	0.08	0.08	0.08	0.50	0.00	0.25	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.04	0.00
An. demeilloni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.6: Human Biting Rate and Endophagic* Index of *Anopheles* Species in Simatou from February–May 2019

Species	February				March				April				May			
	HBR in	HBR out	Total HBR	Endo-phagic index	HBR in	HBR out	Total HBR	Endo-phagic index	HBR in	HBR out	Total HBR	Endo-phagic index	HBR in	HBR out	Total HBR	Endo-phagic index
An. gambiae s.l.	4.17	4.58	4.38	0.48	19.75	23.92	21.83	0.45	24.25	22.33	23.29	0.52	0.33	0.25	0.29	0.57
An. funestus s.l.	0.17	0.25	0.21	0.40	0.50	0.50	0.50	0.50	0.08	0.25	0.17	0.25	0.00	0.08	0.04	0.00
An. ziemanni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.50	0.46	0.45	0.50	0.67	0.58	0.43
An. paludis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. rufipes	0.08	0.00	0.04	1.00	0.25	0.25	0.25	0.50	0.08	0.00	0.04	1.00	0.08	0.00	0.04	1.00
An. pharoensis	31.33	28.83	30.08	0.52	18.92	21.00	19.96	0.47	45.42	39.33	42.38	0.54	41.50	41.00	41.25	0.50
An. coustani	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. welcomei	0.00	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. demeilloni	0.00	0.00	0.00	0.00	0.92	0.58	0.75	0.61	20.08	13.00	16.54	0.61	32.92	27.83	30.38	0.54

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.7: Human Biting Rate and Endophagic Index* of Anopheles Species in Simatou from June–August 2019

Species	June				July				August			
	HBR in	**HBR out**	**Total HBR**	**Endophagic Index**	**HBR in**	**HBR out**	**Total HBR**	**Endophagic Index**	**HBR in**	**HBR out**	**Total HBR**	**Endophagic Index**
An. gambiae s.l.	39.92	43.67	41.79	0.48	284.92	277.50	281.21	0.51	180.25	168.92	174.58	0.52
An. funestus s.l.	2.08	1.50	1.79	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. ziemanni	1.58	1.83	1.71	0.46	0.67	0.92	0.79	0.42	0.42	0.50	0.46	0.45
An. paludis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. rufipes	0.25	0.00	0.13	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. pharoensis	31.08	32.50	31.79	0.49	35.50	33.67	34.58	0.51	34.75	36.58	35.67	0.49
An. coustani	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. welcomei	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. demeilloni	65.17	58.92	62.04	0.53	1.50	0.33	0.92	0.82	0.00	0.00	0.00	0.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.8: Human Biting Rate and Endophagic Index* of Anopheles Species in Simatou in September 2019, and Total from October 2018–September 2019

Species	September				Total (October 2018–September 2019)			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	136.50	136.42	136.46	0.50	58.33	57.32	57.82	0.50
An. funestus s.l.	0.17	0.00	0.08	1.00	0.51	0.45	0.48	0.53
An. ziemanni	0.17	0.75	0.46	0.18	1.60	2.12	1.86	0.43
An. paludis	0.00	0.00	0.00	0.00	0.01	0.00	0.00	1.00
An. rufipes	0.00	0.08	0.04	0.00	0.13	0.10	0.12	0.56
An. pharoensis	20.50	22.17	21.33	0.48	23.15	22.84	22.99	0.50
An. coustani	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.33
An. welcomei	0.00	0.00	0.00	0.00	0.01	0.04	0.02	0.14
An. demeilloni	0.92	0.50	0.71	0.65	10.13	8.43	9.28	0.55

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.9: Human Biting Rate and Endophagic Index* of Anopheles in Mangoum in October & December 2018, February 2019

Species	October 2018				December 2018				February 2019			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	10.42	9.58	10.00	0.52	10.33	8.50	9.42	0.55	27.08	27.00	27.04	0.50
An. funestus s.l.	0.08	0.00	0.04	1.00	0.08	0.00	0.04	1.00	0.17	0.25	0.21	0.40
An. ziemanni	0.17	1.00	0.58	0.14	0.00	0.00	0.00	0.00	0.17	0.00	0.08	1.00
An. paludis	0.00	0.17	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table E.10: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Mangoum in April, June, and August 2019 and Total from October 2018–August 2019

Species	April				June				August				Total (October 2018–September 2019)			
	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index	HBR in	HBR out	Total HBR	Endo-phagic Index
An. gambiae s.l.	45.25	40.50	42.88	0.53	37.92	28.75	33.33	0.57	12.50	10.83	11.67	0.54	23.92	20.86	22.39	0.53
An. funestus s.l.	0.08	0.08	0.08	0.50	0.50	0.25	0.38	0.67	0.00	0.00	0.00	0.00	0.15	0.10	0.13	0.60
An. ziemanni	0.25	0.33	0.29	0.43	0.08	0.08	0.08	0.50	0.33	0.58	0.46	0.36	0.17	0.33	0.25	0.55
An. paludis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.11: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Nyabessang (October 2018–February 2019)

Species	October 2018				December 2018				February 2019			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	8.25	9.58	8.92	0.46	13.67	14.25	13.96	0.49	4.50	4.58	4.54	0.50
An. ziemanni	0.50	1.50	1.00	0.25	0.17	0.42	0.29	0.29	0.75	0.17	0.46	0.82
An. paludis	0.33	0.58	0.46	0.36	0.75	1.83	1.29	0.29	31.67	37.25	34.46	0.46
An. moucheti	15.58	20.67	18.13	0.43	5.92	8.83	7.38	0.40	37.08	31.42	34.25	0.54
An. marshalli	0.50	2.00	1.25	0.20	0.33	0.25	0.29	0.57	0.00	0.17	0.08	0.00
An. nili	12.42	15.08	13.75	0.45	0.17	0.50	0.33	0.25	0.00	0.08	0.04	0.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.12: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Nyabessang in April and June 2019

Species	April 2019				June 2019			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	4.50	3.92	4.21	0.53	5.58	4.00	4.79	0.58
An. ziemanni	0.00	0.25	0.13	0.00	0.00	0.00	0.00	0.00
An. paludis	21.17	30.17	25.67	0.41	2.08	6.25	4.17	0.25
An. moucheti	8.25	6.83	7.54	0.55	11.00	6.67	8.83	0.62
An. marshalli	0.00	0.00	0.00	0.00	0.08	1.33	0.71	0.06
An. nili	2.00	1.50	1.75	0.57	0.33	0.58	0.46	0.36

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.13: Human Biting Rate and Endophagic Index* of Anopheles Species in Nyabessang in August 2019 and Total from October 2018–August 2019

Species	August				Total (October 2018–September 2019)			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	0.83	2.67	1.75	0.24	6.22	6.50	6.36	0.49
An. ziemanni	5.08	7.50	6.29	0.40	1.08	1.64	1.36	0.40
An. paludis	7.75	12.42	10.08	0.38	10.63	14.75	12.69	0.42
An. moucheti	6.92	5.58	6.25	0.55	14.13	13.33	13.73	0.51
An. marshalli	0.00	0.00	0.00	0.00	0.15	0.63	0.39	0.20
An. nili	0.92	0.67	0.79	0.58	2.64	3.07	2.85	0.46

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.14: Human Biting Rate of *Anopheles* Species in Bonabéri from December 2018–August 2019

Species	December 2018		February 2019		April 2019		June 2019		August 2019		Total	
	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR	Total Collected	HBR
An. gambiae s.l.	153	6.38	785	32.71	373	15.54	659	27.46	935	38.96	2,905	24.21
An. ziemanni	0	0.00	0	0.00	0	0.00	0	0.00	1	0.04	1	0.01

Table E.15: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Bonabéri from December 2018–April 2019

Species	December 2018				February 2019				April 2019			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	3.08	9.67	6.38	0.24	16.92	48.50	32.71	0.26	7.50	23.58	15.54	0.24
An. ziemanni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

Table E.16: Human Biting Rate and Endophagic Index* of *Anopheles* Species in Bonabéri from June–August 2019, and Total from December 2018–August 2019

Species	June				August				Total (December 2018–September 2019)			
	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index	HBR in	HBR out	Total HBR	Endophagic Index
An. gambiae s.l.	16.58	38.33	27.46	0.30	22.50	55.42	38.96	0.29	13.32	35.10	24.21	0.3
An. ziemanni	0.00	0.00	0.00	0.00	0.00	0.08	0.04	0.00	0.00	0.02	0.01	0.0

*Note: The endophagic index equals the proportion of mosquitos biting indoors and is calculated by dividing the indoor HBR by the total HBR indoors and outdoors.

ANNEX F: HUMAN BLOOD INDEX OF *ANOPHELES* MOSQUITOS ACROSS SENTINEL SITES

Sites	Host	*An. gambiae* s.l.	*An. funestus* s.l.	*An. ziemanni*	*An. pharoensis*	*An. rufipes*	*An. hancocki*	*An. moucheti*	*An. nili*	*An. demeilloni*	Total	HBI
Gounougou	Human	132	78	0	0	4	0	0	0	0	**214**	29.7%
	Animal	163	23	0	1	62	0	0	0	0	**249**	
	Mix	46	51	1	0	2	0	0	0	0	**100**	
	Not identified	80	35	0	0	42	0	0	0	0	**157**	
	Total	421	187	1	1	110	0	0	0		**720**	
Simatou	Human	51	9	0	12	7	0	0	0	21	**100**	21.7%
	Animal	30	4	0	3	44	3	0	0	5	**89**	
	Mix	15	1	1	0	3	0	0	0	26	**46**	
	Not identified	129	5	1	15	66	7	0	0	2	**225**	
	Total	225	19	2	30	120	10	0	0	54	**460**	

Sites	Host	*An. gambiae* s.l.	*An. funestus* s.l.	*An. ziemanni*	*An. pharoensis*	*An. rufipes*	*An. hancocki*	*An. moucheti*	*An. nili*	*An. demeilloni*	Total	HBI
Mangoum	Human	72	0	0	0	0	0	0	0	0	**72**	74.2%
	Animal	1	0	0	0	0	0	0	0	0	**1**	
	Mix	7	0	0	0	0	0	0	0	0	**7**	
	Not identified	17	0	0	0	0	0	0	0	0	**17**	
	Total	97	0	0	0	0	0	0	0	0	**97**	
Nyabessang	Human	43	0	0	0	0	0	2	1	0	**46**	68.7%
	Animal	0	0	0	0	0	0	0	0	0	**0**	
	Mix	10	0	0	0	0	0	0	0	0	**10**	
	Not identified	11	0	0	0	0	0	0	0	0	**11**	
	Total	194	0	0	0	0	0	2	1	0	**67**	

ANNEX G: PARITY RATE OF *ANOPHELES* MOSQUITOS ACROSS SITES

Sentinel Site	Species	Total Dissected	# Parous	% Parous
Gounougou	*An. gambiae* s.l.	2,244	1,685	75.09
	An. funestus s.l.	291	245	84.19
	An. ziemanni	50	46	92.00
	An. rufipes	2	2	100.00
	An. pharoensis	37	27	72.97
	An. multicinctus	49	38	77.55
	An. tenebrosus	3	2	66.67
	An. smithii	3	3	100.00
Total		**2,679**	**2,048**	**76.45**
Simatou	*An. gambiae* s.l.	2,360	1,585	67.16
	An. funestus s.l.	89	63	70.79
	An. ziemanni	278	108	38.85
	An. paludis	0	0	0.00
	An. rufipes	15	11	73.33
	An. pharoensis	1,469	1,090	74.20
	An. coustani	2	2	100.00
	An. welcomei	13	10	76.92
	An. demeilloni	393	281	71.50
Total		**4,619**	**3,150**	**68.20**
Mangoum	*An. gambiae* s.l.	650	436	67.08
	An. funestus s.l.	1	0	0.00
	An. ziemanni	13	9	69.23
	An. paludis	1	0	0.00
Total		**665**	**445**	**66.92**
Nyabessang	*An. gambiae* s.l.	369	228	61.79
	An. ziemanni	59	38	64.41
	An. paludis	527	270	51.23
	An. moucheti	664	381	57.38

Sentinel Site	Species	Total Dissected	# Parous	% Parous
	An. marshalli	27	15	55.56
	An. pharoensis	2	1	50.00
	An. nili	137	86	62.77
Total		**1,785**	**1,019**	**57.09**
Bonabéri	*An. gambiae* s.l.	1,301	944	72.56
	An. funestus s.l.	2	1	50.00
Total		**1,303**	**945**	**72.52**

ANNEX H: INFECTION RATE OF *ANOPHELES* ACROSS SITES

Species	Simatou			Gounougou			Bonabéri			Mangoum			Nyabessang			Total		
	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection
An. gambiae s.l.	1,174	59	5.02	2,111	79	3.74	1,227	47	3.83	1,407	113	8.0	433	24	5.54	6,352	322	5.1
An. funestus s.l.	61	1	1.64	318	10	3.14	0	0	-	6	0	0.0	0	0	-	385	11	2.9
An. demeilloni	227	7	3.08	0	0	-	0	0	-	0	0	-	0	0	-	227	7	3.1
An. pharoensis	852	12	1.40	99	0	0.00	0	0	-	0	0	-	0	0	-	951	12	1.3
An. ziemanni	401	2	0.49	73	1	1.37	0	0	-	30	1	3.3	56	3	5.36	560	7	1.3
An. multicinctus	0	0	-	90	8	8.89	0	0	-	0	0	-	0	0	-	90	8	8.9
An. moucheti	0	0	-	0	0	-	0	0	-	0	0	-	593	11	1.85	593	11	1.9
An. nili	0	0	-	0	0	-	0	0	-	0	0	-	170	3	1.76	170	3	1.8
An. marshalli	0	0	-	0	0	-	0	0	-	0	0	-	26	3	11.54	26	3	11.5
An. wellcomei	6	0	-	0	0	-	0	0	-	0	0	-	0	0	-	6	0	0.0

Species	Simatou			Gounougou			Bonabéri			Mangoum			Nyabessang			Total		
	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection
An. tenebrosus	0	0	-	5	0	0.00	0	0	-	0	0	-	0	0	-	5	0	0.0
An. smithii	0	0	-	5	0	0.00	0	0	-	0	0	-		0	-	5	0	0.0
An. paludis	0	0	-	0	0	-	0	0	-	0	0	-	368	8	2.17	368	8	2.2
An. rufipes	29	1	3.44	8	0	0.00	0	0	-	0	0	-	0	0	-	37	1	2.7
An. christyi	0	0	-	1	0	0.00	0	0	-	0	0	-	0	0	-	1	0	0.0
An. tenebrosus	0	0	-	2	0	0.00	0	0	-	0	0	-	0	0	-	2	0	0.0
TOTAL	**2,750**	**82**	**2.98**	**2,712**	**98**	**3.61**	**1,227**	**47**	3.83	**1,443**	**114**	**7.9**	**1,646**	**52**	**3.16**	**9,778**	**393**	4.0

ANNEX I: INFECTION RATE OF *ANOPHELES* ACROSS SITES

Species	Simatou			Gounougou			Bonabéri			Mangoum			Nyabessang			Total		
	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection
An. gambiae s.l.	1,174	59	5.02	2,111	79	3.74	1,227	47	3.83	1,407	113	8.0	433	24	5.54	6,352	322	5.1
An. funestus s.l.	61	1	1.64	318	10	3.14	0	0	-	6	0	0.0	0	0	-	385	11	2.9
An. demeilloni	227	7	3.08	0	0	-	0	0	-	0	0	-	0	0	-	227	7	3.1
An. pharoensis	852	12	1.40	99	0	0.00	0	0	-	0	0	-	0	0	-	951	12	1.3
An. ziemanni	401	2	0.49	73	1	1.37	0	0	-	30	1	3.3	56	3	5.36	560	7	1.3
An. multicinctus	0	0	-	90	8	8.89	0	0	-	0	0	-	0	0	-	90	8	8.9
An. moucheti	0	0	-	0	0	-	0	0	-	0	0	-	593	11	1.85	593	11	1.9
An. nili	0	0	-	0	0	-	0	0	-	0	0	-	170	3	1.76	170	3	1.8

Species	Simatou			Gounougou			Bonabéri			Mangoum			Nyabessang			Total		
	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection
An. marshalli	0	0	-	0	0	-	0	0	-	0	0	-	26	3	11.54	26	3	11.5
An. wellcomei	6	0	-	0	0	-	0	0	-	0	0	-	0	0	-	6	0	0.0
An. tenebrosus	0	0	-	5	0	0.00	0	0	-	0	0	-	0	0	-	5	0	0.0
An. smithii	0	0	-	5	0	0.00	0	0	-	0	0	-		0	-	5	0	0.0
An. paludis	0	0	-	0	0	-	0	0	-	0	0	-	368	8	2.17	368	8	2.2
An. rufipes	29	1	3.44	8	0	0.00	0	0	-	0	0	-	0	0	-	37	1	2.7
An. christyi	0	0	-	1	0	0.00	0	0	-	0	0	-	0	0	-	1	0	0.0
An. tenebrosus	0	0	-	2	0	0.00	0	0	-	0	0	-	0	0	-	2	0	0.0
TOTAL	**2,750**	**82**	**2.98**	**2,712**	**98**	**3.61**	**1,227**	**47**	3.83	**1,443**	**114**	**7.9**	**1,646**	**52**	**3.16**	**9,778**	**393**	4.0

ANNEX J: SUSCEPTIBILITY TEST RESULTS

Table J.1: WHO Susceptibility Test Results with Clothianidin across Sites in 2019

Times (hours)	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality
24	88	83	89	78	91	62.9	100	98	100	95
48	88	95.3	89	86.7	91	74.1	100	100	100	100
72	88	100	89	100	91	87.6	-	-	-	-

Table J.2: CDC Bottle Assay Results with Chlorfenapyr (100 µg/bottle) across Sites in 2019

Times (hours)	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality
24	71	97.6	82	94.6	56	12.3	75	100	94	4.3
48	71	100	82	95.7	56	20.5	-	-	94	96.8
72	-	-	82	95.7	56	26.9	-	-	94	100

Table J.3: CDC Bottle Assay Results with Chlorfenapyr (200 µg/bottle) across Sites in 2019

Times (hours)	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality	Total Exposed	% Mortality
24	73	97.2	79	100	62	51.6	73	100	96	0
48	73	100	-	-	62	67.6	-	-	96	100
72	-	-	-	-	62	69.3	-	-	-	-

ANNEX K: FREQUENCY OF TARGET SITE RESISTANCE ALLELES ACROSS SITES

Site	N1575Y			*kdr*-w			*kdr*-e			Ace-1		
	RR	**RS**	**SS**	**RR**	**RS**	**SS**	**RR**	**RS**	**SS**	**RR**	**RS**	**SS**
Gounougou	0	5	95	8	9	83	0	68	32	0	0	93
Mangoum	3	21	77	102	0	0	0	0	98	48	0	41
Nyabessang	1	4	82	53	2	33	0	18	67	10	0	90
Simatou	3	26	71	17	29	47	0	4	95	0	0	99
Total	**7**	**56**	**325**	**180**	**40**	**163**	**0**	**90**	**292**	**58**	**0**	**323**

RR= Resistance homozygote, RS= Resistance heterozygote SS =Susceptible homozygote

ANNEX L: REFERENCES

Brogdon, WG and McAllister JC, 1998. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. J. Am. Mosq. Control Assoc. 14(2):159–64.

Gillies, M.T.; De Meillon, B., 1968: The Anophelinae of Africa South of Sahara, second ed., South African Institute of Medical Research, Johannesburg

Gillies, M.T. and Coetzee, M. (1987) A Supplement to the Anophelinae of Africa South of the Sahara. South African Institute for Medical Research, 55, 1-143.

NMCP 2018: Cameroon 2018 malaria report

Koekemoer L.L., Kamau L., Hunt R.H, Coetzee M., 2002: A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera Culicidae) group.

Kwiatkowska RM, Platt N, Poupardin R, Irving H, Dabire RK, Mitchell S, et al. : Dissecting the mechanisms responsible for the multiple insecticide resistance phenotype in Anopheles gambiae s.s., M form, from Vallee du Kou, Burkina Faso. Gene. 2013 Apr 25; 519(1):98-106

Riveron JM, Yunta C, Ibrahim SS, Djouaka R, Irving H, Menze BD, et al. A single mutation in the GSTe2 gene allows tracking of metabolically based insecticide resistance in a major malaria vector. Genome Biol. 2014 Feb 25; 15(2): R27.

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J. 2008;7:163

WHO (2016): Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd Ed ISBN 978 92 4 151157 5

WHO (2016): A toolkit for integrated vector management in sub-Saharan Africa. ISBN 978 92 4 154965 3

Christophe Antonio‐Nkondjio, Cyrille Ndo, Flobert Njiokou, Jude D. Bigoga, Parfait Awono‐Ambene, Josiane Etang, Albert Same Ekobo, and Charles S. Wondji, 2019: Review of malaria situation in Cameroon: Technical viewpoint on challenges and prospects for disease elimination. Parasites & Vectors, *2019, 12:501.*