Acta Entomology and Zoology

E-ISSN: 2708-0021
P-ISSN: 2708-0013
www.actajournal.com
AEZ 2024; 5(2): 36-44
Received: 02-05-2024
Accepted: 08-06-2024

All the author's details are given below the references section

Contribution of different Anopheles vector complexes and groups to malaria transmission in the Covè, Zagnanado and Ouinhi districts, southern Benin

Boulais Yovogan, Clément Agbangla, Armel Djènontin, Constantin J Adoha, Esdras M Odjo, Roseric Azondekon, Landry Assongba, Juniace Ahoga, Juvénal M Ahouandjinou, Linda Towakinou, Eloi M Honvoh, Olivier Oussou, André Sominahouin, Ramziyath Agbanrin Youssouf, Kèfilath Badirou, Virgile Gnanguenon, Razaki Ossè, Gil G Padonou and Martin C Akogbéto

DOI: https://doi.org/10.33545/27080013.2024.v5.i2a.152

Abstract
Background: Malaria remains a major public health issue in Benin. The present study investigated the contribution of different malaria vector complexes and groups to the disease transmission in the Cove, Zagnanado and Ouinhi health area.
Methods: Between september 2019 and april 2022, mosquito collections through Human Landing Catches were carried out over 9 rounds of collection, in 60 clusters/villages. Morphological identification of the mosquito specimens collected was carried out. A randomly selected subsample of *An. gambiae complex* and *An. funestus* and *An. nili* groups collected indoors and outdoors was screened for *Plasmodium falciparum* sporozoite infection and molecular species identification using ELISA CSP test and PCR methods, respectively.
Results: Of the six *Anopheles* complexes and groups found during the study period, *An. gambiae* s.l. was the majority and represented 92.8% (64530/69527, $p<0.001$). Indoors, the biting rate was higher for *An. gambiae* s.l. (29.6 bites/person/night, p<0,001) compared to *An. funestus* (1.3 bites/person/night) and *An. nili* (0.1 bites/person/night). Similarly, the entomological inoculation rate (EIR) was higher in *An. gambiae* s.l. (1.1 infected bites/person/night) compared to *An. funestus* (0.1 infected bites/person/night) and *An. nili* (0.004 infected bites/person/night). The composition of related species within the three malaria vector was as follows: *An. gambiae* s.l. (64.6% *An. coluzzii* and 35.1% *An. gambiae* s.s.), *An. funestus* (91.9% *An. funestus* s.s., 6.8% *An. leesoni*, and 1.3% *An. rivulorum-like*) and *An. nili* (*An. nili* s.s.).
Conclusion: The present study showed *An. gambiae* s.l. as the main contributor to malaria transmission in the Covè, Zagnanado, Ouinhi, districts. Secondary vector include *An. funestus* and *An. nili* groups.

Keywords: Malaria, transmission, *Anopheles gambiae* complex, *An. funestus* group, *An. nili* group

1. Introduction
Malaria is a leading cause of morbidity and mortality in many tropical and subtropical regions, particularly in sub-Saharan Africa. In 2022, 95% (236 million) of malaria cases and 97% (590, 935) of deaths was occured in African Member States [1]. In Benin, malaria is the leading cause of hospitalisation, with children under 5 and pregnant women being the most vulnerable. In 2021, approximately 3, 163, 648 cases and 2, 956 deaths caused by malaria were recorded in the country [2]. In this context, understanding the dynamics of malaria transmission and the specific contributions of the different vector complexes belonging to Anopheles genus have a vital importance for an effective and sustainable control strategies development.
Five species of *Plasmodium* parasites are responsible for malaria infection [3]. To date, *Plasmodium falciparum* remains the most pathogenic species responsible for deadly forms of the disease [4]. The *Plasmodium* species responsible for human malaria are mainly transmitted by vector species such as *An. gambiae* complex, *An. funestus* group and *An. nili* group [5–7]

Corresponding Author:
Boulais Yovogan
1. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin
2. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Within the *An. gambiae complex*, 8 species have been identified. These are *An. coluzzii, An. gambiae, An. arabiensis, An. bwambae, An. melas, An. merus, An. quadriannulatus and An. quadriannulatus B.* [8]. *An. funestus* group includes 10 species, namely: *An funestus, An. vaneedeni, An. parensis, An. aruni, An. fuscivenosus, An. leesoni, An. confusus, An. rivulorum, An. rivulorum-like* and *An. brucei* [9, 10]. The *Anopheles nili* group is composed of four species, namely *An. nili* s.s, *An. carnevalei, An. ovengensis* and *An. somalicus* [5, 11].

Baseline data collected as part of the New Nets project assessing two types of bi-treated LLINs (Interceptor G2 and Royal Guard) compared to a standard LLIN (Interceptor) in Benin showed an intense transmission of malaria led by *An. gambiae* s.l., the main malaria vector [12]. However, other vector complexes might also be involved in malaria transmission as previously reported in some regions of Benin [13, 14]. The present study aims at assess the presence of different Anopheles complexes in the districts of Covè-Zagnanado-Ouinhi and their potential contribution to the transmission of malaria in this health zone. This study also aims to investigate the impact of PY-PPF LLIN and PY-CFP LLIN on the malaria transmission caused by *An. funestus* and *An. nili*.

Materiels et methods

Study area

The study was conducted from September 2019 to April 2022 in three districts (Covè, Zagnanado and Ouinhi) located in Southern Benin. The average annual rainfall varies between 900 and 1200 mm of water, with vegetative growth varying between 80 and 100 days (INSAE, 2013). The area has two rainy seasons (March-July and October-November) with a malaria infection prevalence of 36.5% for children under [15]. The main activities carried out by the population were agriculture, fishing, hunting, and trade. From the census carried out in June 2019 in the 123 villages of the study area, sixty (60) clusters comprised of 1 village or a group of villages for an average of 200 households (approximately 1200 residents) were formed. The geographical distribution of the clusters within the districts is presented in Figure 1.

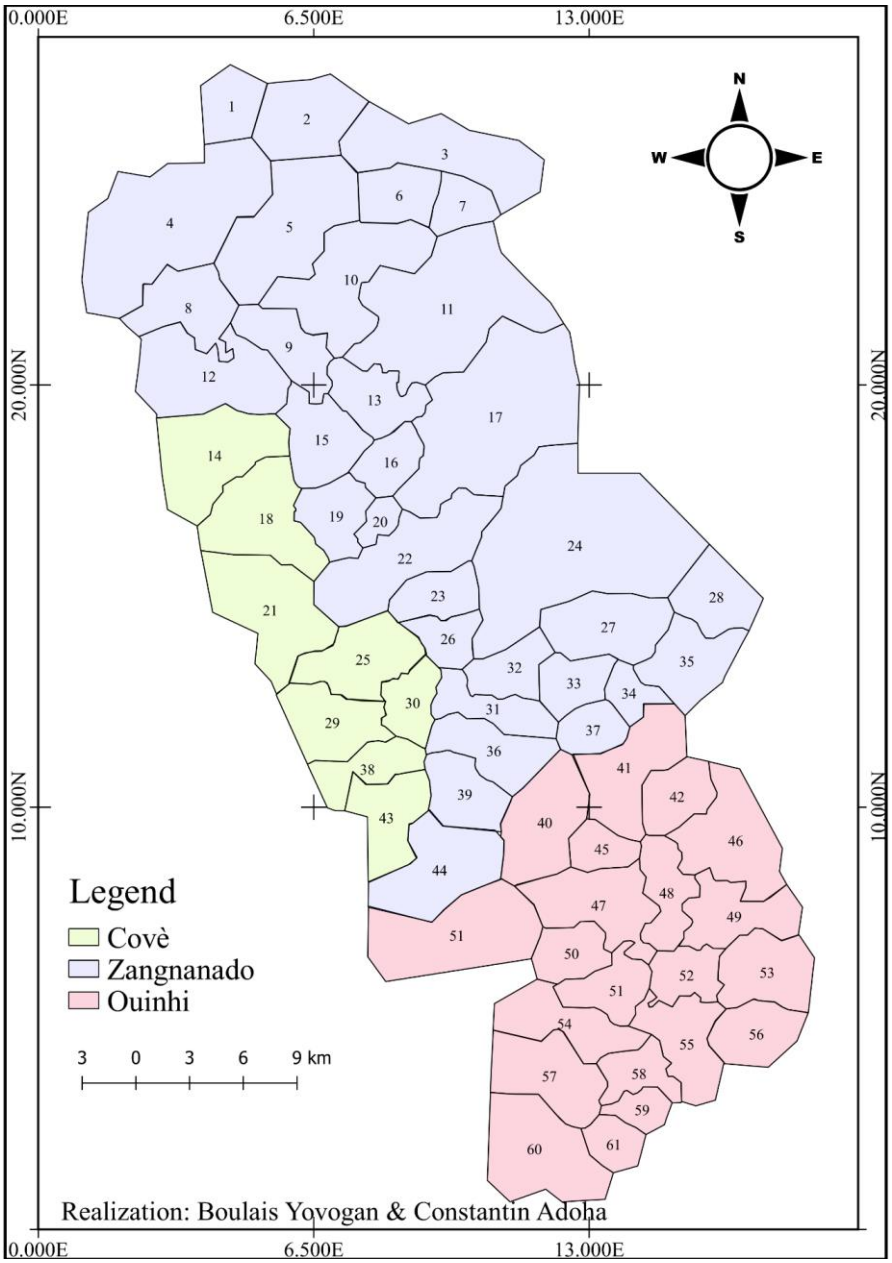

Fig 1: Map of the study area

2.2 Mosquito collection

Human Landing Catch

Mosquitoes were collected from 60 clusters during 9 rounds (September-October 2019 for baseline and June-July 2020, September-October 2020, December 2020-January 2021, Mars-Avril 2021, June-July 2021, September-October 2021, December 2021-January 2022, Mars-Avril 2022 for post intervention) using the human landing catch (HLC) technique. Twenty (20) clusters were randomised selected to each of the following arms: mixture pyrethroid-chlorfenapyr LLINs (Interceptor® G2), mixture pyrethroid-pyriproxyfen LLINs (Royal Guard®), and standard pyrethroid-only LLINs (Interceptor®)[16]. In each cluster, four (04) houses located at a distance of about 15 to 20 meters away from each other was selected from the database of the study census organized in 2019. The collections were conducted from 7 p.m. to 6 a.m. In each house, two collectors (1 indoors and 1 outdoors) were used from 7:00 p.m. to 1:00 a.m. and two other from 1:00 a.m. to 6:00 a.m.

2.3 Morphological and molecular identification of species

Collected mosquitoes were morphologically identified using the taxonomic identification key of Gillies and Meillon [17]. A subsample of *An. gambiae* s.l., *An. funestus* group and *An. nili* group from each cluster collected both indoors and outdoors was randomly selected. Heads and thoraces of Individuals of the three mosquito vector complexes were used for detection of *P. falciparum* sporozoite infection through ELISA-CSP test. Their abdomens, legs and wings, were used for species identification using the PCR protocols of Santolamazza *et al*. [18] (for *An. gambiae* s.l.), Koekemoer *et al*. [19] (for *An. funestus* group), and Kengne *et al*. [20] (for *An. nili* group).

2.4 Data management and analysis

Data were entered twice into databases designed in CS Pro 7.2 software and analysed with Stata 15.0 (Stata Corp., College Station, TX).

Entomological indicators of malaria transmission were determined as shown in the Table 1 below.

The household-level average for Human Biting Rate (HBR), Sporozoite Rate (SR), and Entomological Inoculation Rate (EIR) were used to generate the cluster-level one. The average of the cluster results is presented by species and arms, and their confidence intervals were determined using the Poisson distribution. All statistical analyzes were performed using Stata version 15.0 (Stata Corp., College Station, TX).

Table 1: Formulas for entomological indicators of malaria transmission.

Indicator	Formulas
Nightly Human biting rate (HBR)	Total of *Anpheles* total collected / number of person night
Sporozoite Rate (SR)	Number of infected Mosquito / Total number of mosquito tested through ELISA-CSP
Entomological Inoculation Rate (EIR)	Nightly HBR x SR

3. Results

3. 1 Mosquito composition

A total of 305, 895 mosquito specimens belonging to six different genera were collected indoors (n=130991) and outdoors (n=174904) over the three years of the study. Mosquitoes of the genus *Mansonia* (n=144025, 47.1%, 95%CI=46.9-47.3) were in the majority, followed by the genus *Culex* (n=85723, 28%, 95%CI=27.9-28.2) and *Anopheles* (n=69527, 22.7%, 22.6-22.9). Within the Anopheles complex, *An. gambiae* s.l. was predominant, accounting for 92.8% of the total Anopheles collected (64530/69527, p<0.001). Most of mosquitoes were collected outdoor (Fig. 2).

The other mosquitoes were *An. ziemanni*, *An. pharoensis*, *An. funestus*, *An. nili* and *An. Brohieri*, *An. coustani*, *Aedes spp*, *Coquillettidia* spp, *Eretmapodites spp* were found in very low proportions (≤ 2%) both indoor and outdoor households (Table 2).

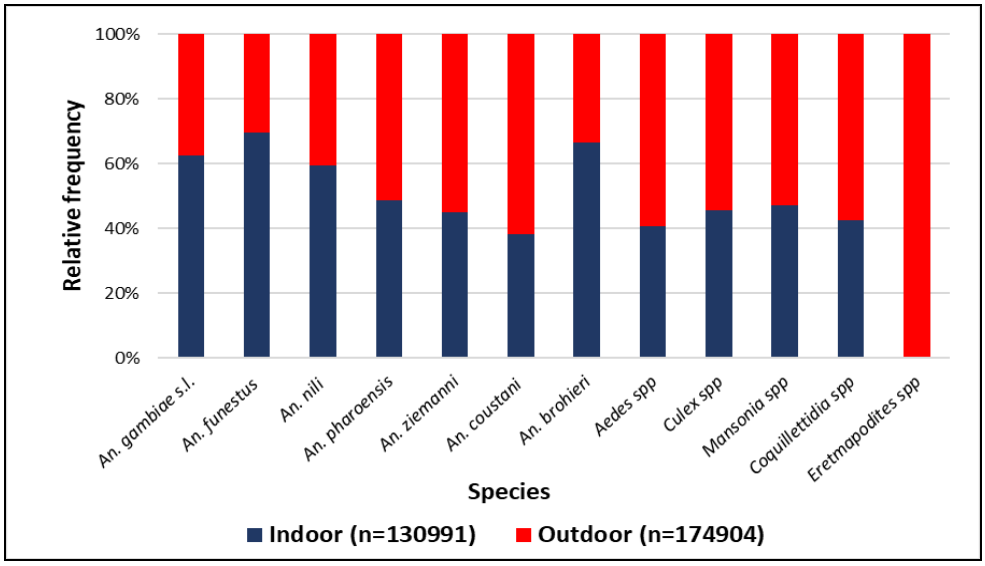

Fig 2: Mosquito species composition

Of the 8042 specimens of *An. gambiae* s.l. subjected to molecular analysis, two species, *An. coluzzii* (64.6%) and *An. gambiae* s.s. (35.1%), and a few hybrids (0.3%) (*An. gambiae/coluzzii*) were identified. Indoors, *An. coluzzii* (64.4%, n=3112, 95% CI: 63.0-65.8) predominated over *An. gambiae* s.s. (35.4%, n=1709, 95% CI: 34.0-36.7) and *An. gambiae/coluzzii* (0.2%, n=11, 95% CI: 0.1-0.4). The same trends were observed outdoors (Figure 3).

Similarly, molecular identification of 473 *An. funestus* group individuals revealed three different species. *An. funestus* s.s. was the most common, comprising 93.1% (n=273, 95% CI: 89.5-95.6) compared to 5.9% (n=18, 95% CI: 3.6-9.3) *An. leesoni* and 1% (n=03, 95% CI: 0.3-3.1) *An. rivulorum-like*. Similar trends were observed indoor and outdoor in the study area (Figure 3).

Within the *An. nili* group, only *An. nili* s.s. was identified.

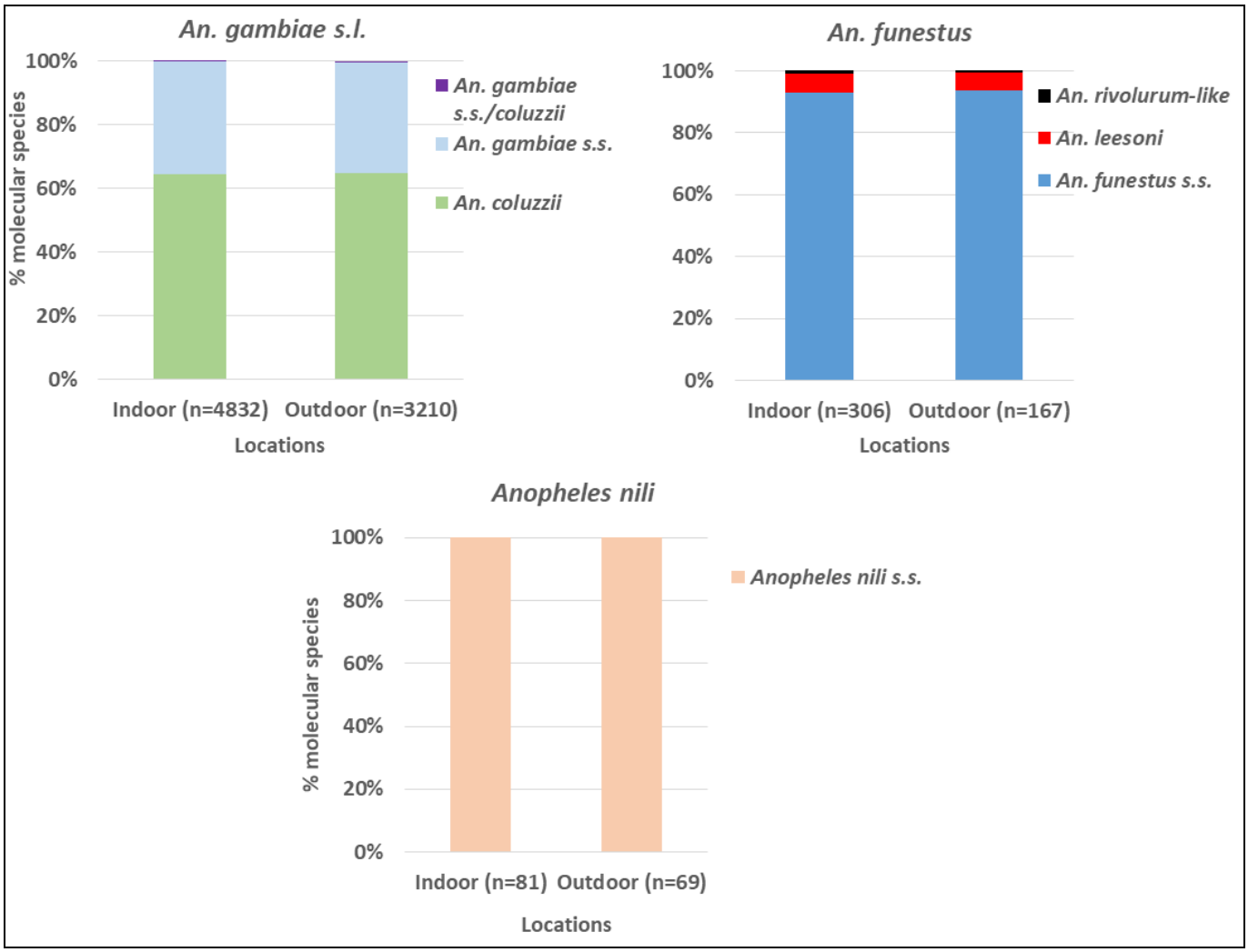

Fig 3: Molecular composition of three Anopheles vectors

3. 2 Human biting rate (HBR), Sporozoite Rate (SR) and Entomological Inoculation Rate (EIR) of *An. gambiae* s.l., *An. funestus* and *An. nili* in the study area

For the whole study area, the indoor biting rate was higher in *An. gambiae* s.l. [29.6 bites/person/night (b/p/n), 95% CI: 23.4-35.8] compared to *An. funestus* (1.3 b/p/n, 95% CI: 0 – 3.2) and *An. nili* (0.15 b/p/n, 95% CI: 0.1-0.2). The same trend was observed outdoors (Table 2.).

Indoor, sporozoite rate (SR) was similar in *An. gambiae* s.l. (3.8%, 95% CI: 2.2-5.4) than in *An. funestus* (3.7%, 95% CI: 0 - 9.3), and *An. nili* (2.9%, 95% CI: 0.2-17.1). Outdoor, the highest SR was observed in *An. funestus* (6.8%, 95% CI: 0 - 16.2), followed by *An. gambiae* s.l. (4.7%, 95% CI: 1.3-8.1). No significant difference was observed between the SR of the three vector complexes indoor as well as outdoor ($p>0.05$) (Table 2).

The indoor EIR was significantly higher in *An. gambiae* s.l. (1.1 infected bites/person/night (ib/p/n), 95% CI: 0.6-1.6) compared to *An. funestus* (0.1 ib/p/n, 95% CI: 0 - 0.2) and *An. nili* (0.004 ib/p/n, 95% CI: 0 - 0.01). The same trend was observed outdoors in *An. gambiae* s.l. (12.2 ib/p/n, 95% CI: 11.2-13.2) vs *An. funestus* (1.9 ib/p/n, 95% CI: 1.7-2.1); but with no significant difference.

Table 2: Contribution of *An. gambiae* s.l., *An. funestus* and *An. nili* to malaria transmission in the study area (baseline)

Locations	*An.* species	N of collected mosquitoes	Person night	HBR (b/p/n)	95%CI	N tested (ELISA)	N Positive	SR (%)	95%CI	EIR (ib/p/n)	95%CI
Indoor	*An. gambiae* s.l.	6373	240	29.6	23.4-35.8	2264	66	3.8	2.2 - 5.4	1.1	0.6 - 1.6
	An. funestus	289	240	1.3	0 - 3.2	289	8	3.7	0 - 9.3	0.1	0 - 0.2
	An. nili	36	240	0.15	0.1- 0.2	34	1	2.9	0.2-17.1	0.004	0 - 0.01
Outdoor	*An. gambiae* s.l.	4434	240	20.6	16.1-25.1	1341	25	4.7	1.3 - 8.1	0.7	0.2 - 1.3
	An. funestus	108	240	0.5	0.1- 0.8	105	3	6.8	0 - 16.2	0.2	0 - 0.5
	An. nili	46	240	0.2	0.1- 0.3	32	0	0	-	0	-

An: Anopheles, N : Number, HBR: Human Biting Rate, SR: Sporozoite Rate, EIR: Entomological Inoculation Rate, b/p/n: bites/person/night, ib/p/n: infected bites/person/night

3. 3 Impact of PY-PPF LLIN and PY-CFP LLIN on the infectivity of *An. coluzzii* and *An. gambiae* s.s.

Two thirds of sporozoite positive *An. gambiae* s.l. found indoors and outdoors were *An. coluzzii*. Species composition in overall *An. gambiae* s.l. population was similar suggesting that sporozoite rate is similar in both molecular species (table 3).

Table 3: Proportion of sporozoite positive *An. coluzzii* and *An. gambiae* s.s.

			Indoor		Outdoor	
			An. gambiae s.s.	*An. coluzzii*	*An. gambiae* s.s.	*An. coluzzii*
Periods	Arms	N positive	% (95% CI), N	% (95% CI), N	% (95% CI), N	% (95% CI), N
Year 1	PY LLIN	42	47.6 (25.9-69.3), 20	52.4 (30.7-74.0), 22	54.6 (28.2-80.8), 12	45.5 (19.2-71.8), 10
	PY-PPF LLIN	15	73.3 (46.5-100), 11	26.7 (0-53.6), 4	70 (30.2-109.8), 7	30 (0-69.77), 3
	PY-CFP LLIN	15	53.3 (27.0-79.7), 8	46.7 (20.3-73.0), 7	33.3 (1.3-65.4), 2	66.67 (34.61-98.72), 4
Year 2	PY LLIN	26	23.1 (3.8-42.3), 6	76.9 (57.7-96.2), 20	47.37 (12.33-82.4), 9	52.63 (17.6-87.7), 10
	PY-PPF LLIN	26	26.9 (0-57.1), 7	73.08 (42.9-103.3), 19	39.13 (4.98-73.3), 9	60.87 (26.72-95.0), 14
	PY-CFP LLIN	20	35 (15.9-54.1), 7	65 (45.9-84.1), 13	40 (2.16-77.8), 4	60 (22.16-97.8), 6
Overall	PY LLIN	68	40.2 (18.6-61.8), 26	59.7 (38.2-91.4), 42	40.3 (17.8-62.8), 21	59.7 (37.2-82.2), 20
	PY-PPF LLIN	41	42.2 (17.3-67.1), 18	57.8 (39.32-76.2), 23	38.9 (11.9-66.1), 16	61.0 (33.9-88.1), 17
	PY-CFP LLIN	35	42.2 (23.7-60.7), 15	59.8 (38.2-81.4), 20	36.1 (13.7-58.5), 6	63.9 (41.5-86.3), 10

An. *Anopheles*, N. number, PY LLIN: pyrethroid LLIN arm, PY-PPF LLIN. pyrethroid-pyriproxyfen LLIN arm, PY-CFP LLIN. Pyrethroid chlorfenapyr LLIN arm

3. 4 Impact of PY-PPF LLIN and PY-CFP LLIN on the transmission of malaria of *An. gambiae* s.l., *An. funestus*, and *An. nili* in indoor (Post intervention)

In indoor households, in *An. gambiae* s.l., reductions of 41% and 56% of the bite rate were observed respectively in the PY-PPF LLIN arm (13.41 b/p/n, 95%CI: 13.12-13.69) and in the PY-CFP LLIN arm (9.85 b/p/n, 95%CI: 9.62-10.10) compared to PY-LLIN (22.73 b/p/n, 95%CI: 22.43-23.17). Similar trends were obtained in *An. funestus* (Table 4). However, in *An. nili*, no reduction in the bite rate was observed in the bi-treated arms (PY-PPF LLIN: 0.05 b/p/n, DR=2.60 with 95%CI =0.51 - 15.25, and in the PY-CFP LLIN arm: 0.06 b/p/n, DR= 2.88 with 95%CI =0.65 - 16.06) compared to the control arm (PY-LLIN: 0.01) (table 4).

In *An. gambiae* s.l., reductions of EIR (59% in PY-PPF LLIN and 64% in PY-CFP LLIN) were observed compared to the PY LLIN arm. On the other hand, in *An. funestus* and *An. nili* no reduction was reported (Table 4).

4. Discussion

This study provided information on the contribution of *An. gambiae*, *An. funestus* and *An. nili* complexes to malaria transmission in the Covè-Zagnanado-Ouinhi health zone where mosquito nets bi-treated with Active Ingredients (AI) were distributed. It showed that *An. gambiae* s.l. was the main vector of transmission, followed by *An. funestus* and *An. nili*. Morphological identification of the mosquitoes revealed the presence of six anopheline complexes and groups, with a marked predominance of the *An. gambiae* complex.

Oveall, of the six (06) species identified by molecular analysis within the three anopheline complexes, *An. coluzzii* is the predominant species, followed by *An. gambiae* s.s. The presence of temporary breeding sites created by rain, permanent and semi-permanent breeding sites created by the many rice-growing sites, and the tributaries of the Ouéme and Zou rivers, most of which were in the area, provide favourable conditions for the development of these species, which have been observed in the three study communes [12]. Previous studies in certain regions of Benin had already reported the presence of *An. funestus* s.s., *An. leesoni* and *An. nili* s.s. [13, 14, 21]. Thus, the presence of permanent or semi-permanent lakes in heavily vegetated areas represent positive breeding sites for *An. funestus* [22–24]. In the communes of Covè, Zagnanado and Ouinhi, there are permanent or semi-permanent sunny streams surrounded by extensive vegetation with small or large forests located not far from the towns. This observation could justify the presence of *An. funestus* and *An. nili* found in the study area. This study also identified, for the first time in Benin, the *An. rivulorum-like* species within the *An. funestus* group, but only in a small proportion (n = 4). A previous study carried out in Tanzania reported that *An. leesoni* and *An. parensis* are involved in the transmission of *Plasmodium falciparum* malaria [25]. In this case, it is necessary to continue research into the different species likely to be involved in malaria transmission and to extend it to other different ecological zones in Benin. The identification of *An. nili* s.s. as the only species of the *An. nili* group was not surprising and corroborates with some work carried out in northern Benin [14] and in some countries of the sub-region [26, 27].

Table 4: HBR, SR and EIR of *An. gambiae* s.l., *An. funestus*, and *An. nili* in indoor (Post intervention)

***Anopheles* species**	**Arms**	**N of collected mosquitoes**	**Person night**	**HBR (b/p/n)**	**95%CI**	**DR (95% IC)**	**N tested (Elisa)**	**n Positive**	**SR (%)**	**95%CI**	**OR (95% IC)**	**EIR (ib/p/n)**	**95%CI**	**DR (95% IC)**
	PY LLIN	14591	640	22.73	22.43-23.17	1 (Ref)	4210	56	1.33	1.05-1.74	1 (Ref)	9.10	9.06-9.12	1 (Ref)
An. gambiae s.l.	PY-PPF LLIN	8583	640	13.41	13.12-13.69	0.59 (0.30-1.17)	2725	35	1.28	0.91-1.8	0.88 (0.58-1.34)	5.16	5-5.21	0.41 (0.23-0.74)
	PY-CFP LLIN	6309	640	9.85	9.62-10.10	0.44 (0.22-0.86)	3104	30	0.9	0.66-1.39	0.70 (0.45-1.09	2.85	2.82-2.87	0.34 (0.18-0.62)
	PY LLIN	110	640	0.17	0.14-0.20	1	76	0	0	0-6.95	ND	0	ND	ND
An. funestus	PY-PPF LLIN	53	640	0.08	0.06-0.11	0.50 (0.24-0.98)	45	2	4.44	0.77-16.36	ND	0.11	0.09-0.13	ND
	PY-CFP LLIN	86	640	0.13	0.11-0.16	0.58 (0.19-1.54)	52	0	0	0-8.57	ND	0	ND	ND
	PY LLIN	8	640	0.01	0.005-0.02	1	6	0	0	0-48.31	ND	0	ND	ND
An. nili	PY-PPF LLIN	34	640	0.05	0.04-0.07	2.60 (0.51 - 15.25)	12	0	0	0-30.12	ND	0	ND	ND
	PY-CFP LLIN	40	640	0.06	0.04-0.08	2.88 (0.65 - 16.06)	29	1	3.44	0.18-19.63	ND	0.06	0.05-0.09	ND

An: Anopheles, N : Number, HBR: Human Biting Rate, SR: Sporozoite Rate, EIR: Entomological Inoculation Rate, DR: Density Ratio, n=number of positive *An. gambiae* s.l., CI: Confidence Interval, OR: Odd Ratio, PY LLIN: pyrethroid LLIN arm, PY-PPF LLIN. pyrethroid-pyriproxyfen LLIN arm, PY-CFP LLIN. Pyrethroid chlorfenapyr LLIN arm, ND: Non Determined, b/p/n: bites/person/night, ib/p/n: infected bites/person/night

Outdoors, similar trends were obtained to those obtained indoors (table 5)

Table 5: HBR, SR and EIR of *An. gambiae* s.l., *An. funestus*, and *An. nili* in outdoor (Post intervention)

***Anopheles* species**	**Arms**	**N of *An.* collected**	**Person night**	**HBR (b/p/n)**	**95% CI**	**DR (95% CI)**	**N tested (Elisa)**	**n Positive**	**SR (%)**	**95%CI**	**OR (95% IC)**	**EIR (ib/p/n)**	**95% CI**	**DR (95% IC)**
	PY LLIN	11940	640	18.66	18.32-14.01	1 (Ref)	3603	30	0.83	0.57-1.20	1 (Ref)	4.65	4.62-4.67	1 (Ref)
	PY-PPF LLIN	7107	640	11.10	10.84-11.36	0.64 (0.34-1.21)	2404	24	0.99	0.65-1.51	0.95 (0.51-1.75)	3.32	3.30-3.34	0.58 (0.3-1.13)
***An. gambiae* s.l.**	PY-CFP LLIN	5193	640	8.11	7.89-8.34	0.47 (0.25-0.88)	2511	11	0.44	0.23-0.81	0.45 (0.22-0.92)	1.06	1.04-1.07	0.3 (0.13-0.67)
	PY LLIN	87	640	0.14	0.11-0.16	1	54	2	3.70	0.64-13.83	1	0.15	0.12-0.17	1
An. funestus	PY-PPF LLIN	57	640	0.09	0.07-0.11	0.60 (0.21-1.69)	36	2	5.56	0.97-20.01	1.66 (0.26 - 10.56)	0.14	0.12-0.18	0.96 (0.53-1.81)
	PY-CFP LLIN	63	640	0.10	0.08-0.12	0.65 (0.32-1.84)	33	0	0	0-12.98	ND	0	ND	ND
	PY LLIN	9	640	0.01	0.006-0.03	1	2	0	0	0-80.21	ND	0	ND	ND
An. nili	PY-PPF LLIN	28	640	0.04	0.03-0.06	2.6 (0.47 - 14.68)	6	0	0	0-48.32	ND	0	ND	ND
	PY-CFP LLIN	24	640	0.04	0.02-0.05	2.9 (0.73 - 15.44)	21	1	4.76	0.25-25.87	ND	0.05	0.03-0.08	ND

An: Anopheles, N : Number, HBR: Human Biting Rate, SR: Sporozoite Rate, EIR: Entomological Inoculation Rate, DR: Density Ratio, n= number of positive *An. gambiae* s.l., CI: Confidence Interval, OR: Odd Ratio, PY LLIN: pyrethroid LLIN arm, PY-PPF LLIN. pyrethroid-pyriproxyfen LLIN arm, PY-CFP LLIN. Pyrethroid chlorfenapyr LLIN arm, ND: Non Determined, b/p/n: bites/person/night, ib/p/n: infected bites/person/night.

In the study area, a person received an average of 22 bites of *An. gambiae* s.l. per night (p/p/n). This rate is significantly higher than the rates for *An. funestus* and *An. nili*, which are less than one (01) bite per person per night (HBR < 1). Similar trends were observed indoor and outdoor households where *An. gambiae* s.l. had a significantly higher biting rate than *An. funestus* and *An. nili*. The low representation of potential breeding sites for *An. funestus* and *An. nili* larvae in the study area is thought to be responsible for the low biting rates of these vectors. The high biting rate of *An. gambiae* s.l. compared with the other species is linked to the abundance of rice-growing areas in the study zone. This is in agreement with the work of Koudou *et al*. [28] who explain the density of *An. gambiae* s.l. vectors compared to *An. funestus* by the presence of rice-growing sites. The sporozoite rates (SR) was slightly higher in *An. funestus* group outdoors compared to *An. gambiae* complex, but there was no significant difference. In northern Benin, although there was no sporozoite infection noted outdoor for *An. funestus*, work has generally shown that there was no significant difference between infection rates within the three complexes, namely *An. gambiae*, *An. funestus* and *An. nili* [14]. In addition, one person received an average of 1.1 (95%CI: 0.6-1.6) infected bites of *An. gambiae* s.l. per night (ib/p/n). This rate is significantly higher compared to *An. funestus* (0.01 ib/p/n; 95%CI: 0-0.2) and *An. nili* (0.004 ib/p/n; 0-0.01). In summary, *An. gambiae* s.l. was the main malaria transmission vector in the study area, followed respectively by *An. funestus* and *An. nili*, which were secondary vectors. These results are similar to some previous work [29, 30]. The low transmission observed in *An. nili* could be due to its low density in the study area.

This study assessed the impact of PY-PPF LLIN and PY-CFP LLIN compared with PY LLIN on the density, sporozoite index and entomological inoculation rate of the *An. gambiae* complex, *An. funestus* and *An. nili* groups, the main malaria vectors in the study area. A previous study in Benin on the *An. gambiae* complex had already documented the impact of these LLINs on malaria transmission [31]. Our results showed a reduction in the biting rate of *An. funestus* of 50% and 42% respectively in the PY-PPF LLIN and PY-CFP LLIN arms compared to the PY LLIN arm. In contrast, no reduction in the bite rate was observed in *An. nili*. The low proportions of *An. funestus* and *An. nili* collected after the distribution of the study LLINs did not allow statistical analyses to observe the effect of PY-PPF LLIN and PY-CFP LLIN on the sporozoite index and on the entomological inoculation rate in the study area. In summary, PY-PPF LLIN and PY-CFP LLIN caused a reduction in *An. funestus* density and could also be responsible for the low proportions of *An. nili* and *funestus* collected after the distribution of the study LLINs.

At baseline, sporozoite infection data collected in all three study arms suggested that *An. gambiae* s.s. was likely to be more infective than *An. coluzzii* [12]. However, two (02) years after study LLINs distribution, two molecular species seems to be infective similarly, as previously observed in the rural areas of Bouaké in Côte d'Ivoire [32]. This could be because of the relative time in the age structure of the vector population during baseline collection that occurred over only one time-point sampling night, contrary to the post intervention data collection that was performed over eight rounds of collection.

In general, *An. funestus* and *An. nili* were involved in malaria transmission in the study area. To our knowledge, this study is the first to assess the involvement of *An. nili* in malaria transmission in the communes of Covè-Zagnanado-Ouinhi. Further studies to examine in depth the behaviour of these secondary vector complexes are required for effective control. Similarly, the numbers of *An. pharoensis* and *An. ziemanni* collected are not negligible and further studies are needed to also assess their involvement in malaria transmission in the region.

5. Conclusion

The study showed for the first time the presence of *An. rivulorum-like* within the *An. funestus* group in Benin. *An. gambiae* s.l. was the main vector involved in malaria transmission, followed by *An. funestus* and *An. nili* which are secondary vectors. The future deployment of any control strategy should take into account the involvement of these secondary vector complexes for better control of the disease.

Ethical Considerations

Ethical approvals for this study were obtained from Benin's National Ethics Committee for Health Research (N°30/MS/DC/SGM/DRFMT/CNERS/SA, Approval n°6 of 04/03/2019) and the Ethics Committee of the London School of Hygiene and Tropical Medicine (16237-1) after reviewing the study protocol. All study participants provided informed consent prior to their involvement. Only trained collectors who were capable of capturing mosquitoes before being bitten participated in the study. Before the study began, these collectors were vaccinated against yellow fever and received treatment if they tested positive for malaria infection. During the trial, they received care at the local health facility whenever they exhibited symptoms similar to those of malaria.

Acknowledgements

We thank the PNLP of Benin, the people of the Zou region and their community leaders for their cooperation in the implementation of this study. This research is supported by a grant to the London School of Hygiene and Tropical Medicine from UNITAID and the Global Fund through the Innovative Vector Control Consortium (IVCC). We would like to thank the team at the London School of Hygiene and Tropical Medicine: Drs Natacha Protopopoff, Jackie Cook, Arthur Sovi, Louisa A. Messenger, Corine Ngufor, and Manfred Accrombessi for all their contributions to this study. The funding partners played no role in the design of the study, the collection and analysis of the data, the decision to publish, or the preparation of the manuscript.

References

1. Africa-Malaria-Progress-Report-2023. Malaria Progress Report. African Union Commission, African Leaders Malaria Alliance, and RBM Partnership to End Malaria; c2023. p. 14. Available from: https://alma2030.org/wp-content/uploads/2024/02/africa-malaria-progress-report-2023-en.pdf
2. Alliance des Dirigeants Africains Contre le Paludisme. Bénin - Rapport trimestriel d'ALMA – 1er trimestre 2023. c2023. p. 3. Available from: https://alma2030.org/wp-content/uploads/2024/01/benin-quarterly-report-2023-q1-fr.pdf

3. WHO. Malaria. Cited 2024 Jul 11. Available from: https://www.who.int/health-topics/malaria
4. Otto TD, Gilabert A, Crellen T, Böhme U, Arnathau C, Sanders M, *et al*. Genomes of all known members of a Plasmodium subgenus reveal paths to virulent human malaria. Nat Microbiol. 2018;3:687-697.
5. Fontenille D, Simard F. Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. Comp Immunol Microbiol Infect Dis. 2004;27:357-375.
6. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, *et al*. Mosquitoes and their Control. Heidelberg: Springer; c2010.
7. Wiebe A, Longbottom J, Gleave K, Shearer FM, Sinka ME, Massey NC, *et al*. Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. Malar J. 2017;16:85.
8. Coetzee M, Hunt RH, Wilkerson R, Torre AD, Coulibaly MB, Besansky NJ. Anopheles coluzzii and Anopheles amharicus, new members of the Anopheles gambiae complex. Zootaxa. 2013;3619:246-274.
9. Cohuet A, Simard F, Toto JC, Kengne P, Coetzee M, Fontenille D. Species identification within the Anopheles funestus group of malaria vectors in Cameroon and evidence for a new species. Am J Trop Med Hyg. 2003;69:200–205.
10. Coetzee M, Coosemans M, Manguin S, Garros C, Koekemoer LL. A single multiplex assay to identify major malaria vectors within the African Anopheles funestus and the Oriental An. minimus groups. Am J Trop Med Hyg. 2004;70:583–590.
11. Awono-Ambene HP, Kengne P, Simard F, Antonio-Nkondjio C, Fontenille D. Description and bionomics of Anopheles (Cellia) ovengensis (Diptera: Culicidae), a new malaria vector species of the Anopheles nili group from south Cameroon. J Med Entomol. 2004;41:561–568.
12. Yovogan B, Sovi A, Padonou GG, Adoha CJ, Akinro B, Chitou S, *et al*. Pre-intervention characteristics of the mosquito species in Benin in preparation for a randomized controlled trial assessing the efficacy of dual active-ingredient long-lasting insecticidal nets for controlling insecticide-resistant malaria vectors. PLoS One, 2021, 16.
13. Djouaka R, Akoton R, Tchigossou GM, Atoyebi SM, Irving H, Kusimo MO, *et al*. Mapping the distribution of Anopheles funestus across Benin highlights a sharp contrast of susceptibility to insecticides and infection rate to Plasmodium between southern and northern populations. Wellcome Open Res. 2016;1:28.
14. Ossè RA, Tokponnon F, Padonou GG, Sidick A, Aïkpon R, Fassinou A, *et al*. Involvement of Anopheles nili in Plasmodium falciparum transmission in North Benin. Malar J. 2019;18:152.
15. Institut National de la Statistique et de l'Analyse Économique (INSAE) et ICF. Enquête Démographique et de Santé au Bénin, 2017–2018: Indicateurs Clés [Internet]. Cotonou, Bénin et Rockville, Maryland, USA: INSAE et ICF; c2019. Available from: https://insae.bj/images/docs/insae-statistiques/sociales/Sante/Enqu%C3%AAte%20D%C3%A9mographique%20et%20de%20Sant%C3%A9%20au%20B%C3%A9nin%20(EDSB)%20de%202017-2018.pdf
16. Accrombessi M, Cook J, Ngufor C, Sovi A, Dangbenon E, Yovogan B, *et al*. Assessing the efficacy of two dual-active ingredients long-lasting insecticidal nets for the control of malaria transmitted by pyrethroid-resistant vectors in Benin: study protocol for a three-arm, single-blinded, parallel, cluster-randomized controlled trial. BMC Infect Dis. 2021;21:194.
17. Gillies MT, De Meillon B. The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region). Oxford: Oxford University Press; 1968. Available from: https://www.cabdirect.org/cabdirect/abstract/19692900946
18. 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.
19. Koekemoer LL, Kamau L, Hunt RH, Coetzee M. A cocktail polymerase chain reaction assay to identify members of the Anopheles funestus (Diptera: Culicidae) group. Am J Trop Med Hyg. 2002;66:804–11.
20. Kengne P, Awono-Ambene P, Nkondjio CA, Simard F, Fontenille D. Molecular identification of the Anopheles nili group of African malaria vectors. Med Vet Entomol. 2003;17:67–74.
21. Djènontin A, Bio-Bangana S, Moiroux N, Henry MC, Bousari O, Chabi J, *et al*. Culicidae diversity, malaria transmission and insecticide resistance alleles in malaria vectors in Ouidah-Kpomasse-Tori district from Benin (West Africa): A pre-intervention study. Parasites Vectors. 2010;3:83.
22. Gimnig JE, Ombok M, Kamau L, Hawley WA. Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. J Med Entomol. 2001;38:282–8.
23. Mwangangi JM, Mbogo CM, Muturi EJ, Nzovu JG, Githure JI, Yan G, *et al*. Spatial distribution and habitat characterisation of Anopheles larvae along the Kenyan coast. J Vector Borne Dis. 2007;44:44–51.
24. Nambunga IH, Ngowo HS, Mapua SA, Hape EE, Msugupakulya BJ, Msaky DS, *et al*. Aquatic habitats of the malaria vector Anopheles funestus in rural south-eastern Tanzania. Malar J. 2020;19:219.
25. Temu EA, Minjas JN, Tuno N, Kawada H, Takagi M. Identification of four members of the Anopheles funestus (Diptera: Culicidae) group and their role in Plasmodium falciparum transmission in Bagamoyo coastal Tanzania. Acta Trop. 2007;102:119-25.
26. Dia I, Diop T, Rakotoarivony I, Kengne P, Fontenille D. Bionomics of Anopheles gambiae Giles, An. arabiensis Patton, An. funestus Giles and An. nili (Theobald) (Diptera: Culicidae) and transmission of Plasmodium falciparum in a Sudano-Guinean zone (Ngari, Senegal). J Med Entomol. 2003;40:279–83.
27. Adja AM, N'goran EK, Koudou BG, Dia I, Kengne P, Fontenille D, *et al*. Contribution of Anopheles funestus, An. gambiae and An. nili (Diptera: Culicidae) to the perennial malaria transmission in the southern and western forest areas of Côte d'Ivoire. Ann Trop Med Parasitol. 2011;105:13–24.

28. Koudou BG, Adja AM, Matthys B, Doumbia M, Cissé G, Koné M, *et al*. Agricultural activities and malaria transmission in two eco-epidemiological settings in central Côte d'Ivoire. Bulletin de la Société de Pathologie Exotique. 2007;100(2):124–6.
29. Antonio-Nkondjio C, Awono-Ambene HP, Etang J, Fontenille D, Ndo C, Simard F, *et al*. Epidemiological importance of the Anopheles nili group of malaria vectors in equatorial villages of Cameroon, Central Africa. Bulletin de Liaison et de Documentation de l'Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC); c2009;13–20.
30. Yokoly FN, Zahouli JBZ, Small G, Ouattara AF, Opoku M, de Souza DK, *et al*. Assessing Anopheles vector species diversity and transmission of malaria in four health districts along the borders of Côte d'Ivoire. Malaria Journal. 2021;20(1):409.
31. Accrombessi M, Cook J, Dangbenon E, Yovogan B, Akpovi H, Sovi A, *et al*. Efficacy of pyriproxyfen-pyrethroid long-lasting insecticidal nets (LLINs) and chlorfenapyr-pyrethroid LLINs compared with pyrethroid-only LLINs for malaria control in Benin: a cluster-randomised, superiority trial. The Lancet. 2023;401(10377):435–46.
32. Zoh DD, Yapi A, Adja MA, Guindo-Coulibaly N, Kpan DMS, Sagna AB, *et al*. Role of Anopheles gambiae s.s. and Anopheles coluzzii (Diptera: Culicidae) in human malaria transmission in rural areas of Bouaké, Côte d'Ivoire. Journal of Medical Entomology. 2020;57(5):1254–61.

Author Details

Boulais Yovogan
1. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin
2. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Clément Agbangla
Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin

Armel Djènontin
1. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin
2. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Constantin J Adoha
1. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin
2. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Esdras M Odjo
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Roseric Azondekon
1. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin
2. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Landry Assongba
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Juniace Ahoga
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Juvénal M Ahouandjinou
1. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin
2. Ecole de Gestion et d'Exploitation des Systèmes d'Elevage, Université Nationale d'Agriculture, Kétou, Benin

Linda Towakinou
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Eloi M Honvoh
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Olivier Oussou
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

André Sominahouin
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Ramziyath Agbanrin Youssouf
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Kèfilath Badirou
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Virgile Gnanguenon
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Razaki Ossè
1. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin
2. Ecole de Gestion et d'Exploitation des Systèmes d'Elevage, Université Nationale d'Agriculture, Kétou, Benin

Gil G Padonou
1. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Abomey-Calavi, Benin
2. Centre de Recherche Entomologique de Cotonou, Cotonou, Benin

Martin C Akogbéto
Centre de Recherche Entomologique de Cotonou, Cotonou, Benin