# Involvement of *Anopheles nili* in *Plasmodium falciparum* transmission in North Benin

Razaki A. Ossè[1,2*], Filémon Tokponnon[2,3], Germain Gil Padonou[2,4], Aboubakar Sidick[2], Rock Aïkpon[2,5], Arsène Fassinou[2,4], Come Z. Koukpo[2,4], Wilfrid Sèwadé[2], Bruno Akinro[2], Arthur Sovi[2,6], Melchior Aïssi[7] and Martin C. Akogbéto[2]

## Abstract

**Background:** Several studies carried out in Benin have shown the involvement of *Anopheles gambiae* sensu lato (s.l.), the *Anopheles funestus* group in malaria transmission, but none of them reported the contribution of the *Anopheles nili* group to the transmission of this disease. The current study investigated the question through an entomological cross-sectional survey performed in Northern Benin.

**Methods:** Mosquito samplings were performed in September and October 2017 in 4 villages located in two districts: Bambaba and Wodara (Kérou district) and, Péhunco 2 and Béké (Péhunco district). The collections were carried out indoors and outdoors using human landing catches (HLC) to assess the human biting rate (HBR) and pyrethrum spray catches (PSC) to evaluate the blood feeding rate and the blood meal origin using the ELISA test. All collected mosquitoes were morphologically identified and, the polymerase chain reaction (PCR) technique was used for molecular identification of sibling species of *An. gambiae* s.l., *An. funestus* group and *An. nili* group sporozoite index (SI) was also assessed by the ELISA test.

**Results:** Overall, *An. gambiae* s.l., *An. funestus* group and *An. nili* group were the three vectors found in the study area. A significantly higher human biting rate (HBR) was recorded in *An. nili* group (5 bites/human/night) compared to *An. funestus* group (0.656 bites/human/night) in the Kérou district (p < 0.0001). *Anopheles gambiae* s.l. displayed the highest HBR (26.19 bites/human/night) in the same district. The entomological inoculation rate (EIR) was 1.875 infected bites/human/month in *An. nili* group against 13.05 infected bites/human/month in *An. gambiae* s.l. and 0.938 infected bites/human/month in *An. funestus* group in Kérou. In Péhunco, the EIR was 1.02 infected bites/human/month in *An. gambiae* s.l. PCR results showed that *An. nili* sensu stricto (s.s.) and *An. funestus* s.s. were the only species of the *An. nili* and *An. funestus* groups, respectively. The anthropophagic character of *An. gambiae* s.l. was also highlighted.

**Conclusion:** This study provides useful information on the contribution of *An. nili* group as secondary vector to malaria transmission in northern Benin. Broader studies must also be carried out in a larger study area to assess the involvement of other *Anopheles* species to malaria transmission. This will aid to better plan malaria vector control interventions.

**Keywords:** Malaria, EIR, *Anopheles nili*, Contribution, Benin

*Correspondence: ossraz@yahoo.fr
[2] Centre de Recherche Entomologique de Cotonou (CREC),
06 BP 2604 Cotonou, Benin
Full list of author information is available at the end of the article

Ossè *et al. Malar J*    (2019) 18:152

Page 2 of 10

## Background

A wide range of human and animal diseases are transmitted by mosquitoes and those diseases remain a major source of death worldwide [1]. Malaria remains one of the most serious vector-borne diseases, affecting half of the world's 7.4 billion people [2]. The proliferation of mosquitoes is promoted not only by ecological changes due to human activities (deforestation, public works, construction of dams, rice paddies, irrigation), but also by environmental parameters (rainfall, temperature and relative humidity), which also play a fundamental role in the level of transmission and the epidemiology of diseases [3, 4]. In intertropical Africa, malaria transmission is very heterogeneous due to eco-climatic variations [5]. Currently, five species of the parasite of the *Plasmodium* genus have been identified as responsible for malaria infection in humans [6]. Among them *Plasmodium falciparum* remains the most virulent species causing the deadly forms of malaria [7]. The *Plasmodium* species responsible for human malaria are mainly transmitted by primary vector species, such as *Anopheles gambiae* sensu lato (s.l.), *Anopheles funestus* group and *Anopheles nili* group [8, 9]. The existence of those different species complexes in an area represents a great challenge for malaria control programmes. *Anopheles nili* group has a wide geographical distribution in most of tropical Africa [10] and its preferential habitats are fast-flowing, upright-flowing streams, large rivers or dense shade along streams [7]. It is a group that includes four species, including *An. nili* sensu stricto (s.s.), *Anopheles carnevalei*, *Anopheles somalicus* and *Anopheles ovengensis* [8, 11].

In Benin, over the past two decades, the roles of *An. gambiae* s.l. and *An. funestus* group in the transmission of *P. falciparum* in several regions of the country have been studied by several authors [12–19]. Few of these studies have shown the presence of *An. nili* group in the different surveyed areas. Moreover, none of them has demonstrated its involvement in malaria transmission in Benin. It is in this context that a large population of *An. nili* group was collected during this cross-sectional survey conducted in the districts of Kérou and Péhunco located in the Atacora region. Thus, the possible involvement of this *Anopheles* species in the transmission of *P. falciparum* was investigated in the study area.

This study aims at providing information on vector species composition involved in malaria transmission in the areas of Kérou and Péhunco (northwestern Benin) where there is a lack of data.

## Methods

### Study area

This study was conducted from September to October 2017 in the districts of Kérou and Péhunco, both located in the north-western Benin (Fig. 1). The two districts have a Sudano-Guinean climate characterized by a rainy season, from mid-April to mid-October, and a dry season from mid-October to mid-April. They belong to an agro-ecological zone characterized by an average annual rainfall of 1000 mm. The average temperature varies between 25 in August and 31 °C in April.

The district of Kérou is irrigated by two large permanent rivers, the Mekrou and the Pendjari as well as of many tributaries from the Alibori river. Péhunco is drained by the river Mekrou and also by many rivers. The most important economic activity of these two districts is agriculture, especially with the cultivation of cotton, sorghum and cashew nuts [20].

### Mosquito sampling and processing on the field

In each district, two villages were selected for mosquito collections, Wodara and Bambaba (respectively central and peripheral village of the Kérou district) and, Péhunco 2 and Béké (respectively central and peripheral village of the Péhunco district). Collections of mosquitoes were performed using both human landing catches (HLC) and pyrethrum spray catches (PSC).

HLCs were carried out in two houses per village. Four sessions of night collections were organized each month with one human sitting inside and another one outside of each house, bringing to a total of 16 human-nights/village/month and a total of 32 human-nights/district/month.

For PSCs, 10 bedrooms were surveyed in each district (5 in the central village and the remaining 5 in the peripheral village) to collect early in the mornings, all mosquitoes that have entered the houses the night before. Thus, aerosol bombs (Rambo®) containing 0.25% transfluthrin and 0.20% permethrin were sprayed in the houses and white canvas were spread on the floor for the collection of fallen mosquitoes.

Mosquitoes caught by both methods were morphologically identified using a mosquito identification key [21] and, the ovaries of the *Anopheles* vectors were dissected to determine the parity rate [22]. *Anopheles* vectors collected by PSCs were classified according to the physiological state of their abdomens (unfed, fed, half gravid, gravid) to determine the blood feeding rate. The *Anopheles* vectors collected with the two sampling methods were then stored in tagged eppendorf tubes containing silica gel and cotton for subsequent laboratory analysis.

### Laboratory analysis

539 head-thoraxes of *Anopheles* vectors collected indoor and outdoor through HLC were crushed and then analysed by the ELISA tests for the determination of the circumsporozoite protein (CSP) positivity of *Plasmodium*
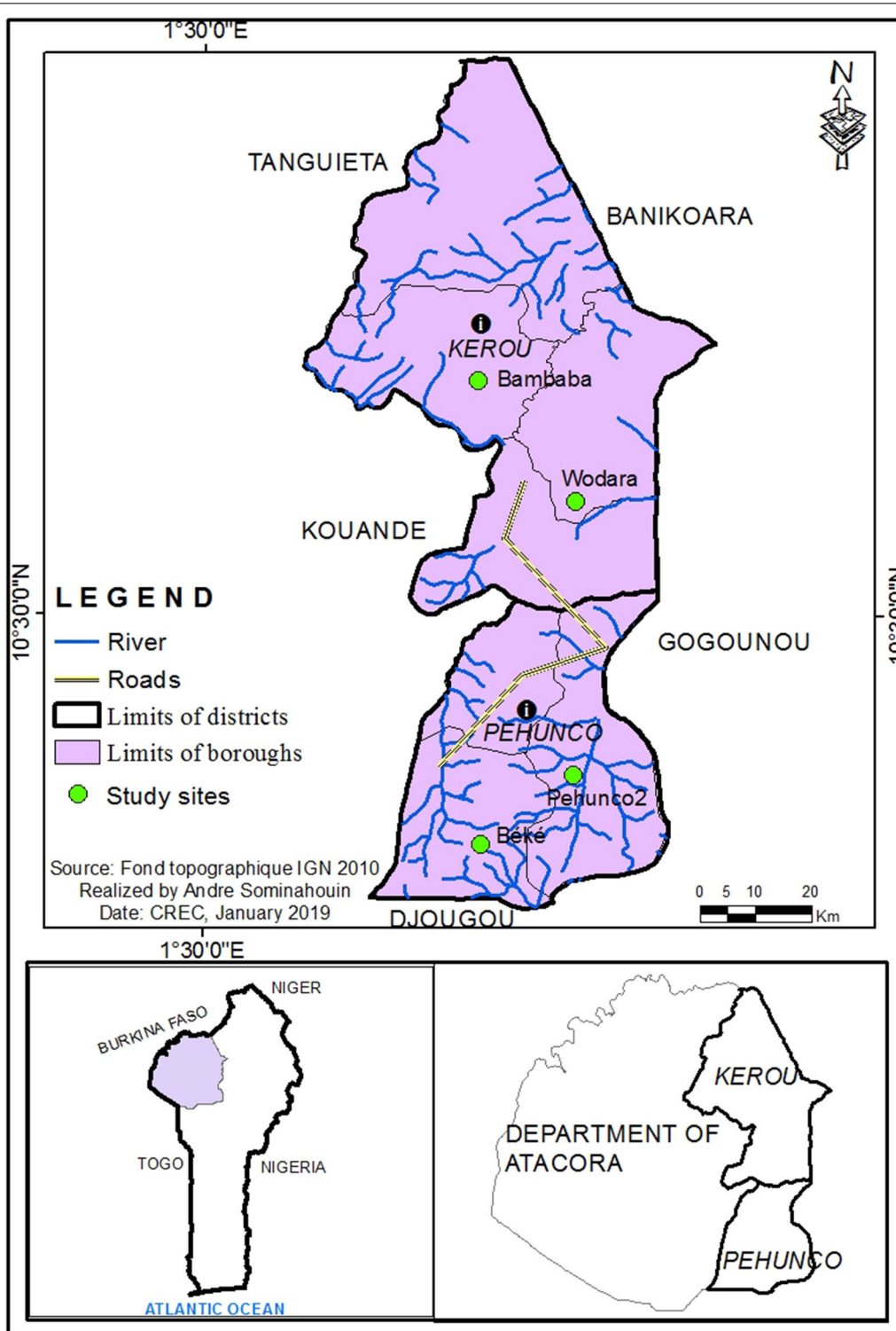
**Fig. 1** Map of the study area

Ossè *et al. Malar J*    (2019) 18:152

Page 4 of 10

*falciparum* using the protocol of Wirtz et al. [23]. This allows the determination of the sporozoite index (SI). The legs, wings, and abdomens of these mosquitoes were used for DNA extraction to perform molecular species identification.

The PCR technique based on the protocols of Santomalazza et al. [24], Koekemoer et al. [25] and Kengne et al. [26] was used to identify the sibling species of *An. gambiae* complex, *An. funestus* group and *An. nili* group, respectively. In *An. gambiae* s.l., the presence of L1014F *kdr* and G119S *ace-1* mutations was determined following the methods of Martinez-Torres et al. [27] and Weill et al. [28], respectively. Moreover, the blood meal origin was sought in the blood fed *Anopheles* mosquitoes collected by PSC, using a direct enzyme immunoassay (ELISA) according to the method of Beier et al. [29] with human, cattle, sheep, chicken and pig antibodies.

### Data analysis

The results were processed and analysed using the R Core Team software (Version 3.5.1-2018) and Excel spreadsheets. The human biting rates (HBR = number of collected vectors/number of humans/number of nights), infection rates (Number of infected mosquitoes/total tested), entomological inoculation rates ($EIR_{night}$ = HBR × infection rate; $EIR_{month}$ = $EIR_{night}$ × 30) and parity rates (Number of parous mosquitoes/total tested) were calculated and compared between species of the same district. The Poisson test allowed us to compare the HBRs and EIRs between species in the district of Kérou. The comparison of parity rates, infectivity and allelic frequencies of L1014F *kdr* and G119S *ace-1* mutations by species and by site were made with the Chi square comparison test.

### Results

#### Mosquito species composition and molecular identification of sibling species of *Anopheles gambiae* complex, *Anopheles funestus* group and *Anopheles nili* group

The mosquito fauna collected in both surveyed districts was very diverse (Table 1). A total of 1558 mosquitoes divided into 12 different species were collected with a predominance of *Anopheles* species (83.25%: 1297/1558).

In Péhunco, out of the collected mosquitoes, 36.36% (128/352) were *An. gambiae* s.l. and 0.3% (1/352) *An. funestus* group. In the Kérou district, 9 species were collected. Among those species, *An. gambiae* s.l. was the most abundant (69.48%: 838/1206) followed by *An. nili* group (13.27%: 160/1206), *Anopheles brohieri* (8.37%: 101/1206), *Anopheles ziemanni* (3.90%: 47/1206) and *An. funestus* group (1.74%: 21/1206).

**Table 1 Mosquito species composition in Péhunco and Kérou between September and October 2017**

| Species | Péhunco | | Kérou | | Total |
|---|---|---|---|---|---|
| | Indoor | Outdoor | Indoor | Outdoor | |
| *Anopheles gambiae* s.l. | 74 | 54 | 430 | 408 | 966 |
| *Anopheles funestus* group | 0 | 1 | 9 | 12 | 22 |
| *Anopheles nili* group | 0 | 0 | 51 | 109 | 160 |
| *Anopheles pharoensis* | 0 | 0 | 0 | 1 | 1 |
| *Anopheles ziemanni* | 0 | 0 | 14 | 33 | 47 |
| *Anopheles brohieri* | 0 | 0 | 19 | 82 | 101 |
| Anophelinae (sub total 1) | 74 | 55 | 523 | 645 | 1297 |
| *Aedes aegypti* | 0 | 0 | 1 | 0 | 1 |
| *Aedes vitatus* | 0 | 0 | 0 | 2 | 2 |
| *Culex quinquefasciatus* | 127 | 91 | 18 | 16 | 252 |
| *Culex nebulosus* | 0 | 2 | 0 | 0 | 2 |
| *Culex tigripes* | 1 | 0 | 0 | 0 | 1 |
| *Mansonia africana* | 1 | 1 | 0 | 1 | 3 |
| Culicinae (sub total 2) | 129 | 94 | 19 | 19 | 261 |
| Grand total | 203 | 149 | 542 | 664 | 1558 |

PCR results showed the presence of *An. gambiae* and *An. coluzzii* as sibling species of the *An. gambiae* complex in both Péhunco and Kérou. *Anopheles gambiae* was found in majority (96.43% and 78.43% at Péhunco and Kérou, respectively). In addition, all mosquito specimens of the *An. funestus* group (Péhunco and Kérou) and the *An. nili* group (Kérou) were found to be *An. funestus* s.s. and *An. nili* s.s., respectively (Fig. 2).

#### Biting behaviour, HBR, SI and EIR

In Kérou, the *An. nili* group was exophagic as the proportion of this species caught outdoors (68.13%: 109/160) was significantly higher than that recorded indoors (31.87%: 51/160) (p < 0.0001). In the same district the indoor (51.31%: 430/838) and the outdoor (48.69%: 408/838) biting behaviour of *An. gambiae* s.l. was similar (p = 0.282), as was also observed for the *An. funestus* group [indoors proportion: 42.86% (9/21); outdoors proportion: 57.14% (12/21); p = 0.35]. However, in Pehunco, *An. gambiae* s.l. was endophagic [Indoors proportion: 57.81% (74/128); Outdoors proportion: 42.19% (54/128); p = 0.011] (Table 1).

In the district of Kérou, the biting rate of *An. gambiae* s.l. (26 bites/human/night) was significantly higher than that of the *An. nili* group (5 bites/human/night) (p < 0.0001). The biting rate of *An. funestus* group (0.656 bites/human/night) was significantly lower than that of *An. gambiae* s.l. and *An. nili* group (p < 0.0001). The trend was the same between *An. funestus* group and *An. gambiae* s.l. in Péhunco (p < 0.0001) (Table 2).

Ossè *et al. Malar J*      (2019) 18:152

Page 5 of 10



**Fig. 2** Distribution of sibling species within the *An. gambiae* complex and the *An. funestus* and *An. nili* groups in the study sites
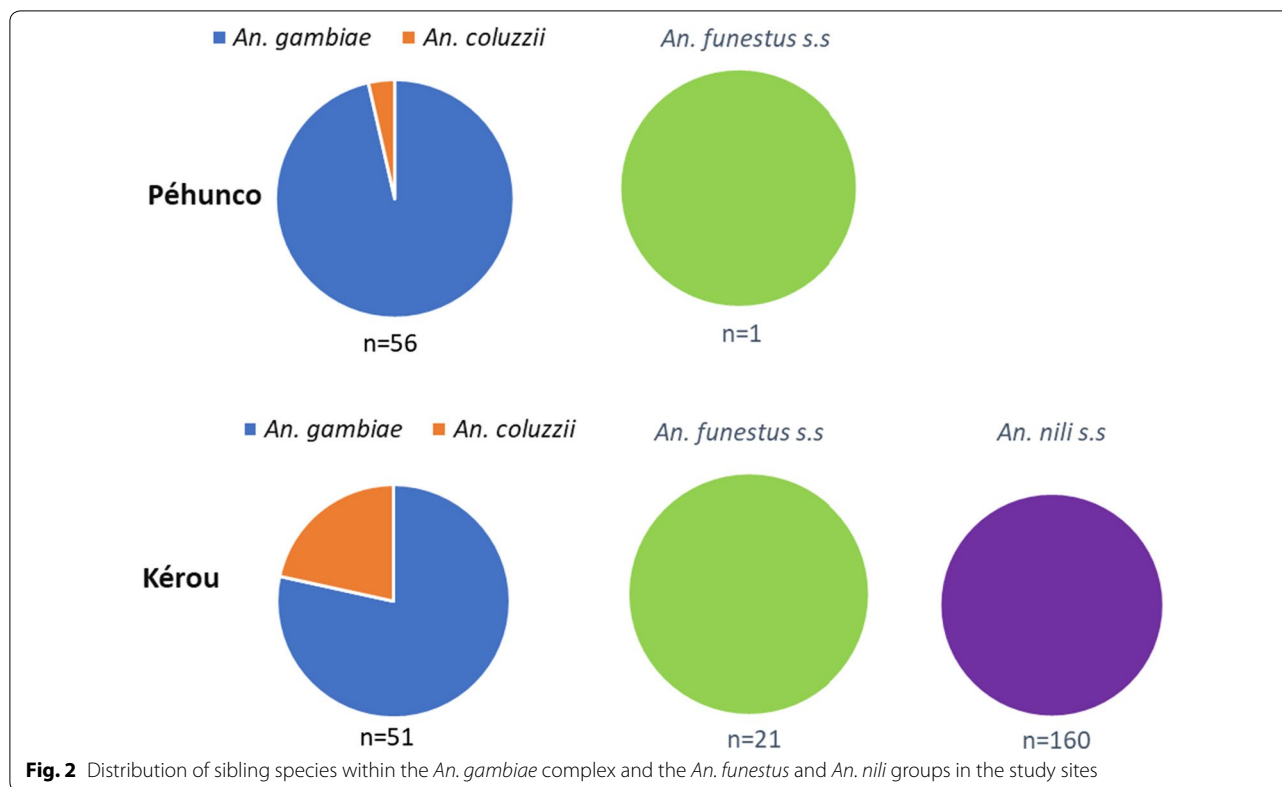
**Table 2 Entomological inoculation rate (EIR) in *Anopheles gambiae* s.l., *Anopheles funestus* group and *Anopheles nili* group in the Péhunco and Kérou districts**

| | Biting locations | Number of collected mosquitoes | | HBR (b/h/night) | | Sporozoite index (N tested) | | EIR (ib/h/night) | | EIR (ib/h/month) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Kérou | Péhunco | Kérou | Péhunco | Kérou | Péhunco | Kérou | Péhunco | Kérou | Péhunco |
| *An. gambiae* s.l. | Indoors | 430 | 74 | 26.875[a] | 4.625[a] | 0.025 (121)[a] | 0.014 (66)[a] | 0.672 | 0.065 | 20.156[a] | 1.943[a] |
| | Outdoors | 408 | 54 | 25.5[a] | 3.375[a] | 0.008 (120)[a] | 0 (50)[a] | 0.204 | 0 | 6.120[b] | 0[b] |
| | Total | 838 | 128 | 26.19* | 4* | 0.017* (241) | 0.009 (116) | 0.435 | 0.034 | 13.05* | 1.02 |
| *An. funestus* group | Indoors | 9 | 0 | 0.5625[b] | 0[a] | 0.111 (9)[a] | 0 | 0.062 | 0 | 1.873[c] | 0 |
| | Outdoors | 12 | 1 | 0.75[b] | 0.0625[a] | 0 (12)[a] | 0 (1) | 0 | 0 | 0[d] | 0 |
| | Total | 21 | 1 | 0.656[‡] | 0.031[‡] | 0.048 (21)* | 0 (1) | 0.031 | 0 | 0.9375[‡] | 0 |
| *An. nili* group | Indoors | 51 | 0 | 3.1875[c] | 0 | 0.02 (51)[a] | 0 | 0.064 | 0 | 1.913[e] | 0 |
| | Outdoors | 109 | 0 | 6.8125[d] | 0 | 0.009 (109)[a] | 0 | 0.061 | 0 | 1.839[e] | 0 |
| | Total | 160 | 0 | 5[¥] | 0 | 0.0125 (160)* | 0 | 0.063 | 0 | 1.875[¥] | 0 |

b/h, bite/human; ib/h, infected bite/human; N, number of mosquitoes

[a,b,c,d,e] Values with different superscripts at the two biting locations of the same district are significantly different (p < 0.05)

[*,‡,¥] The total values with different superscripts in a same district are significantly different (p < 0.05)

For the SI, no significant difference was recorded between the three vectors in the Kérou district (p = 0.496). In Péhunco, only *An. gambiae* s.l. was found positive for *P. falciparum* antigen (Table 2).

In Kérou, the indoors EIR of *An. gambiae* s.l. (20.156 infected bites/human/month) and *An. funestus* group (1.873 infected bites/human/month) were significantly higher than that recorded outdoors [*An. gambiae*

Ossè *et al. Malar J*    (2019) 18:152

Page 6 of 10

s.l.: 6.12 infected bites/human/month (p < 0.0001); *An. funestus* group: 0 infected bites/human/month (p = 0.0005)]. In the same district, the indoor (1.913 infected bites/human/month) and the outdoor (1.839 infected bites/human/month) EIR of *An. nili* group was similar (p = 1). Furthermore, in the district of Kérou, the malaria transmission risk by *An. gambiae* s.l. (13.05 infected bites/human/month) was significantly higher than the malaria transmission risk of the *An. nili* group (1.875 infectious bites/human/month) (p = 0.0009) and the *An. funestus* group (0.938 infected bites/human/month) (p < 0.0001). It should be noted that the EIR of *An. nili* group was significantly higher than that of *An. funestus* group in Kérou (p < 0.0001) during the study period. In Péhunco, the indoor EIR for *An. gambiae* s.l. (1.943 infected bites/human/month) was significantly higher than that noted outdoor (0 infected bites per month) (p < 0.0001) (Table 2).

## Parity rate of *An. gambiae* s.l., *An. funestus* group and *An. nili* group

Figure 3 shows the different parity rates with 88% [78.99–97.01], 57.14% [35.97–78.31] and 46% [32.19–59.81] for *An. gambiae* s.l., *An. funestus* group and *An. nili* group in the Kérou district, respectively. The parity rate in *An. gambiae* s.l. was significantly higher than that of *An. funestus* group (p = 0.004) and *An. nili* group (p < 0.0001). In Pehunco, the parity rate recorded for *An. gambiae* s.l. was 72% [59.55–84.45].

## Blood feeding rate and blood meal source in *An. gambiae* s.l

The blood feeding rate of *An. gambiae* s.l. collected inside houses after PSC was 63.16% in Péhunco against 60.53% in Kérou (p = 0.847). In both districts, the majority of *An. gambiae* s.l. vectors took their blood meal on human (91.30% in Kérou against 100% in Péhunco). In Kérou, 4.35% (1/23) of the collected *An. gambiae* s.l. blood fed on beef and, the same proportion on sheep (Table 3).
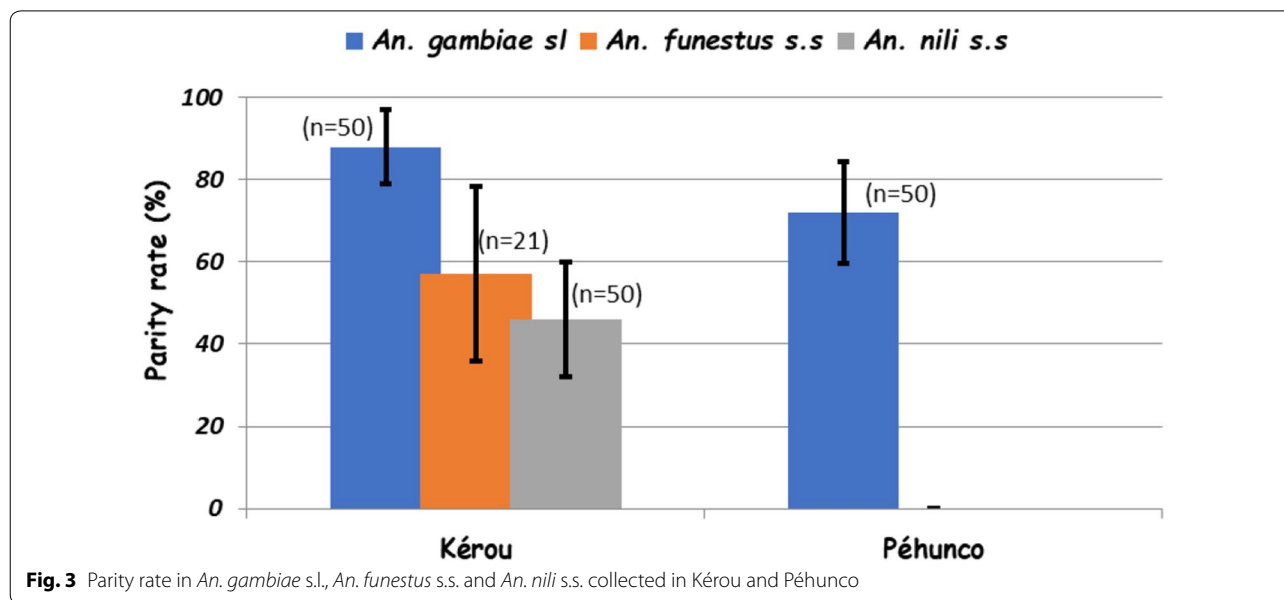


**Fig. 3** Parity rate in *An. gambiae* s.l., *An. funestus* s.s. and *An. nili* s.s. collected in Kérou and Péhunco

**Table 3 Blood meal origin in *Anopheles gambiae* s.l. collected indoors in Kérou and Péhunco**

| Districts | T. collected | T. Blood fed | Blood feeding rate (%) | Blood meal origin in *An. gambiae* s.l. | | | |
|---|---|---|---|---|---|---|---|
| | | | | N. Human (%) | N. Beef (%) | N. Sheep (%) | N. Pig (%) |
| Péhunco | 19 | 12 | 63.16 | 12 (100) | | | 0 |
| Kérou | 38 | 23 | 60.53 | 21 (91.30) | 1 (4.35) | 1 (4.35) | 0 |
| Total | 57 | 35 | 61.40 | 22 (62.86) | 1 (2.86) | 1 (2.86) | 0 |

T, total of mosquitoes; N., number of mosquitoes having taken their blood meal on

Ossè *et al. Malar J*    (2019) 18:152

Page 7 of 10

### L1014F *kdr* and G119S *ace-1* allele frequencies in *An. gambiae* and *An. coluzzii*

The L1014F *kdr* mutation was found at very high frequencies in both Péhunco (86% and 100% respectively in *An. gambiae* and *An. coluzzii)* and Kérou (83% and 73% respectively in *An. gambiae* and *An. coluzzii)* (Table 4). In each district, the allelic frequencies of the L1014F *kdr* mutation were similar in *An. gambiae* and *An. coluzzii* (p > 0.05).

The G119S *ace-1* mutation was also recorded in the two districts but at low levels with allelic frequencies of 5% and 0%, respectively, in *An. gambiae* and *An. coluzzii* in Péhunco and, 7.3% and 5%, respectively, in *An. gambiae* and *An. coluzzii* in Kérou (Table 4). No significant difference was also recorded between the allelic frequencies of the G119S *ace-1* mutation of *An. coluzzii* and *An. gambiae* in both districts (p > 0.05).

### Discussion

The study of malaria vectors in an area is a prerequisite not only to understand the epidemiology of the disease but also to implement a strategy for effective and targeted control of those vectors. In the current study, 12 mosquito species including 6 anophelinae were identified in the two surveyed districts. Among these 6 *Anopheles* species, two (*An. gambiae* s.l. and *An. funestus* group) have already been implicated in malaria transmission in Benin [13, 17, 18]. During this cross-sectional study, they were found in both study districts but, *An. nili* group was found only in Kérou. The three vector species (*An. gambiae* s.l., *An. funestus* group and *An. nili* group) identified in Kérou live in sympatry in this savannah zone. This finding is similar to those of several authors in West Africa [30, 31].

In Péhunco and Kérou, *An. gambiae* and *An. coluzzii* are the only members of the *An. gambiae* complex recorded with a predominance of *An. gambiae* (96.43% and 78.43%, respectively, in Péhunco and Kérou). No species of *An. arabiensis* was identified after PCR in both districts. This could be due to anthropogenic actions such as deforestation and urbanization that destroy its natural habitats causing its gradual disappearance in

some areas of northern Benin as reported by Aïkpon et al. [32] and Salako et al. [33]. All the specimens of the *An. nili* and *An. funestus* groups identified by PCR were *An. nili* s.s. and *An. funestus* s.s. in the district of Kérou. These results corroborate those of Djouaka et al. [19, 34] who found *An. funestus* s.s. as the only species of the *An. funestus* group in the areas of Pahou and Kpomè in southern Benin. The results are also similar to works by Carnevale et al. [35], Dia et al. [36] and Adja et al. [31], who found *An. nili* s.s. as the only species in forest zone respectively in Cameroon, Senegal and Côte d'Ivoire.

The number of *An. funestus* group collected in both districts was low during the study period as was also reported by some authors in the area and around [32, 37, 38]. This could be justified by the scarcity of breeding sites favorable to the development of this species. Moreover, contrary to *An. gambiae* s.l., *An. nili* group was found to be exophagic (significantly higher biting activity outdoors compared to indoors) in Kérou. This low biting behaviour of *An. nili* group inside the dwellings had also been reported in Nigeria [39]. Kérou being a savanna area, this behaviour of *An. nili* group confirms the results of other authors who have shown that savanna populations of *An. nili* group are more exophagic and exophilic [40, 41].

In Kérou, each inhabitant receives 5 bites of *An. nili* group per night. This biting rate was significantly higher than the one of *An. funestus* group and not negligible for just four nights of collection. This reflects the presence of breeding sites favorable to the development of this species in the area. In fact, Kérou is close to several rivers, and the water level of these rivers is kept high for a good part of the year by the rain. This flow of water contributes to the development of several permanent mosquito breeding sites. This could probably explain the high density of the populations of the *An. nili* group in Kérou. As a result, in an area with multiple vectors, variation in ecological, spatial and temporal parameters directly influences the relative role of each species in malaria transmission [42]. The biting rate of *An. gambiae* s.l. was very high in Kérou (26.19 bites/human/night) and in Péhunco (4 bites/human/night) compared to the other

**Table 4 Allelic frequencies of L1014F *kdr* and G119S *ace-1* mutations in *Anopheles gambiae* and *Anopheles coluzzii* in the Péhunco and Kérou districts**

| | Species | N tested | L1014F *Kdr* | | | | *p* value | G119S *Ace-1* | | | | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RR | RS | SS | F(L1014F *Kdr*) | | RR | RS | SS | F(G119S *Ace-1*) | |
| Péhunco | *An. gambiae* | 54 | 42 | 9 | 3 | 0.86 | 0.957 | 0 | 5 | 49 | 0.05 | 1 |
| | *An. coluzzii* | 2 | 2 | 0 | 0 | 1 | | 0 | 0 | 2 | 0 | |
| Kérou | *An. gambiae* | 41 | 30 | 8 | 3 | 0.83 | 0.439 | 0 | 6 | 35 | 0.073 | 1 |
| | *An. coluzzii* | 11 | 7 | 2 | 2 | 0.73 | | 0 | 1 | 10 | 0.05 | |

Ossè *et al. Malar J*     (2019) 18:152

Page 8 of 10

species and allows this vector to maintain the transmission of malaria in both districts.

Another important aspect of the current study is the involvement of *An. nili* group in malaria transmission in Benin. Indeed, the SI of *An. nili* group was 1.25% in Kérou. This index is similar to that recorded by Carnevale et al. [35] in Cameroon and lower than the one of Elissa et al. [43] in Gabon and Adja et al. [31] in Côte d'Ivoire. The sporozoite indexes of *An. gambiae* s.l. in Kérou (1.7%) and Péhunco (0.9%) were lower than those reported by previous studies in different areas of Benin [16, 33, 37]. This could be due to the low number of tested mosquitoes in the short collection period covered by the current study as compared to previous records which considered a longer time period.

This study also showed malaria transmission by different mosquito species in the Kérou district. *An. gambiae* s.l. and *An. nili* group share in the indoors and outdoors malaria transmission. In *An. funestus* group where EIR has been observed indoors, no definitive conclusion can be drawn as to its participation in outdoor transmission since the collection period in this study is short. As previously found in several sites in Benin [13–18], *An. gambiae* s.l. also remains the primary vector of malaria transmission in Kérou (13.05 infected bites/human/month), followed by *An. nili* group (1.87 infected bites/human/month) and *An. funestus* group (0.94 bites/human/month).

The EIR by *An. gambiae* s.l. in Kérou was higher than those reported in a longitudinal study by Salako et al. [37] in similar bioecological areas (Kandi and Gogounou) in the department of Alibori, Benin. In Péhunco, each inhabitant receives 1.02 infected bites of *An. gambiae* s.l. per month. The high transmission by *An. gambiae* s.l. in Kérou compared to both *An. funestus* group and *An. nili* group may be due to its significantly higher physiological age compared to the one of the other mosquito species ($p < 0.05$). The small number of *An. funestus* group collected in each district could explain its low level of malaria transmission. Findings of this study shows that no specimen of *An. nili* group and *An. funestus* group was collected indoors after PSC. This could be due to the low sampling of mosquitoes performed during our survey. *An. gambiae* s.l. which was the most frequent vector in the two districts takes mostly its blood meal on humans, thus confirming its anthropophagic nature [33].

Regarding the presence of resistance mechanisms in *An. gambiae* s.l., the L1014F *Kdr* mutation was found at very high frequencies in its two detected sibling species (*An. gambiae* and *An. coluzzii*) in the two districts. Contrary to the works of Gnanguenon et al. [44], Yahouédo et al. [45] and Akogbeto et al. [33] in some locations of southern and northern Benin, no significant difference was noted between the allelic frequencies of the L1014F *kdr* mutation of *An. gambiae* and *An. coluzzii* in Kérou and Péhunco. This result corroborates those of Salako et al. [46] who also found no significant difference between the L1014F *kdr* frequencies of *An. gambiae* and *An. coluzzii* in Djougou, Ségbana and Copargo, northern Benin. The trend was the same for the relatively low allelic frequencies of the G119S *ace-1* mutation of *An. gambiae* and *An. coluzzii* of the two study districts. However, the highest allelic frequencies of the G119S *Ace-1* mutation was recorded in *An. gambiae* in each zone with values ranging from 5 to 7.3%. In *An. coluzzii*, this frequency varied from 0% in Péhunco to 5% in Kérou.

## Conclusion

In the present study, *An. gambiae* s.l., *An. funestus* group and *An. nili* group were the three most important vectors that transmit malaria in the Kérou district. This is the first report on the contribution of the *An. nili* group as a secondary vector of malaria transmission in Benin. This suggests broader studies involving other districts to determine if other *Anopheles* species also contribute to malaria transmission. This will help a better orientation of vector control interventions.

## Authors' contributions
RAO, FT, GGP, RA and MCA designed the study. RAO, AF and CK performed the fields activities. RAO, AS and WS carried out the laboratory's activities. RAO drafted the manuscript and analysed the data. MCA, MA, GGP, ADS, BA and RA critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

## Author details
[1] Ecole de Gestion et d'Exploitation des Systèmes d'Elevage, Université Nationale d'Agriculture, Kétou, Benin. [2] Centre de Recherche Entomologique de Cotonou (CREC), 06 BP 2604 Cotonou, Benin. [3] National Malaria Control Programme, Ministry of Health, Cotonou, Benin. [4] Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Abomey-Calavi, Benin. [5] Ecole Normale Supérieure de Natitingou, Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques, Natitingou, Benin. [6] Faculté d'Agronomie, Université de Parakou, Parakou, Benin. [7] Conseil National de Lutte contre le SIDA, la Tuberculose, le Paludisme, les Hépatites et les épidémies, Cotonou, Benin.

## Competing interests
The authors declare that they have no competing interests.

Ossè *et al. Malar J* (2019) 18:152

Page 9 of 10

## Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References
1. Calzolari M. Mosquito-borne diseases in Europe: an emerging public health threat. Reports Parasitol. 2016;5:1–12.
2. WHO. World Malaria report 2017. Geneva: World Health Organization; 2017.
3. Rogier MPC, Parzi MCD, Spiegel MAC. Histoire naturelle et épidémiologie du paludisme. In: Saissy JM, editor. Paludisme grave. Paris: Arnette; 2001.
4. Kazembe LN, Kleinschmidt I, Holtz TH. Spatial analysis and mapping of malaria risk in Malawi using point-referenced prevalence of infection data. Int J Health Geograph. 2006;5:41.
5. Antonio-Nkondjio C, Awono-Ambene P, Toto JC, Meunier JY, Zebaze-Kemleu S, Nyambam R, et al. High malaria transmission intensity in a village close to Yaounde, the capital city of Cameroon. J Med Entomol. 2002;39:350–5.
6. White NJ. *Plasmodium knowlesi*: the fifth human malaria parasite. Clin Infect Dis. 2008;46:172–3.
7. Carnevale P, Robert V. Les anophèles, biologie, transmission du paludisme et lutte antivectorielle. IRD Editions. 2009.
8. Fontenille D, Simard F. Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. Comp Immun Microbiol Infect Dis. 2004;27:357–75.
9. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, et al. Mosquitoes and their Control. 2nd ed. Berlin: Springer; 2010.
10. Hamon J, Mouchet J. Les vecteurs secondaires du paludisme humain en Afrique. Méd Trop. 1961;21:643–60.
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;41:561–8.
12. Akogbéto M. Lagoonal and coastal malaria at Cotonou: entomological findings. Santé. 2000;10:267–75.
13. Djènontin A, Bio-Bangana S, Moiroux N, Henry M-C, 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. Parasit Vectors. 2010;3:83.
14. Padonou GG, Sezonlin M, Gbedjissi GL, Ayi I, Azondekon R, Djènontin A, et al. Biology of *Anopheles gambiae* and insecticide resistance: entomological study for a large scale of indoor residual spraying in South East Benin. J Parasitol Vector Biol. 2011;3:59–68.
15. Ossè RA, Aïkpon R, Gbédjissi GL, Gnanguenon V, Sèzonlin M, Govoétchan R, et al. A shift from indoor residual spraying (IRS) with bendiocarb to Long-Lasting Insecticidal (mosquito) Nets (LLINs) associated with
16. Sovi A, Govoétchan R, Tokponnon F, Hounkonnou H, Aïkpon R, Agossa F, et al. Impact of land-use on malaria transmission in the plateau region, southeastern Benin. Parasit Vectors. 2013;6:352.
17. Aïkpon R, Sèzonlin M, Tokponon F, Okè M, Oussou O, Oké-Agbo F, et al. Good performances but short lasting efficacy of Actellic 50 EC Indoor Residual Spraying (IRS) on malaria transmission in Benin. West Africa. Parasit Vectors. 2014;7:256.
18. Gnanguenon V, Govoetchan R, Agossa FR, Ossè R, Oke-Agbo F, Azondekon R, et al. Transmission patterns of *Plasmodium falciparum* by *Anopheles gambiae* in Benin. Malar J. 2014;13:444.
19. Djouaka R, Riveron JM, Yessoufou A, Tchigossou G, Akoton R, Irving H, et al. Multiple insecticide resistance in an infected population of the malaria vector *Anopheles funestus* in Benin. Parasit Vectors. 2016;9:453.
20. Afrique Conseil. Monographie de la commune de Kérou et de Péhunco. 2006.
21. Gillies MT, Coetzee M. A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region). Publ South Afr Inst Med Res. 1987;1987:55.
22. Detinova TS, Gillies MT. Observations on the determination of the Age composition and epidemiological importance of populations of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in Tanganyika. Bull World Health Organ. 1964;30:23–8.
23. Wirtz R, Zavala F, Charoenvit Y, Campbell G, Burkot T, Schneider I, et al. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. Bull World Health Organ. 1987;65:39.
24. Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, Torre A. Insertion polymorphisms of SINE200 retrotranposons within speciation island of *Anopheles gambiae* molecular forms. Malar J. 2008;7:163.
25. 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.
26. 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.
27. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et al. Molecular characterization of pyrethroids knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol Biol. 1998;7:179–84.
28. Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol Biol. 2004;13:1–7.
29. Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargam TPII, et al. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA) tested on Anopheles (Diptera: Culicidae) in Kenya. J Med Entomol. 1988;25:9–16.
30. Fofana D, Koné AB, Konan YL, Konan KL, Doannio JMC, N'Goran KE. Diversité spécifique et nuisance culicidienne dans les villages de N'gatty et d'Allaba en milieu côtier lagunaire de Côte-d'Ivoire. Cahiers Santé. 2010;20:73–9.
31. 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.
32. Aïkpon R, Osse R, Govoetchan R, Sovi A, Oke-Agbo F, Akogbeto MC. Entomological baseline data on malaria transmission and susceptibility of *Anopheles gambiae* to insecticides in preparation for indoor residual spraying (IRS) in Atacora, (Benin). J Parasitol Vector Biol. 2013;5:102–11.
33. Akogbéto MC, Salako AS, Dagnon F, Aïkpon R, Kouletio M, Sovi A, et al. Blood feeding behaviour comparison and contribution of *Anopheles coluzzii* and *Anopheles gambiae*, two sibling species living in sympatry, to malaria transmission in Alibori and Donga region, northern Benin, West Africa. Malar J. 2018;17:307.
34. Djouaka R, Irving H, Tukur Z. Exploring mechanisms of multiple insecticide resistance in a population of the malaria vector *Anopheles funestus* in Benin. PLoS ONE. 2011;6:e27760.
35. Carnevale P, Le Goff G, Toto JC, Robert V. *Anopheles nili* as the main vector of human malaria in villages of southern Cameroon. Med Vet Entomol. 1992;6:135–8.

changes in malaria transmission indicators in pyrethroid resistance areas in Benin. Parasit Vectors. 2013;6:73.

Ossè *et al. Malar J*     (2019) 18:152

Page 10 of 10

36. Dia I, Diop T, Rakotoarivony L, 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.

37. Salako AS, Ahogni I, Kpanou C, Sovi A, Azondekon R, Sominahouin AA, et al. Baseline entomologic data on malaria transmission in prelude to an indoor residual spraying intervention in the regions of Alibori and Donga, Northern Benin, West Africa. Malar J. 2018;17:392.

38. Aïkpon R, Ossè R, Ahogni I, Dagnon F, Tokponon F, Lyikirenga L, et al. Residual malaria transmission assessment: quantification of the relative risk of malaria transmission at different night-time and location in Atacora region in Benin, West Africa. J Entomol Zool Studies. 2018;6:1026–32.

39. Oyewole IO, Ibidapo CA, Okwa OO, Oduola AO, Adeoye GO, Okoh HI, et al. Species composition and role of Anopheles mosquitoes in malaria transmission along Badagry axis of Lagos Lagoon, Lagos, Nigeria. Int J Insect Sci. 2010;2:51–7.

40. Awono-Ambene P, Antonio-Nkondjio C, Toto J, Ndo C, Etang J, Fontenille D, et al. Epidemological importance of the *Anopheles nili* group of malaria vectors in equatorial villages of Cameroon, Central Africa. Sci Med Afr. 2009;1:13–20.

41. Antonio-Nkondjio C, Kerah C, Simard F, Awono-Ambene H, Mouhamadou C, Tchuinkam T, et al. Complexity of malaria vectorial system in Cameroon: contribution of secondary vectors to malaria transmission. J Med Entomol. 2006;43:1215–21.

42. Bigoga JD, Manga L, Titanji VP, Coetzee M, Leke RG. Malaria vectors and transmission dynamics in coastal south–western Cameroon. Malar J. 2007;6:5.

43. Elissa N, Karch IS, Bureau PH, Ollomo B, Lawoko IM, Yangari IP, et al. Malaria transmission in a region of savanna-forest mosaic, Haut-Ogooué, Gabon. J Am Mosq Control Assoc. 1999;15:15–23.

44. Gnanguenon V, Agossa FR, Badirou K, Govoetchan R, Anagonou R, OkeAgbo F, et al. Malaria vectors resistance to insecticides in Benin: current trends and mechanisms involved. Parasit Vectors. 2015;8:223.

45. Yahouédo GA, Cornelie S, Djégbé I, Ahlonsou J, Aboubakar S, Soares C, et al. Dynamics of pyrethroid resistance in malaria vectors in southern Benin following a large-scale implementation of vector control interventions. Parasit Vectors. 2016;9:385.

46. Salako AS, Ahogni I, Aïkpon R, Sidick A, Dagnon F, Sovi A, et al. Insecticide resistance status, frequency of L1014F Kdr and G119S Ace-1 mutations, and expression of detoxification enzymes in *Anopheles gambiae* (s.l.) in two regions of northern Benin in preparation for indoor residual spraying. Parasit Vectors. 2018;11:618.