Assessing *Anopheles* Vector Species Diversity and Transmission of Malaria in Cross-Border Areas of Côte d'Ivoire

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Abstract

Background Although malaria and *Anopheles* mosquito vectors are highly prevalent in Côte d'Ivoire, data are still lacking on disease transmission dynamics in cross-border areas. To address this lack of information, we assessed the *Anopheles* mosquito vector species diversity, the *An. gambiae* complex members and the transmission of malaria in four cross-border areas of Côte d'Ivoire.

Method From July 2016 to December 2017, we collected adult *Anopheles* mosquitoes in four cross-border health districts of Côte d'Ivoire (Aboisso, Bloléquin, Odienné and Ouangolodougou) using standardized window exit trap (WET) and pyrethrum knockdown spray collection (PSC) methods. We identified collected mosquitoes morphologically at species level and *An. gambiae* complex members using short interspersed nuclear element-based polymerase chain reaction (SINE-PCR). We analyzed *An. gambiae*, *An. funestus* and *An. nili* specimens for malaria *Plasmodium* parasite infection using the cytochrome oxidase I gene (COX-I), and malaria prevalence among human population through local Ministry of Health (MoH) statistical yearbooks.

Results In total, 281, 754, 1,319 and 2,443 specimens of *Anopheles* adult females were collected in Aboisso, Bloléquin, Odienné and Ouangolodougou, respectively. We found seven *Anopheles* species dominated by *An. gambiae* s.l. (94.8%-99.1%), followed by *An. funestus* (0.4%-4.3%) and *An. nili* (0%-0.7%). Among *An. gambiae s.l.*, *An. coluzzii* predominated in Aboisso (89.2%) and Bloléquin (92.2%), while *An. gambiae* s.s. was present at the highest frequency in Odienné (96.0%) and Ouangolodougou (94.2%). The *Plasmodium* sporozoite infection rate in *An. gambiae s.l.* was highest in Odienné (11.0 %; n = 100) followed by Bloléquin (7.8%, n = 115), Aboisso (3.1%; n = 65) and Ouangologoudou (2.5%; n = 120). In *An. funestus*, *P. falciparum* sporozoite infection rate was estimated at 6.2% (n = 32) in Bloléquin, 8.7% (n = 23) in Odienné. No *An. funestus* specimens were found infected with *P. falciparum* sporozoite infection in Ouangolodougou and Aboisso. No *P. falciparum* sporozoite was detected in *An. nili* specimens in the four health districts. Among the local human populations, malaria prevalence rate was higher in Odienné (39.7%; n = 45,376) and Bloléquin (37.6%; n = 150,205) compared with that in Ouangolodougou (18.3%; n = 131,629) and Aboisso (19.7%; n = 364,585).

Conclusion In cross-border health districts of Côte d'Ivoire, *Anopheles* vector species diversity and abundance and *Plasmodium* sporozoite infection were high, thus resulting in high transmission of malaria to local populations. *An. gambiae* and *An. funestus* were found to be highly *Plasmodium*-infected in the health districts of Bloléquin and Odienné where malaria prevalence among humans was particularly high. This study provides important information that can be used to guide national malaria control programme strategies in Côte d'Ivoire, mainly in cross-border settings.

Introduction

Malaria continues to be the deadliest tropical infectious disease, with higher incidences in Africa [1]. In 2018, malaria has caused over 228 million cases and 405,000 deaths worldwide, of which 93% has occurred in sub-Saharan Africa [1]. In Côte d'Ivoire, malaria is still a major public health challenge and the leading reason for consultations in health services. It is responsible for up to 43% of morbidity, 11.8% of mortality, 40% of school absenteeism, 50% of loss of agricultural income and 62% of hospitalizations [2]. According to the world malaria report, the entire Ivorian population is at risk of malaria, with the most vulnerable being children under five years of age and pregnant women [3].

Malaria transmission in Africa is very heterogeneous due to eco-climatic variations across the continent [4]. Currently, five species of the *Plasmodium* genus have been identified as being responsible for malaria infection in humans [5, 6]. Of these, *P. falciparum* remains the most prevalent and most virulent species causing the deadly forms of malaria [6, 7]. The *Plasmodium* species responsible for human malaria are mainly transmitted by primary vector species, such as *Anopheles gambiae sensu lato* (*s.l.*) [8], *Anopheles funestus* group and *Anopheles nili* group [9–11].

In West Africa, two molecular forms have been identified in *An. gambiae s.l.*, formerly known as M and S. Recently, they have been identified as distinct species belonging to the *An. gambiae* complex and named *An. coluzzii* for the M form and *An. gambiae s.s*. for the S form [9]. These species display strong anthropophilic host-seeking behaviour, and have a long life expectancy, thus causing large numbers of secondary malaria cases from one infected individual [12]. *Anopheles* larvae are aquatic and found in a variety of breeding site types in terms of size, permanence, vegetation and water cleanliness [13]. Overall, the larvae of *An. gambiae* grow in small, shallow, relatively clean and sunny water reservoirs (puddles of water, stagnant water) [14, 15], and is more frequent in rain-dependent temporary breeding sites. *An. coluzzii* is associated to permanent breeding sites and those resulting from human activity and prefers urban water collections and adapts quickly to pollution [16, 17]. According to Mourou *et al*. [18], the breeding habitats of this species are known to increase in number and productivity during the rainy season, but almost disappear during the dry season. Deploying major vector control interventions, such as the rapid scale-up of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), without a detailed understanding of the species composition, distribution and behaviour dynamics of the local vectors can lead to limited impact or failure of these interventions [19, 20]. In addition, the main role of *An. gambiae* s.s. in the malaria parasites transmission and the molecular forms, that occurs in these districts were not yet elucidated. This may complicate impact monitoring of malaria control interventions [21].

In Côte d'Ivoire, the malaria vectors *An. gambiae* s.s. and *An. coluzzii* are widespread across the country [22, 23], whilst *An. funestus* and *An. nili* act as secondary vectors [24]. These species are well-adapted to diverse types of breeding sites (e.g., permanent breeding sites or temporary rain pools such as puddles, shallow wells, footprints, or in rice and vegetable fields) in both rural and urban areas [25]. Côte d'Ivoire shows considerable bio-climatic variations from north to south, leading to the subdivision of the country into different ecological zones [26]. Thus, the south-east of Côte d'Ivoire is marked by coastal inland lagoons [27]. The southern region, especially the south-west, is covered with dense tropical rainforest. The Guinean forest-savannah mosaic belt extends across the middle of the country from east to west and the northern part belongs to the Sudanian savannah. All these ecological conditions contribute to the proliferation of many species of mosquitoes responsible for malaria transmission malaria across the country [28]. This contributes to the maintenance of malaria transmission throughout the year, with peaks during the rainy season [29]. Identification of these species and their involvement in malaria transmission in these different ecological zones by molecular methods could provide essential information for use in the planning and implementation of vector control measures. However, data are still lacking on disease transmission dynamics in cross-border areas. This study was at assessing the species diversity of *Anopheles* mosquito vectors, *An. gambiae* complex members and transmission of malaria in four cross-border areas of Cote d'Ivoire, namely Aboisso, Bloléquin, Odienné and Ouangolodougou.

Methods

Study sites

This study was conducted in the four health district of Côte d'Ivoire. The health districts are namely Aboisso (5° 28' N, 3° 12' W) and Bloléquin (6° 34' N, 8° 00' W) Odienné (9° 30' N, 7° 33' W) and Ouangolodougou (9° 58' N, 5° 09' W) (Figure 1).

The district of Aboisso is located in the primary rainforest of Côte d'Ivoire on southwestern border with Ghana. The climate is humid tropical type, characterized by abundant rainfall with an average annual height of about 1,500 mm of rain. The average annual temperatures are between 25 and 27 °C. This district covers an area of over 4,662 km² with a population size of 307,852 people and, thus, a density of 66 inhabitants per km². Within this district, *Anopheles* mosquitoes were collected in the villages of Affiénou (5° 25' N; 2° 56' W) and Appouesso (5° 57' N; 3° 10' W). Both villages are drained by numerous streams and lowlands, and have modern and traditional housing, a health centre, electricity and water supply. Coffee, cocoa, rubber and palm oil are the main cash crops while vegetable, taro and banana are the main food crops in the area.

The district of Bloléquin is situated in the dense forest zone in the west of Côte d'Ivoire on the border with Liberia. The population is estimated at 123,336 inhabitants. The climate is mountainous type, with annual average rainfall sometimes exceeding 2,000 mm per year and annual temperatures ranging from 15 to 33 °C. It covers an area of 2,962 km^2 with a population density of approximately 41 inhabitants per km^2. The district is irrigated by important tributaries coming from the Sassandra and Cavally rivers, favouring the establishment of various permanent water courses, puddles and small dams. The study sites in this district covered the villages of Zéaglo (6° 34' N, 7° 47' W) and Dépouta (6° 54' N, 7° 57' W). Only Zéaglo has a health centre, electricity, water supply and modern housing. The agricultural activity is mainly based on coffee, cocoa and rubber culture. Food crops are dominated by banana, cassava, maize and vegetables, and included flooded lowland rice paddy and rainfed rice cultivation.

The district of Odienné is in the savannah zone in north-west of Côte d'Ivoire and shares a border in its western part with the Republic of Guinea. It covers an area of 14 000 km² with a population of 193,364, giving a density 13.8 inhabitants per km². The climate is tropical sub-humid type with annual rainfall varying between 1,400 and 1,600 mm per year and annual temperatures ranging between 25.4 and 33 °C. The vegetation is essentially dominated by savannah type vegetation, with trees or shrubs with a grassy tendency. In some places there are patches of forest and also forest galleries along the watercourses**.** The district is fed by tributaries of the Sassandra river such as the Bagoué and Tienba rivers. Our surveys were conducted in Gbéléban (09° 36' N, 08° 08' W) and Niénésso (09° 21' N, 07°36' W). Gbéléban is bordered to the south by the Gbanala river and has modern housing, a health centre, electricity and water, while Niénésso has no health centre and is bordered to the east by an undeveloped lowland which provides watering for cattle. Most of the local inhabitants are farmers and their staple crops include cereals, tubers, cotton and cashew nuts.

The health district of Ouangolodougou is in the savannah zone in the north of Côte d'Ivoire. It is bordered in the northern part by Burkina Faso and covers an area of 5,380 km^2, with an estimated population of 260,519 habitants, giving a density of 48.4 inhabitants per km^2. The district is characterized by a Sudanese climate with a unimodal rainfall regimen from May to November. The annual rainfall varies from 1000 to 1400 mm, while the mean annual temperature ranges from 21 to 35 °C. The minimum temperatures can drop to 16 °C due to the Harmattan wind during December and January. The natural vegetation is mainly a mixture of savannah and open forest characterized by trees and shrubs that are approximately 8–15 m in height. The soil is highly conducive to agriculture and most of the local inhabitants are farmers with staple crops including rice, maize, and cotton. Rice is mainly cultivated during the rainy season in flooded soils. The study area included the villages of Broundougou (9° 59' N; 05°09'95"W) and Satolo (10° 10' N; 05°27'W). Broundougou has a health centre, electricity, water supply and modern houses, whilst Satolo has no modern infrastructure.

Before this study, we explored the local malaria prevalence from 2013 to 2015 recorded in the yearbooks in each district to select the study sites. The prevalence of malaria varied from 22.5% to 23.5% in Aboisso and was estimated to be between 16.8% and 19.5% in Ouangolodougou. The prevalence varied from 38.2% to 41.7% in Bloléquin and 41.2% to 42.9% in Odienné.

Study design

Entomological surveys were conducted in two villages in each of the four cross-border health districts of Côte d'Ivoire identified above. We conducted the first phase of entomological collections in July-December 2016 and a second phase of collections during July-December 2017 to capture seasonal variations in mosquito species diversity and abundance and malaria transmission.

Adult mosquito collections

Adult mosquitoes were sampled from July 2016 to December 2017 using window exit traps (WETs) and pyrethrum knock-down spray collections (PSCs). In each site, 15 WETs were installed on the windows of inhabited houses for two consecutive days per survey. Mosquitoes in the traps were collected every morning between 6 a.m. and 9 a.m. PSCs were performed early each morning from 6 a.m. and 8 a.m., before opening the windows, in ten rooms selected in different dwellings during two days per site per district. PSCs were performed in households that were different from those used for WET collections. In case of unavailability or refusal of participants, mosquitoes were collected from neighbouring houses.

Field mosquito processing

Anophelines were sorted from collected culicines using morphological identification keys [30] Anopheline species were then determined morphologically [31]. We dissected the ovaries of the females of *Anopheles* vectors (*An. gambiaes.l.*, *An. funestus*, and *An. nili*) and observed the degree of coiling of ovarian tracheoles to determine their parity status [32]. All collected anopheline females were stored individually in Eppendorf tubes containing desiccant, labelled with the study site, point and date of collection, and stored at -20 °C for further molecular analysis in the laboratory at the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire in Abidjan, Côte d’Ivoire.

Molecular identification of *Anopheles gambiae* complex members

DNA was extracted from the legs of *An. gambiae* mosquitoes using the boiling preparation method**s** [33]. Briefly, three legs of each female *Anopheles* were cut and crushed in 100 mL of distilled water and boiled at 95°C for 10 min. The supernatant was pipetted and transferred into new labelled Eppendorf tubes which were stored at -20 °C and used as template for the polymerase chain reaction **(**PCR**)**.

An. gambiae complex members were identified according to the SINE-PCR molecular method described by Santolamazza *et al*. [34]. The primer F6.1A with the sequence 5'-TCGCCTTAGACCTTGCGTTA-3' was used to distinguish *An. coluzzii* and the primer R6.1B with the sequence 5'-CGCTTCAAGAATTCGAGATAC-3' to distinguish *An. gambiae* s.s. A LongGene® thermocycler (A200 Gradient Thermal cycler; LongGene Scientific Instruments Co., Ltd Hangzhou, P.R. China) was used with the following programme: 37 °C for 30 min, 94 °C for 30 s, and 59 °C for 30 s; 72 °C for 1 min repeated 35 times; and a final step at 72 °C for 10 min to finish the reaction. An agarose gel was prepared with 2% agarose in TBE (Tris/borate/EDTA) containing ethidium bromide at 10 mg/ml. The PCR product was loaded onto the agarose gel and allowed to migrate under a voltage of 100 V for 70 min. The result was visualized with a UV illuminator (TOYOBO Trans Modele TM-20).

Determination of sporozoite rates in *Anopheles gambiae*

DNA was extracted from the head and thorax of the adult females of *Anopheles* vector mosquitoes in each district [35] and screened for *Plasmodium* DNA using the fast COX-I PCR method described in Echeverry *et al*. [36]. This is a very sensitive and rapid method which uses a set of primers, COX-IF (5'AGAACGAACGCTTTTAACGCCTG 3') and COX-IR (3' ACTTAATGGTGGAT ATAAAGTCCATCCwGT 5'), to amplify a polymorphic fragment of the COX-(I) gene using a recombinant DNA polymerase. The thermocycler (TaKaRa PCR Thermal Cycler Dice TP600) program was 94 °C for 5 min followed by 40 cycles of 94 °C for 1 min, 62 °C for 1 min and 72 °C for 90 s, and a final elongation step at 72 °C for 10 min. Five microlitres of the PCR product was visualized on 1% agarose gel in order to confirm amplification of the expected ~540 bp product (*Plasmodium* genus positive).

Malaria infection prevalence

In each health district, data on the malaria prevalence in the local populations were collected from the MoH statistical yearbooks. The total number of consultations was compared with the number of people examined with malaria from 2016 to 2017.

Data analysis

Data were double entered in Microsoft Excel 2013 software and transferred to STATA 14 (Stata Corp, College Station, Tx, USA) for analysis. The Kruskal–Wallis (KW) test was used to compare the differences in mosquito densities. The parity rate (PR) was calculated as the number of parous females multiplied by 100 and divided by the total number of females dissected. The *P. falciparum* sporozoite infection rate in each vector species population was calculated by dividing the number of *Plasmodium*-positive mosquitoes by the total number of mosquitoes tested, and this was expressed as a percentage (%). The χ^2 test was used to compare sporozoite and PR between the collection sites and the health districts. All differences were considered significant at $p < 0.05$.

Results

Mosquito species composition

Table 1 shows the species composition of vector species among the anopheline fauna collected in the cross-border health districts of Aboisso, Bloléquin, Odienné and Ouangolodougou. During the 1-year study, a total of 4,797 *Anopheles* female adults were collected by WET and PSCs. Of these, *An. gambiae*, *An. funestus* and *An. nili* accounted for 99.4% of anophelines collected. In Aboisso, Bloléquin, Odienné and Ouangolodougou districts, these three *Anopheles* species together accounted for 100% (n = 281), 99.2% (n = 748), 99.3% (n = 1,309) and 99.5% (n = 2,431) of anopheline fauna, respectively. *An. gambiae s.l.* was predominant in all study sites, with particularly higher abundance in the northern districts of Odienné and Ouangolodougou in savannah area compared to the districts located in forest area (χ^2 = 22.85; df = 4, $p < 0.001$). *An. funestus* was found in relatively low in abundance in three of the four districts: Bloléquin 4.3% (n = 754); Odienné 1.8% (n = 1,319); Aboisso 0.7% (n = 281); and Ouangolodougou 0.4% (n = 2,443). *An. nili* was not found in Ouangolodougou, where *An. gambiae s.l.* was found in high abundance, but collected as a very low proportion in the three other districts.

Table 1
Species composition of Anopheles mosquitoes collected in four cross-border districts of Côte d'Ivoire from July 2016 to December 2017

Species	Aboisso		Bloléquin		Odienné		Ouangolodougou		Total	
	n	%	n	%	n	%	n	%	n	%
Anopheles gambiae	277	98.6	715	94.8	1,282	97.2	2,420	99.1	4,694	97.9
Anopheles funestus	2	0.7	32	4.3	23	1.8	11	0.4	68	1.5
Anopheles nili	2	0.7	1	0.1	4	0.3	0	0	7	0.1
Anopheles coustani	0	0	0	0	0	0	7	0.3	7	0.1
Anopheles pharoensis	0	0	1	0.1	3	0.2	2	0.1	6	0.1
Anopheles. cinctus	0	0	0	0	3	0.2	3	0.1	6	0.1
Anopheles domicolus	0	0	1	0.1	4	0.3	0	0	5	0.1
Anopheles paludis	0	0	4	0.6	0	0	0	0	4	0.1
Total	281	100	754	100	1,319	100	2,443	100	4,797	100

n: number, %: percentage

Distribution and parity rate of *Anopheles gambiae* s.l. in the four districts

Anopheles mosquito density varied significantly from one collection site to another (KW = 11.03, df = 7, p = 0.012). The highest *Anopheles* density was observed in Ouangolodougou (16.7 females/house/day (f/h/d)) and the lowest density in Aboisso (1.9 f/h/d). The average densities observed in the collection of Aboisso and Bloléquin sites were similar, being 0.9 f/h/d and 2.5 f/h/d, respectively. These densities were lower than those for Odienné (4.7 f/h/d) and Ouangolodougou (8.3 f/h/d). *Anopheles* mosquito densities in the four districts were significantly different (KW test = 7.06; df = 3; p = 0.002).

Table 2 presents the parity rates of *An. gambiae s.l.* collected in the four cross-border health districts. The majority of *An. gambiae s.l.* dissected were old with an average parity rate of 56.7 (n = 4,651). The highest parity rate was observed in *An. gambiae s.l.* in Odienné (69.0%; n = 1,258), followed by Aboisso (63.8%; n = 276), Bloléquin (51.1%; n = 735), and Ouangolodougou (49.2%; n = 2,382). The proportions of parous and nulliparous females of *An. gambiae* s.l. were significantly different in Odienné (χ^2 = 6.46; df = 1; p = 0.01), and Ouangolodougou (χ^2 = 15.30; df = 1; $p < 0.001$). Significant variation was observed in *An. gambiae* parity rates between health districts ($p < 0.001$).

Table 2
Parity rates of Anopheles gambiae s.l. collected in four cross-border districts of Côte d'Ivoire from July 2016 to December 2017

Districts	Total dissected	No. parous	Parity rate		p
	n	n	(%)	95%CI (%)	
Aboisso	276	176	63.8	[57.7–69.4]	0.44
Bloléquin	735	420	57.1	[53.4–60.7]	0.78
Odienné	1,258	868	69.0	[66.3–71.5]	0.01*
Ouangolodougou	2,382	1,172	49.2	[47.1–51.2]	<0.001*
Total	4,651	2,636	56.7	[56.1–63.2]	0.31
n: number, CI: confidence interval, *: significant difference, *p* = p-value					

Distribution of molecular forms of *An. gambiaes.l.* species.

Figure 2 illustrates the different molecular forms recorded in the study areas within the health districts of Aboisso, Bloléquin, Odienné and Ouangolodougou. All *An. gambiae s.l.* specimens analyzed were *An. gambiae s.s*. molecular forms M (*An. coluzzii*) or S (*An. gambiae s.s.*). However, the proportions of the molecular forms (M form and S form) substantially varied among the health districts. The M forms predominated in the health districts of Aboisso and Bloléquin located in the forest zone exhibiting respective proportion of 89.2% (n = 65) and 92.2% (n = 115) while the S forms exhibited a high frequency in the districts of Odienné (96.0%; n = 100) and Ouangolodougou (94.2%; n = 120) located in the savannah zone.

Plasmodium infection rate in *Anopheles* mosquito species

Table 3 exhibits the *P. falciparum* sporozoite rates detected in the *Anopheles* species in the cross-border districts of Aboisso, Bloléquin, Odienné and Ouangolodougou. Only *An. gambiae* and *An. funestus* was found to be infected with *P. falciparum* sporozoites, while no *P. falciparum* sporozoites being detected in the collected *An. nili* specimens. In total, 29 mosquitoes were found infected and the infection rate of malaria vectors ranged from 2 to 10%. Overall *P. falciparum* sporozoite rate was 3.1% (n = 65, %CI: 0.4–10.7) in Aboisso, 7.8% (n = 115, %CI: 3.6–14.3) in Bloléquin, 11.0% (n = 100, %CI: 5.6–18.8) in Odienné and 3.3% (n = 120, %CI: 0.9–8.3) Ouangolodougou. There was no significant difference in *P. falciparum* sporozoite rate in *An. gambiae s.l.* between the cross-border health districts ($\chi^2 = 7.33$; d f = 3, p = 0.065). In *An. funestus*, the *P. falciparum* sporozoite rate was 6.2% (2/32, %CI: 0.7–20.8) in Bloléquin and 8.7% (2/23, %CI: 1.1–28.0) in Odienné, but *P. falciparum* sporozoites were note detected in this species in Aboisso (0/2) or Ouangolodougou (0/11).

Table 3
Plasmodium falciparum sporozoite rates in Anopheles mosquito species collected in four cross-border districts of Côte d’Ivoire from July 2016 to December 2017

Species	Aboisso		Bloléquin		Odienné		Ouangolodougou	
	No. tested (infected)	SR (%) CI (95%)	No. tested (infected)	SR (%) CI (95%)	No. tested (infected)	SR (%) CI (95%)	No. tested (infected)	SR (%) CI (95%)
An. gambiae s.l.	65 (2)	3.1 [0.4–10.7]	115 (9)	7.8 [3.6–14.3]	100 (11)	11.0 [5.6–18.8]	120 (3)	2.5 [0.5–7.1]
An. funestus	2 (0)	0.0 [0.0-84.2]	32 (2)	6.2 [0.7–20.8]	23 (2)	8.7 [1.1–28.0]	11 (0)	0.0 [0.0-28.5]
An. nili	2 (0)	0.0 [0.0-84.2]	1 (0)	0.0 [0.0-97.5]	4 (0)	0.0 [0.0-60.2]	0.0 (0)	0.0 [0–0]
Total	69 (2)	2.9 [0.3–10.1]	148 (11)	7.4 [3.8–12.9]	127 (13)	10.2 [5.6–16.9]	131 (3)	2.3 [0.5–6.5]

No. tested = Number of mosquitoes tested, SR = Sporozoite rate, % = Percentage, CI = Confidence interval

Malaria prevalence among local human populations

Figure 3 indicates the malaria prevalence rate among people in the four cross-border districts from 2016 to 2017. The prevalence rate was high in Odienné (39.7%; n = 45,376) and Bloléquin (37.6%; n = 150,205) health districts, compared with Ouangolodougou (18.3%; n = 131,629), and Aboisso (19.7%; n = 364,585).

Discussion

Our study aiming to assess *Anopheles* mosquito vectors and malaria transmission in cross-border health district of Côte d’Ivoire is a prerequisite not only for understanding the epidemiology of the disease, but also for developing an effective and targeted vector control strategy [37]. In this study, we have highlighted the relative diversity and abundance of *Anopheles* mosquitoes in four cross-border districts of Côte d'Ivoire and assessed their implications in malaria transmission to local communities. Our results have shown that *An. gambiae* s.l. was most the abundant vector of malaria in the four districts. Moreover, *Plasmodium* sporozoite infection rate in *An. gambiae s.l*. and *An. funestus* was higher in Odienné (11.0% and 8.27%, respectively) and Bloléquin (7.8% and 6.2%, respectively), compared with Aboisso (3.1% and 0%, respectively) and Ouangolodougou (2.5% and 0%, respectively). Similarly, malaria prevalence rate among people in Odienné (39.7%) and Bloléquin (37.6%) health districts was higher compared with that in Ouangolodougou (18.3%), and Aboisso (19.7%) health districts. These observations may not only inform future research perspectives but should hopefully also guide future decision making on malaria control strategies, especially in these cross-border areas of Côte d'Ivoire.

This study recorded a high diversity of *Anopheles* mosquito species with seven *Anopheles* species identified, among which three (*An. gambiae*, *An. funestus* and *An. nili*) have previously been incriminated in malaria transmission in Côte d'Ivoire [22, 38]. Moreover, *An. gambiae s.l.* was the predominant species in all study sites, with particularly higher abundance in the northern health districts of Ouangolodougou and Odienné located in savannah zones compared with the districts of Aboisso and Bloléquin situated in forest zones. The high diversity and variation in the relative abundance of *Anopheles* mosquito species might result from a combination of ecological and climatic factors favouring the larval development of some species. Indeed, permanent water courses, puddles and small dams are probably abundant in these areas [21, 39].

The diversity and variation in *Anopheles* mosquito species composition may be related to human activities (e.g., rice farming, vegetable crops, etc.) and with vector control interventions, as well [40, 41]. *An. gambiae* s.l. breeds generally in temporarily sunny freshwater and permanent water such as rice paddies [42–43]. Breeding of *An. funestus* is typically associated with permanent and semi-permanent water bodies with emergent vegetation [44], with larvae being found in marshes, large sunny ponds, lake shores and rice fields. The preferred habitats of *An. nili* are fast-flowing streams, large rivers or in dense shade along streams [41]. The relative abundance of *Anopheles* recorded in savannah areas could be due to the vegetation and with frequency of the rains with the small, uncovered puddles being present at the onset of the rainy season, especially near dwellings, which are the preferred breeding sites for *Anopheles*. Odienné and Ouangolodougou are rural districts in which several crops are cultivated including rice, maize, yam, vegetable crops, cashew nuts and cotton. Rice paddies and vegetable crops were strongly associated with high densities of malaria vectors [45, 46]. In addition, we found that *An. gambiae s.l.* collected exhibited high parity rates in all districts, thus suggesting that a significant proportion of the local vector populations that have sufficient lifespan allowing for the completion of *Plasmodium* parasite lifecycle and transmission to humans. Similar findings have previously been reported in Côte d'Ivoire [46, 48].

This study has shown that *An. coluzzii* (previously the M-form of *An. gambiae*) was the dominant species in forest area, whilst *An. gambiae s.s.* (previously the S-form of *An. gambiae*) was the dominant species in savannah. The relative dominance of these two species may be associated with specific and characteristic breeding sites. The presence of *An. gambiae s.s.* and *An. coluzzii* has been previously reported in Côte d'Ivoire [21, 49]. The abundance of *An. coluzzii* in our samples from forest areas could be related to the type of breeding sites and the climatic conditions in these study sites [50–51]. Several studies carried out in Côte d'Ivoire have shown a predominance of *An. coluzzii* in forest area especially in the western [22] and south-eastern [52] parts. The highest abundance of *An. gambiae s.s.* was observed in the savannah area where relatively abundant precipitation provides more favorable humidity and temperature conditions [53, 54]. The predominance of *An. gambiae s.s.* in savannah zones has been observed by Touré *et al.* [55] and Tia *et al.* [49], suggesting that environmental conditions in savannah zones are unfavourable for the reproduction and the survival of *An. coluzzii*.

Our improved understanding of malaria transmission at the local level is essential for the development and implementation of effective vector control strategies. Thus, to identify potential vector species of malaria in our various study sites, individual females of *An. gambiae* were tested using molecular PCR for the presence of *P. falciparium* sporozoites. The finding showed that only two species were involved in malaria transmission across the four cross-border health districts, with *An. gambiae s.l.* being the main malaria vector in all districts. In addition, *An. gambiae s.l*, and *An. funestus* were efficient vectors of *P. falciparum* in these two cross-border districts. This observation is consistent with findings from previous studies showing that the high capacity of malaria vectors ensures high transmission in an ecological area is intimately related with environmental conditions [21, 22]. Although infection rates were almost similar between study sites, the intensity of transmission was very heterogeneous. Malaria transmission is lower in Aboisso and Ouangolodougou health districts. On contrary, it remains relatively high in Bloléquin and Odienné districts where two additional Anopheles vectors are involved in malaria transmission. This high transmission of malaria could possibly be due to the high infection rate of malaria vectors and the lack, failure or limited impacts of vector control measures in these districts. In addition, the presence of several vectors in the same area could significantly increase the risk of malaria transmission. Our study suggests that malaria vector control interventions should be strengthened in Bloléquin and Odienné in order to reduce or eliminate the burden of malaria in these districts.

The low prevalence of malaria observed (< 20%) in the health districts of Aboisso and Ouangolodougou suggests that both districts are in areas of moderate transmission [56]. In contrast, high prevalence rates of *P. falciparum* infections (ranging from 38.6–39.7%) in Bloléquin and Odienné may imply that these districts are areas of high malaria transmission. This variation of *Plasmodium* transmission from one health district to another may be related to climatic conditions. Several previous studies have shown that *Plasmodium* infections are influenced by environmental factors such as temperature, rainfall, humidity and altitude [57, 58]. These factors directly or indirectly influence the development and appearance of *Anopheles* mosquitoes and, thus, the geographical distribution of the malaria infection and disease. The heterogeneity of malaria transmission observed in the present study is consistent with previous studies [27, 38]. This may also be the effect of differences in intervention strategies coordinated by the National Malaria Control Programme (NMCP), especially free distribution of LLINs to vulnerable population and the use of ACTs for the early treatment of malaria cases.

Despite repeated mass distribution of LLINs, malaria transmission remains high and heterogeneous across cross-border health districts of Côte d'Ivoire with the presence of several vectors species: *An. funestus, An. nili* and *An. gambiae s.l.* The latter is the most species encountered. Our study showed a high diversity and abundance of *Anopheles* mosquitoes, which could contribute to malaria transmission persistence over time. Moreover, high sporozoite infection and parity rates were recorded in all four heath districts and highlighted the high transmission of malaria within local populations. These finding suggest either that the LLINs distributed are not being used properly by the populations or the local vector populations are developing resistance to the insecticides use in the LLINs. Currently, the vector control strategy of NMCP of Côte d'Ivoire is based on LLIN distribution. Recently in August 2020 the MoH have experimented indoor residual spraying (IRS) in pilot site (Sakassou). LLINs are already proved is efficacity in personal and community protection against malaria [59, 60]. However, in the recent past, pyrethroids were the single insecticide class used for impregnation of LLINs, owing to their rapid action, excito-repellent effects, effectiveness at low doses and low toxicity to humans [61]. Unfortunately, pyrethroid resistance in malaria vectors has emerged and spread rapidly in Côte d'Ivoire [62] and several parts of Africa [63–65]. Therefore, efforts should be made to evaluate the effectiveness of insecticide-treated LLINs with different modes of action to which there is no cross-resistance and to evaluate promising tools to be used in combination with LLINs in highly endemic areas. Since mosquito capture has been done indoors, it is important to rapidly roll out the implementation of IRS nationally using next generation formulations, which has already proven its effectiveness in combination with LLIN [66].

Conclusion

The specific richness and abundance of anopheline vectors were high in the cross-border health districts of Côte d'Ivoire. *Plasmodium* sporozoite infection rates were also high, leading to continued and high transmission of malaria in local populations. *An. gambiae s.l.* was the main malaria vector, with *An. funestus* playing a secondary vector role in the health districts of Bloléquin and Odienné. Malaria transmission remained high in the health districts of Odienné and Bloléquin where two additional vectors were involved, compared to the districts of Aboisso and Ouangolodougou. *An. Coluzzii* was most common in forest areas while *An. gambiae s.s.* predominated in the savannah areas of Côte d'Ivoire. Therefore, it is necessary to regularly monitor the bionomics of local *Anopheles* vector species and follow up possible change in malaria transmission dynamics to strengthen national vector control strategies taking into account the cross-border regions of Côte d'Ivoire.

Abbreviations

ACT: Artemisinin-based combination therapy; COX-I: Cytochrome oxidase I; CSRS: Centre Suisse de Recherches Scientifiques; DNA: deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; IRS: Indoor residual spraying; KW: Kruskal–Wallis, LLINs: Long-lasting insecticidal nets; MoH: Ministry of Health, NMCP: National Malaria Control Programme; PCR: Polymerase chain reaction; PR = Parity rate; PSC: Pyrethrum spray collection; SINE-PCR: Short interspersed Nuclear element-based polymerase chain reaction; TBE: Tris Borate EDTA; UV: Ultraviolet; WET: Window exit traps.

Declarations

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Authors' contribution

FNY, JBZZ and BGK designed surveys. FNY and JBZZ carried out the surveys. FNY, MO and DKdS conducted PCR assays. AFO analysed data. FNY drafted the manuscript. JBZZ, SG, AFO and BGK critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this article and are available from the corresponding author.

Ethics Statement

Ethical clearance for this study was obtained from the National Ethics Review Committee of Côte d'Ivoire (001//MSHP/CNER-kp). This study also received an approval from the health authorities of each district. In each study area, permission to work was granted by the community leaders or chiefs of villages. Community members and house owners get clear information on objectives, procedures, potential risks of harm and benefits related to the study and then oral informed consent and permission to conduct activities was provided. Participation in mosquito collection was strictly based on volunteering and all collectors were trained on the specific mosquito collection methods prior to initiation of the study.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. World malaria report 2019. Geneva: World Health Organization; 2019. https://www.who.int/publications/i/item/world-malaria-report-2019.
2. Centers for Disease Control and Prevention in Côte d'Ivoire, 2020. https://www.cdc.gov/globalhealth/countries/cote-d-ivoire/pdf/Cote-D-Ivoire_Factsheet.pdf.
3. World Malaria Report 2016 Geneva, Switzerland, 2016. http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1.
4. Antonio-Nkondjio C, Ndo C, Njiokou F, Bigoga JD, Awono-Ambene P, Etang J, et al. Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination. Parasit Vectors. 2019;12(1):501.
5. Hoo R, Zhu L, Amaladoss A, Mok S, Natalang O, Lapp SA, et al. Integrated analysis of the *Plasmodium* species transcriptome. EBioMedicine. 2016;7:255–66.
6. Dayananda K, Achur R, Gowda Dc. Epidemiology, drug resistance, and pathophysiology of *Plasmodium vivax* J Vector Borne Dis. 2018;55(1):1–8.
7. 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(6):687–97.
8. 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(1):85.
9. Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, Besansky NJ. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* Zootaxa. 2013;3619:246–74.
10. Sinka ME, Golding N, Massey NC, Wiebe A, Huang Z, Hay SI, et al. Modelling the relative abundance of the primary African vectors of malaria before and after the implementation of indoor, insecticide-based vector control. Malar J. 2016;15(1):142.
11. 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(1): 152.
12. World Malaria Report 2018. Geneva: World Health Organization; 2018. https://apps.who.int/iris/bitstream/handle/10665/275867/9789241565653-eng.pdf.
13. Ong'wen F, Onyango PO and Bukhari T. Direct and indirect effects of predation and parasitism on the *Anopheles gambiae* Parasit Vectors. 2020;13(1): 43.
14. Kudom AA. Larval ecology of *Anopheles coluzzii* in Cape Coast, Ghana: water quality, nature of habitat and implication for larval control. Malar J. 2015.;14:447.
15. Getachew D, Balkew M and Tekie, H. *Anopheles* larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. Malar J. 2020;19(1): 65.
16. Mbida Mbida A, Etang J, Akono Ntonga P, Eboumbou Moukoko C, Awono-Ambene P, Tagne D et al. Nouvel aperçu sur l'écologie larvaire d'*Anopheles coluzzii* Coetzee et Wilkerson, 2013 dans l'estuaire

du Wouri, Littoral-Cameroun. Bull Soc de Pathol Exot. 2017;110:92–101.

17. Ossè RA, Bangana SB, Aïkpon R, Kintonou J, Sagbohan H, Sidick A, et al. Adaptation of *Anopheles coluzzii* larvae to polluted breeding Sites in Cotonou: A strengthening in urban malaria transmission in Benin. Vector Biol J. 2019;4:1.
18. Mourou J-R, Coffinet T, Jarjaval F, Cotteaux C, Pradines E, Godefroy L et al. Malaria transmission in Libreville: results of a one-year survey. Malar J. 2012;11:40.
19. Killen GF. Characterizing, controlling and eliminating residual malaria transmission. Malar J. 2014;13:330.
20. Deressa W, Loha E, Balkew M, Hailu A, Gari T, Kenea O, et al. Combining long-lasting insecticidal nets and indoor residual spraying for malaria prevention in Ethiopia: study protocol for a cluster randomized controlled trial. Trials. 2016;17(1):20.
21. Coetzee M, Craig M, le Sueur D. Distribution of African Malaria Mosquitoes Belonging to the *Anopheles gambiae* Parasitol Today. 2000;16(2):74-7.
22. Koffi AA, Alou LPA, J-PK K, N'Guessan R, Pennetier C. Re-visiting insecticide resistance status in *Anopheles gambiae* from Côte d'Ivoire: A nation-wide informative survey. PloS One. 2013;8:e82387.
23. Zogo B, Soma DD, Tchiekoi BN, Somé A, Ahoua-Alou LP, Koffi AA et al. *Anopheles* bionomics, insecticide resistance mechanisms, and malaria transmission in the Korhogo area, northern Côte d'Ivoire: a pre-intervention study. Parasit. 2019;26:40.
24. Adja AM, N'Goran EK, Koudou BG, Dia I, Kengne P, Fontenille D et al. Contribution of *Anopheles funestus*, *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(1):13–24.
25. Gbalegba TE, M'Bra NGC, Kaba KR, Boby A, Koné OAM, Chouaibou M, et al. Étude du niveau de production larvaire d'*Anopheles gambiae s.l.* (Diptera: Culicidae) dans différents types de gîtes à Oussou-yaokro au Centre-Ouest et à Korhogo, au Nord (Côte d'Ivoire). J App Bioscience. 2016;105(1):10170.
26. Edi CA, Koudou BG, Bellai L, Adja AM, Chouaibou M, Bonfoh B, et al. Long-term trends in *Anopheles gambiae* insecticide resistance in Côte d'Ivoire. Parasites Vectors. 2014 Dec;7(1):500.
27. Raso G, Schur N, Utzinger J, Koudou BG, Tchicaya ES, Rohner F et al. Mapping malaria risk among children in Côte d'Ivoire using Bayesian geo-statistical models. Malar J. 2012;11:160.
28. Fofana D, Koné AB, Konan YL, Konan KL, Doannio JMC, N'goran EK. Transmission des plasmodies humaines dans les villages de N'gatty et d'Allaba en milieu lagunaire de Côte d'Ivoire. Cahiers de Santé. 2010;20(2):73–9.
29. Texier G, Machault V, Barragti M, Boutin JP, Rogier C. Environmental determinant of malaria cases among travellers. Malar J. 2013;12:87.
30. Harbach R, 1988. The mosquitoes of the subgenus *Culex* in the Southwester Asia and Egypt (Diptera: Culicidae). Contrib Amer Ent Inst.; 24:240.

31. Gillies M and Coetzee M, 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region), Pub South Afr Inst Med Res.
32. Detinova TS, 1963. Méthodes à appliquer pour classer par groupes d'âges les diptères présentant une importance médicale. Notamment certains vecteurs du paludisme. WHO Monog Ser.; 47 :220.
33. de Souza DK, Koudou BG, Bolay FK, Boakye DA, Bockarie MJ. Filling the gap 115 years after Ronald Ross: the distribution of the *Anopheles coluzzii* and *Anopheles gambiae s.s* from Freetown and Monrovia, West Africa. PLoS One. 2013;8:e64939.
34. Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A, 2008. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J.; 7(1):163.
35. Obenauer PJ, Abdel-Dayem MS, Stoops CA, Villinski JT, Tageldin R, Fahmy NT, et al. Field responses of *Anopheles gambiae* complex (Diptera: Culicidae) in Liberia using yeast-generated carbon dioxide and synthetic lure-baited light traps. J Med Entomol, 2013; 50(4), 863-870.
36. Echeverry DF, Deason NA, Makuru V, Davidson J, Xiao H, Niedbalski J, et al. Fast and robust single PCR for *Plasmodium* sporozoite detection in mosquitoes using the cytochrome oxidase I gene. Malar J. 2017;16(1):230.
37. Fontenille D, Cohuet A, Awono-Ambene PH, Antonio-Nkondjio C, Wondji C, Kengne P, et al. «Systematics and biology of *Anopheles* vectors of *Plasmodium* in Africa, recent data». Méd Trop: Rev Du Corps De Santé Colonial. 2003;63(3): 247-53.
38. Assouho KF, Adja AM, Guindo-Coulibaly N, Tia E, Kouadio AMN, Zoh DD, et al. Vectorial Transmission of malaria in major districts of Côte d'Ivoire. J Med Entomol. 2020;57(3): 908-14.
39. Betsi AN, Tchicaya ES, Koudou BG. Forte prolifération de larves d' *gambiae* et *An. funestus* en milieux rizicoles irrigués et non irrigués dans la région forestière ouest de la Côte-d'Ivoire. Bull Soc Pathol Exot . 2012;105(3):220-9.
40. Ouldabdallahi Moukah M, Ba O, Ba H, Ould-Khairy ML, Faye O, Bogreau H, et al. Malaria in three epidemiological strata in Mauritania. Malar J. 2016;15:204.
41. Musiime AK, Smith DL, Kilama M, Rek J, Arinaitwe E, Nankabirwa JI, et al. Impact of vector control interventions on malaria transmission intensity, outdoor vector biting rates and *Anopheles* mosquito species composition in Tororo, Uganda. Malar J. 2019;18(1):445.
42. Carnevale P, Robert V, Manguin S, Corbel V, Fontenille D, Garros C, et al. Les anophèles - biologie, transmission du Plasmodium et lutte antivectorielle. IRD ed: Marseille. 2009;391 p.
43. Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2010;3(1):117.
44. Klinkenberg E, Takken W, Huibers F, Touré YT. The phenology of malaria mosquitoes in irrigated rice fields in Mali. Acta Trop. 2003;85:71-82.
45. Fodjo BK, Koudou BG, Tia E, Saric J, N'dri PB, Zoh MG. Insecticides resistance status of *gambiae* in areas of varying agrochemical use in Côte d'Ivoire. BioMed Research Inter. 2018;2018: 1-9.

46. Koudou BG, Adja AM, Matthys, Doumbia M, Cissé G, Koné M, et al. Pratiques agricoles et transmission du paludisme dans deux zones éco-épidémiologiques au centre de la Côte d'Ivoire. Bull Soc Pathol Exo. 2007;100(2):124-6.
47. Koudou BG, Tano Y, Doumbia M, Nsanzabana C, Cisse G, Girardin O et al. Malaria transmission dynamics in central Côte d'Ivoire: the influence of changing patterns of irrigated rice agriculture. Med Vet Entomol. 2005;19(1):27-37.
48. Diakité NR, Guindo-Coulibaly N, Adja AM, Ouattara M, Coulibaly JT, Utzinger J et al. Spatial and temporal variation of malaria entomological parameters at the onset of a hydro-agricultural development in central Côte d'Ivoire. Malar J. 2015;14: 340.
49. Tia E, Chouaibou M, Gblégba NGC, Boby OAM, Koné M, Kadjo KA. Distribution des espèces et la fréquence du gène Kdr chez les populations de *Anopheles gambiae s.s*. et de *Anopheles coluzzii* dans cinq sites agricoles de la Côte d'ivoire. Bull Soc Path Exc. 2017; 110:130-4.
50. Kamdem C, Tene Fossog B, Simard F, Etouna J, Ndo C, Kengne P, et al. Anthropogenic Habitat Disturbance and Ecological Divergence between Incipient Species of the Malaria Mosquito *Anopheles gambiae*. Pinto J, editor. PLoS One. 2012;7(6):e39453.
51. Etang J, Mbida Mbida A, Ntonga Akono P, Binyang J, Eboumbou Moukoko CE, Lehman LG, et al. *Anopheles coluzzii* larval habitat and insecticide resistance in the island area of Manoka, Cameroon. BMC Infect Dis. 2016;16(1):217.
52. Ahoua Alou LP, Koffi AA, Adja MA, Assi SB, Kouassi PK, N'Guessan R. Status of pyrethroid resistance in *Anopheles gambiae s. s*. M form prior to the scaling up of Long-Lasting Insecticidal Nets (LLINs) in Adzopé, Eastern Côte d'Ivoire. Parasit Vectors. 2012;5:289.
53. Depinay J-MO, Mbogo CM, Killeen G, Knols B, Beier J, Carlson J, et al. A simulation model of African *Anopheles* ecology and population dynamics for the analysis of malaria transmission. Malar J. 2004;3(1):29.
54. Coulibaly B, Koné R, Barry MS, Emerson B, Coulibaly MB, Niare O, et al. Malaria vector populations across ecological zones in Guinea Conakry and Mali. West Africa. Malar J. 2016;15:191.
55. Touré M, Carnevale P, and Chandre F. Impact retardé des moustiquaires imprégnées de lambdacyhalothrine sur la fréquence de la mutation kdr chez *Anopheles gambiae s.s*. (diptera: Culicidae) au nord de la Côte d'Ivoire. Bull Soc Path Exc. 2012; 105:305-10.
56. Rougier C, Henry MC, Trape JF. Evaluation épidémiologique du paludisme en zone d'endémie. Med Trop. 2009;69:123-42.
57. Stresman GH. Beyond temperature and precipitation: Ecological risk factors that modify malaria transmission. Acta Trop. 2010;116(3):167-72.
58. Lefevre T, Ohm J, Dabiré KR, Cohuet A, Choisy M, Thomas MB, et al. Transmission traits of malaria parasites within the mosquito: Genetic variation, phenotypic plasticity, and consequences for control. Evol Appl. 2017;11(4): 456-69.
59. Damien GB, Djènontin A, Chaffa E, Yamadjako S, Drame PM, Ndille EE, et al. Effectiveness of insecticidal nets on uncomplicated clinical malaria: a case–control study for operational evaluation.

Malar J. 2016;15(1):102.

60. Hounkonnou C, Djènontin A, Egbinola S, Houngbegnon P, Bouraima A, Soares C, et al. Impact of the use and efficacy of long lasting insecticidal net on malaria infection during the first trimester of pregnancy - a pre-conceptional cohort study in southern Benin. BMC Public Health. 2018;18(1):683.
61. Sovi A, Govoétchan R, Ossé R, Koukpo CZ, Salako AS, Syme T, et al. Resistance status of *Anopheles gambiae s.l.* to insecticides following the 2011 mass distribution campaign of long-lasting insecticidal nets (LLINs) in the Plateau Department, south-eastern Benin. Malar J. 2020;19(1):26.
62. Camara S, Koffi AA, Ahoua Alou LP, Koffi K, Kabran J-PK, Koné A, et al. Mapping insecticide resistance in *Anopheles gambiae (s.l.)* from Côte d'Ivoire. Parasit Vectors. 2018;11(1):19.
63. Aïkpon RY, Padonou G, Dagnon F, Ossè R, Ogouyemi Hounto A, Tokponon F, et al. Upsurge of malaria transmission after indoor residual spraying withdrawal in Atacora region in Benin, West Africa. Malar J. 2020;19(1):3.
64. Chukwuekezie O, Nwosu E, Nwangwu U, Dogunro F, Onwude C, Agashi N, et al. Resistance status of *Anopheles gambiae (s.l.)* to four commonly used insecticides for malaria vector control in South-East Nigeria. Parasit Vectors. 2020;13(1):152.
65. Wat'senga F, Agossa F, Manzambi EZ, Illombe G, Mapangulu T, Muyembe T, et al. Intensity of pyrethroid resistance in *Anopheles gambiae* before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. Malar J. 2020;19(1):169.
66. Oxborough RM, Seyoum A, Yihdego Y, Dabire R, Gnanguenon V, Wat'senga F, et al. Susceptibility testing of *Anopheles* malaria vectors with the neonicotinoid insecticide clothianidin; results from 16 African countries, in preparation for indoor residual spraying with new insecticide formulations. Malar J. 2019;18(1):264.

Figures

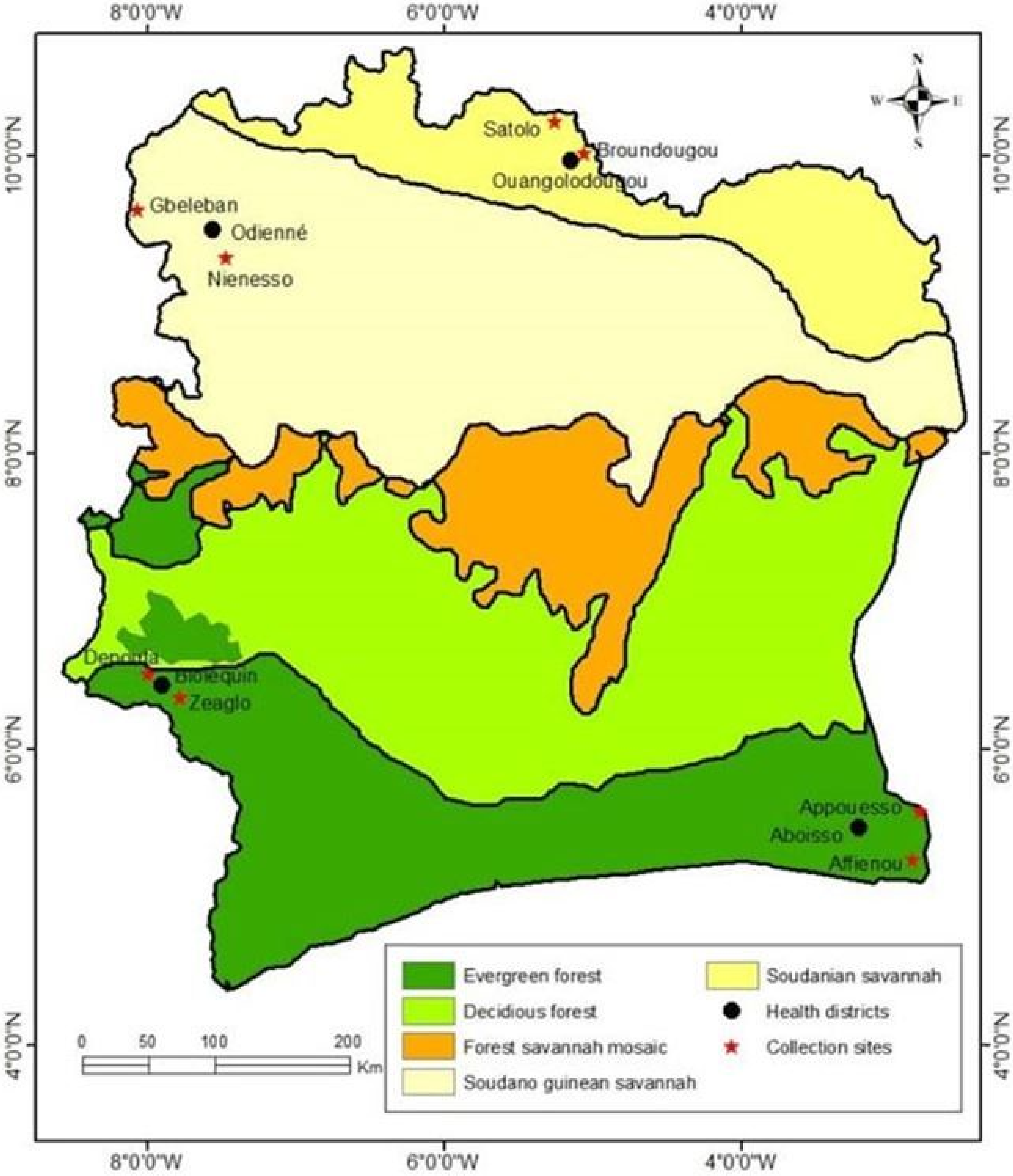

Figure 1

Map of Côte d'Ivoire showing the study sites in the cross-border health districts of Aboisso, Bloléquin, Odienné and Ouangolodougou

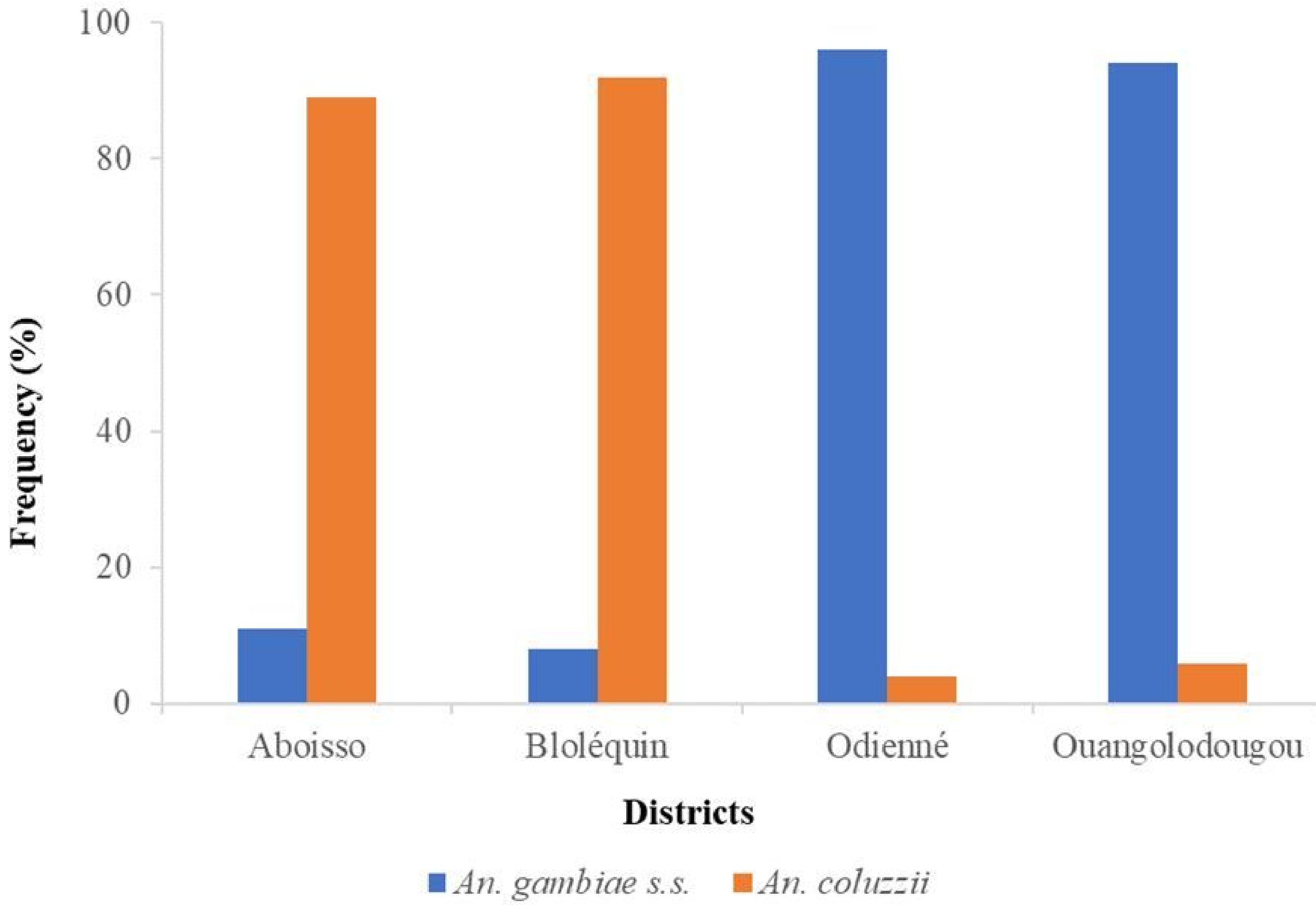

Figure 2

Distribution of members of Anopheles gambiae s.l. complex in four cross-border districts of Côte d'Ivoire from July 2016 to December 2017.

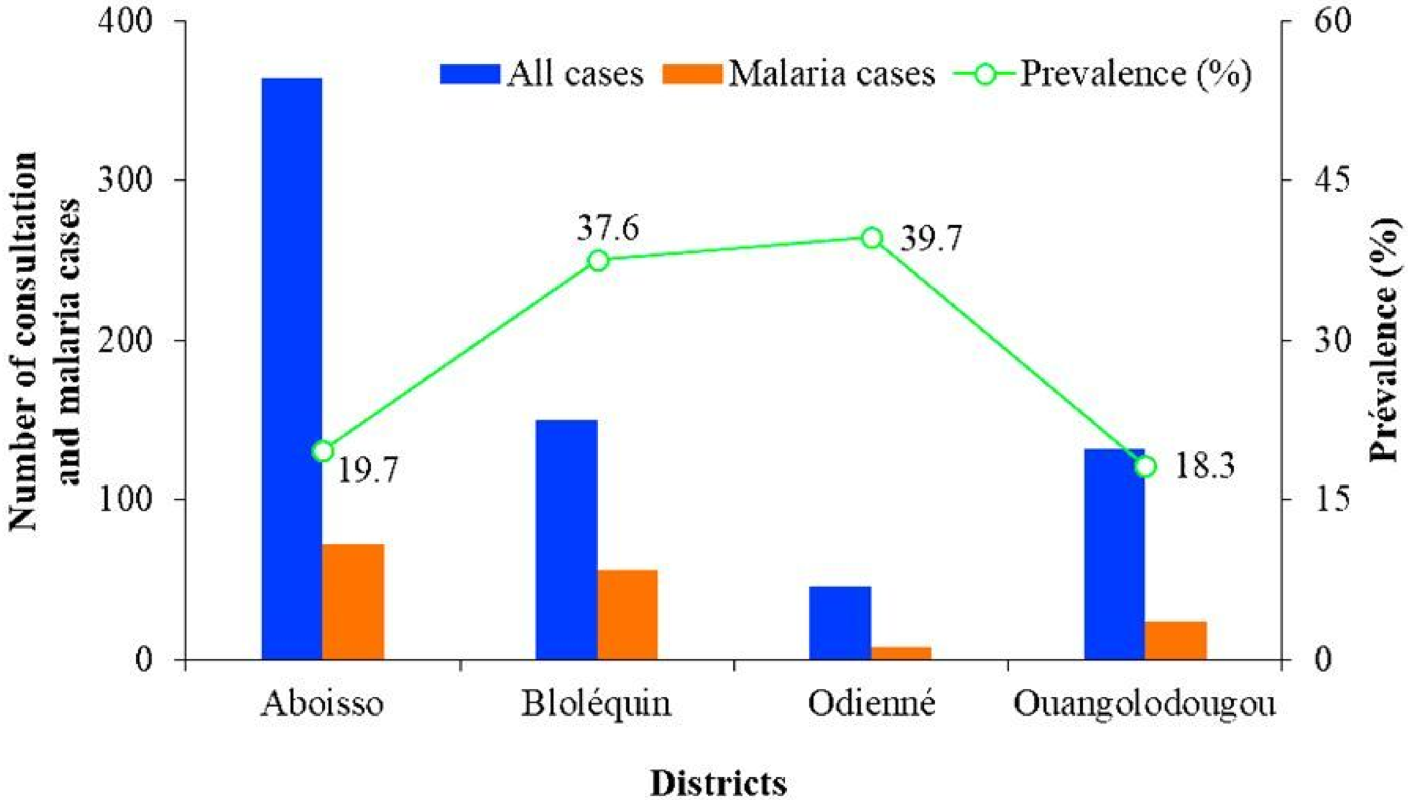

Figure 3

Prevalence of malaria among people in four cross-border districts of Côte d'Ivoire from July 2016 to December 2017.