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## Mosquito (Diptera: Culicidae) diversity and malaria prevalence in Kovié, prefecture of Zio, Togo

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### Abstract

Vector-borne diseases are diseases caused by parasites and viruses transmitted by vectors. Africa is home to a rich diversity of vector species which can transmit various diseases including malaria. Human adaptation strategies to climate change such as irrigation and the development of hydro-agricultural areas create a favorable environment that reinforces the development and proliferation of vectors, particularly Culicidae. To determine the composition of mosquito fauna and the malaria prevalence, series of larval surveys and adult mosquito collections (by Spray catch and Human Landin catch) were conducted in Kovié, a peri-urban locality located in the River Zio basin characterized with irrigated rice production. Results showed that the mosquito fauna at Kovié is primarily made up of Anophelinae (78%) and Culicinae (22%), belonging to 4 genera: *Mansonia*, *Aedes*, *Culex*, and *Anopheles*. The identification of 813 specimens of *Anopheles gambiae s.l.* using PCR revealed that *Anopheles coluzzii* was the only species collected. Epidemiological surveys in Kovié medical center showed that malaria was the only mosquito-borne disease recorded in the area. Malaria prevalence in the locality was more or less high among children under 5 years old and pregnant women.

**Keywords:** Kovié, mosquito fauna, PCR, malaria prevalence

### Introduction

Vector-borne diseases are infectious diseases transmitted by vectors which are mostly hematophagous or non-hematophagous arthropods that transmit pathogens, from one vertebrate to the other [1]. These arthropods are mainly insects and mites that transmit parasitic diseases such as malaria, Chagas disease, plague, dengue, chikungunya, Rift Valley fever, and West Nile disease. There is a diversity of vector species in Africa. These species can redistribute in new habitats created by microclimates which are generated by climate change [2]. This redistribution could lead to a new disease mapping. The most common vector-borne disease is malaria, which is caused by a blood parasite of the genus *Plasmodium* transmitted by mosquitoes of *Anopheles* genus. Globally, in 2019, malaria cases and deaths were approximately 229 million and 409,000, respectively [3]. Five *Plasmodium* species including *Plasmodium falciparum*, *P. malariae*, *P. vivax*, *P. ovale*, and *P. knowlesi* are present on all the continents. In 2014, reports indicated that in mosquito-infested areas, approximately 120 million people were infected with filaria, 50 million with arboviruses, and 198 million with plasmodium [4,5]. In many African countries, the food self-sufficiency ambition led to the development of hydro-agriculture including irrigated crops, especially rice [6]. Togo has many irrigated areas of which the most important is the irrigated rice-growing area of the Valley of Zio [7]. This zone includes four villages: Mission-Tové, Kovié, Assomé, and Ziovonou with a total surface area of 660 ha [8]. However, only 373 ha are developed for rice production which favors mosquito proliferation. This study is to investigate mosquito diversity and the diseases transmitted in rice-growing environment. The specific objectives are to list the different mosquito of medical importance, to identify malaria vectors, and to establish a link between the presence of malaria vectors and malaria prevalence.

## Materials and Methods

### Study site

The study was conducted in four villages of Kovié (Fig. 1), located at 30 km northwest of Lomé in the prefecture of Zio. Kovié (06°34'38"N–01°11'47"E) is a peri-urban area located in the valley of Zio in Togo. Geographically, this area presents a favorable ecosystem for the development of insect

vector species such as *Anopheles gambiae s.l.* and *Aedes sp.* Kovié enjoys a tropical climate with two dry seasons and two rainy seasons. The main dry season is from December to March and the short dry season from July to August. The main rainy season runs from late March to June and the short rainy season from mid-September to late October<sup>[8]</sup>. Irrigated rice cultivation is the main activity in this area<sup>[9]</sup>.

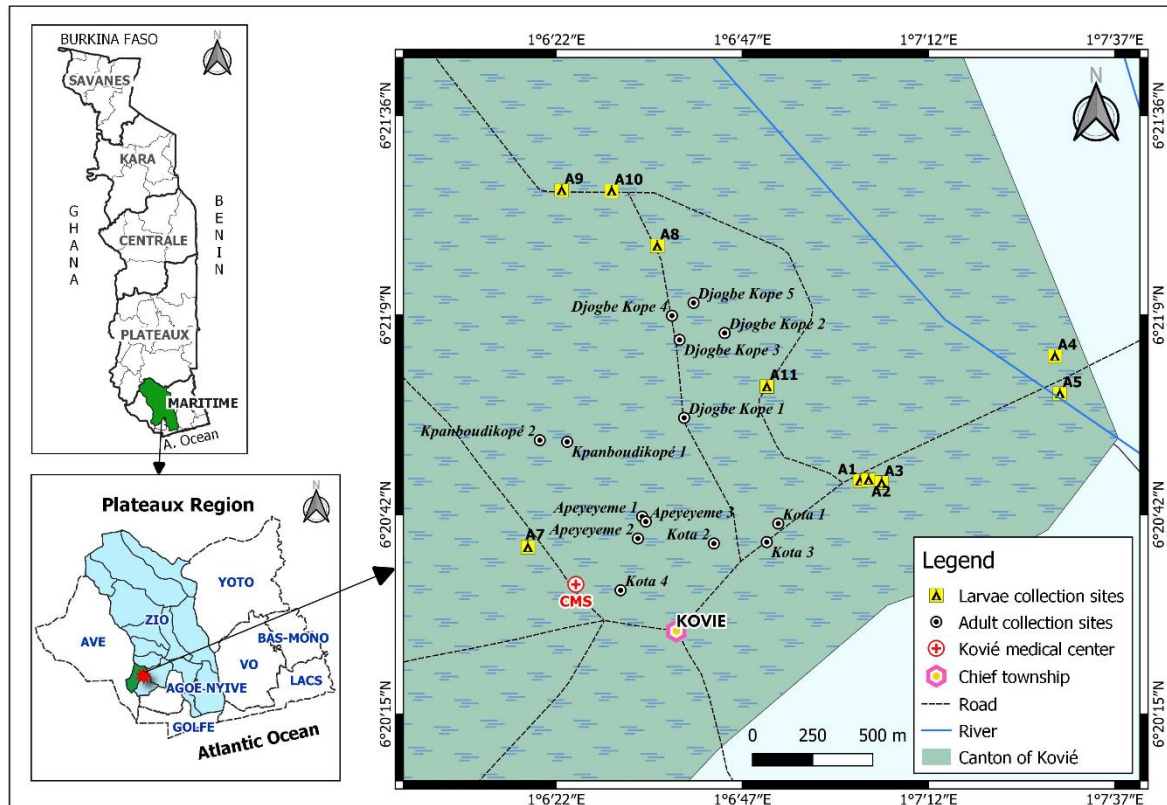


Fig 1: Map of the study site

### Mosquito collection and identification

Pre-imaginal mosquitoes were collected in gutters, rice fields, car tire pits, containers, and puddles in the neighborhoods of Kovié and especially in the rice-growing areas. Larvae and pupae were collected by dipping technique<sup>[10]</sup> in positive breeding sites. Collection was carried out twice a month for 4 months (June to September 2020). The larvae and pupae collected were sorted by genus and reared ( $T = 27 \pm 2^\circ\text{C}$ ,  $\text{RH} = 80 \pm 5\%$ ) to adulthood using the method of Desfontaine *et al.*<sup>[11]</sup> at the insectary of the Laboratory of Ecology and Ecotoxicology, University of Lomé. Adult mosquitoes were also collected using the methods of Human Landing Catch and Spray-catch, from September 14 to 17, 2020 in habitations where people sleep under mosquito nets in the study areas. Samples were then conditioned in Eppendorf tubes containing silicagel and kept at  $-4^\circ\text{C}$  and identified using the keys of Gillies & De Meillon<sup>[12]</sup>, Gillies & Coetzee<sup>[13]</sup> for Anophelinae, and Edwards<sup>[14]</sup> for Culicinae.

Sibling species of *Anopheles gambiae s.l.* were identified by Polymerase Chain Reaction-Restriction according to the protocol of Scott *et al.*<sup>[14]</sup>. DNA extraction was performed using the method of Collins *et al.*<sup>[16]</sup>, resuspended in sterile deionized water and kept at  $-80^\circ\text{C}$ . The following steps were undertaken to run the PCR according to the protocol of Favia *et al.*<sup>[17]</sup>: an initial step of 10 min at  $94^\circ\text{C}$  was followed by 25 cycles with denaturation at  $94^\circ\text{C}$  for 30 s, annealing at 63

$^\circ\text{C}$  for 30 s, and extension at  $72^\circ\text{C}$  for 30 s; the products were extended for 7 min at  $72^\circ\text{C}$ , after the final cycle.

### Epidemiological data collection

Epidemiological data were collected at the health center of Kovié. To identify the vector-borne diseases which were the primary cause of consultation in the local population, retrospective data from 2018 to 2020 were considered. We focused only on the data of thick blood smears results as reported in the medical registry of the health center.

### Statistical Analysis

The prevalence rate was calculated as the number of positive blood smears over the total number of blood smears. Data were processed with Excel 2010 (Microsoft Corp) spreadsheet and the analyses of variances (ANOVA) were performed with the PAST software version 3.23. The significance level was 5%.

## Results

### Mosquito diversity

A total of 1,038 mosquitoes belonging to two sub-families: Anophelinae, 78% and Culicinae, 22% (Fig. 2) were collected at Kovié. The two subfamilies are divided into four genera: *Anopheles*, *Culex*, *Aedes*, and *Mansonia* (Fig. 3). Only *Mansonia* was collected at adult stage and *Aedes* was at larval

stage. *Anopheles* and *Culex* were caught at both larval and adult stages.

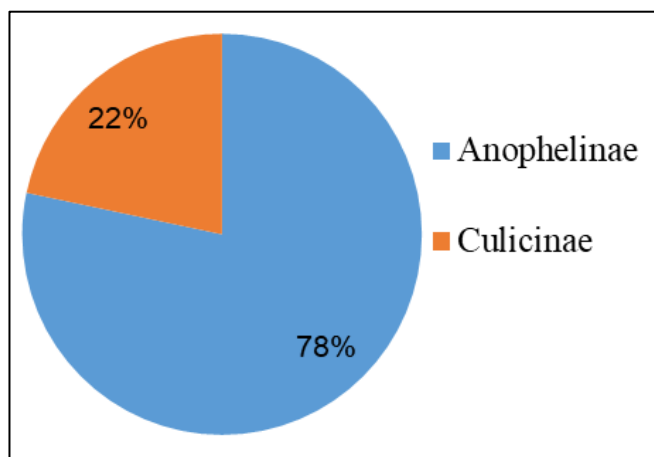


Fig 2: Distribution of mosquito sub-families identified

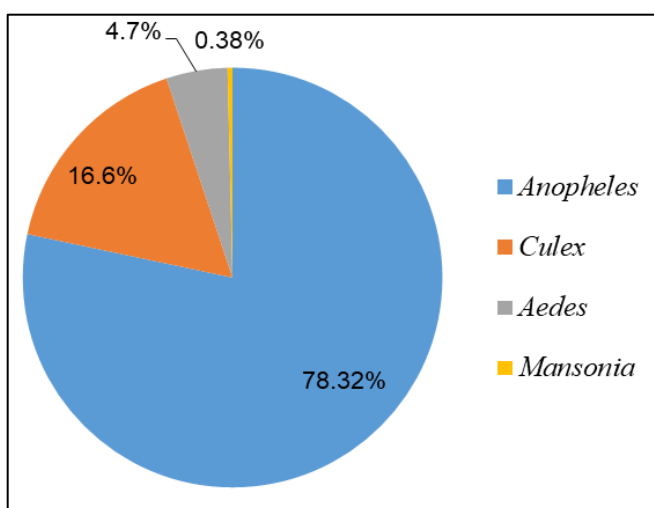


Fig 3: Distribution of mosquito genera identified

Culicinae sub-family (5 species) was more species-rich than Anophelinae (1 species). *Culex*, *Aedes*, and *Anopheles* were the main genera recorded. (Table 1). The *Anopheles* (n = 813, 78.32%) of Kovié was the most represented with the main species being *An. coluzzii*, followed by *Culex* (n = 172, 17%), *Mansonia* and *Aedes* which were less represented.

Table 1: Mosquito species composition at Kovié

Sub-families	Genera	Species	Number (%)
Culicinae	<i>Aedes</i>	<i>Ae. aegypti</i>	49 (4.7)
	<i>Culex</i>	<i>Cx. pipiens</i>	45 (4.33)
	<i>Culex</i>	<i>Cx. sp</i>	33 (3.2)
	<i>Culex</i>	<i>Cx. poicilipes</i>	94 (9.05)
	<i>Mansonia</i>	<i>Ma. uniformis</i>	4 (0.4)
Anophelinae	<i>Anopheles</i>	<i>An. coluzzii</i>	813 (78.32)

**Resting behavior**

A total of 483 female mosquitoes from 5 different species were caught in 20 bedrooms (Table 2). The average resting density was 4.87 females per bedroom per day (FBD). The highest resting density was observed in *An. coluzzii* (20.6 FBD) and followed distantly by *Cx. Pipiens* (2.25 FBD). Low resting densities were observed in *Cx. poicilipes* (0.95 FBD), *Culex* sp (0.15 FBD), and *Ma. uniformis* (0.4 FBD) (Table 2).

Table 2: Resting density of mosquito species at Kovié

Species	Number collected	Resting density (FBD)
<i>Cx. pipiens</i>	45	2.25
<i>Culex. sp</i>	3	0.15
<i>Cx. poicilipes</i>	19	0.95
<i>Ma. uniformis</i>	4	0.4
<i>An. coluzzii</i>	412	20.6
Mean	96.6	4.87

**Epidemiological survey**

Information relating to medical consultations for vector-borne diseases were obtained at the Kovié Health Center. Information was exclusively about malaria (Table 3). The variations in positive thick blood smear (TBS) by year and age group are shown in Figure 3. Results showed that malaria cases were higher in ≥5 years: 579 cases in 2018, 594 cases in 2019, and 165 cases in 2020. However, in <5 years and PW, the TBS revealed that the highest number of malaria infections was recorded in 2019. Analyses showed a significant difference (F= 9.88623; ddf = 2; p = 0.02831) between the values collected in <5 years, ≥5 years, and PW per year. Malaria prevalence by age group is shown in Table 3.

Table 3: Malaria prevalence between 2018 and 2020

Years	Age groups	Positive TBS	Negative TBS	Total	Prevalence (%)
2018	<5 years	100	51	151	66
	≥5 years	579	235	814	71
	PW	81	53	134	60
2019	<5 years	88	55	143	62
	≥5 years	594	248	842	71
	PW	54	114	168	32
2020	<5 years	26	39	65	40
	≥5 years	165	108	273	60
	PW	19	30	49	39

PW: pregnant women, TBS: thick blood smear

**Discussion**

In this study, mosquito fauna in Kovié is predominantly comprised of *Anopheles*, followed by *Culex*. Similar trends were reported by Djegbe *et al.* [18] in Benin and Adja *et al.* [19] in Côte d'Ivoire. *Aedes* and *Mansonia* were less represented. The presence of mosquitoes is aggravated by irrigated rice cultivation related breeding sites. Indeed, Kovié is a peri-urban locality located in a lowland. Rice cultivation in lowland has created a very favorable ecosystem for the proliferation of *An. gambiae s.l.* Several studies have shown the impact of irrigated rice cultivation on the proliferation of *Anopheles* mosquitoes, especially *An. gambiae* [20,21]. The diversity of mosquito fauna at Kovié is very low as indicated by Simpson's diversity index value (D̂) = 0.4. This low index could be explained by the short period of collection. Despite such a low index, all the genera of medical importance (*Anopheles*, *Culex*, and *Aedes*) are represented; this represents a threat to the health of the local population. The presence of *Culex* in the breeding sites could be explained by the presence of ions and organic compounds in the breeding sites because of the use of organic fertilizers in the rice fields. It has been shown that *Culex* prefers breeding sites rich in organic compounds and adapts to polluted environments with high ionization [22-24]. In addition, *An. coluzzii* could adapt to organic-rich and highly mineralized breeding sites [25]; this

could explain the proliferation of this species in Kovié. The absence of *Mansonia* mosquitoes during the larval surveys, although collected during the SC is probably related to the Dipping method <sup>[10]</sup> used in collecting the larvae. In fact, this collection method is not appropriate for the collection of *Mansonia* larvae which tend to cling to plants. Hence the need to combine adult collection methods to increase the probability of collection. The high number of female mosquitoes recorded is an important factor in the epidemiology and transmission of mosquito-borne diseases given their role as vectors. The fact that *An. coluzzii*, *Cx. poicilipes*, *Cx. pipiens*, and *Ma. uniformis* were collected in houses explains the anthropophilic behavior of these species. *Anopheles coluzzii* is highly anthropophilic as reported by Dossou-Yovo <sup>[26]</sup>. Most of the houses in Kovié have rooms with ventilation openings, which contribute to increasing the number of mosquitoes entering rooms, thus increases the likelihood of infectious bites. In Kovié, malaria is the main cause of consultation, especially among children <5 years, considered as vulnerable population together with PW. The National Malaria Control Program (NMCP) reported that malaria is the leading cause of mortality in pediatric services <sup>[27]</sup>. In addition, 2020 data indicated a slightly low prevalence rate in PW and <5 years; this could be explained by the special attention the local community gives to malaria prevention by avoiding mosquito bites through an effective use of mosquito nets, the main vector control tool adopted by the NMCP in the country. High prevalence of malaria (especially in 2018 and 2019) at Kovié could be attributed to the high number of *An. coluzzii* – the only representative sibling of *Anopheles gambiae s.l.* – recorded in the area and to the reduction of LLINs effectiveness. Indeed, Diuk-Wasser *et al.* <sup>[28]</sup> reported that there is a quadratic relationship between mosquito abundance and malaria transmission. *Anopheles coluzzii* was reported in 2013 and 2016 by Amoudji *et al.* <sup>[29]</sup> in our study area. However, two other members of the complex including *An. gambiae* and *An. arabiensis*, the main malaria vectors, have been recently reported in Togo <sup>[29-31]</sup>.

## Conclusion

Mosquitoes collected at Kovié were predominantly represented by *Anopheles coluzzii*. Data from the health center showed that malaria is the main cause of consultation, especially among children <5 years and PW. Though, malaria prevalence was slightly low in 2020, it was high in 2018 and 2019.

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