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Preliminary investigation on aggressive culicidae fauna and malaria transmission in two wetlands of the Wouri river estuary, Littoral-Cameroon

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Abstract
A study of the malaria transmission was carried out for the first time in the Manoka Island and Youpwè mainland area in Douala. Mosquitoes were sampled by human landing catches simultaneously indoor and outdoor in December 2013 and April 2014. Collected mosquitoes were morphologically identified and anopheles ovaries were dissected for parity determination. Legs and head-thorax of each dissected anopheles specimen belonging to *Anopheles gambiae* complex were used for molecular identification and infectivity status determination. A total of 2,827 female mosquitoes belonging to *Anopheles, Culex* and *Aedes* genera were collected in both localities. *Anopheles gambiae* complex and *Culex pipiens* was dominant respectively in Manoka (81.1%) and Youpwè (77.9%). In both localities, *Anopheles gambiae* s.l. had a high tendency to exophagy. Only *Anopheles coluzzii* was found infected by *Plasmodium falciparum*. Between Manoka and Youpwè, there was no significant difference in the infection rate and the entomological inoculation rate.

Keywords: Aggressive *Culicidae* fauna, *Anopheles coluzzii*, malaria transmission, littoral, Cameroun

1. Introduction
In Cameroon, malaria remains a major public health problem and the leading cause of morbidity and mortality [1]. Eco-climatic variations influence the transmission of the disease [1, 2]. The vector of this disease, the female anopheles mosquito, is constituted of groups or complexes with morphologically similar species, colonizing different environments, whose vector competence and vector capacity are very varied. Knowledge of the vector system in a given context allows establishing more appropriate control measures against the disease [3]. In Cameroon, a large part of the country (south-north transect) lies between the equatorial epidemiological facies corresponding to the forest zone of the south and the tropical area of northern savanna zone; both facies and their transition zones have already been subjected to many entomological studies [4-9]. However, no entomological data are available in the literature on peninsular and insular areas as the island of Manoka and its surroundings along the Wouri river estuary on the Atlantic South-Western coast of Cameroon. This island does commercial activities with the city of Douala and Youpwè quarter serves as the hub for the transportation of human populations and goods between the two areas; these movements could allow passive transport of anopheles and parasite exchanges. This article provides information on the diversity of aggressive culicidae fauna, feeding behavior of vectors and malaria transmission to improve vector control measures and reducing culicidae nuisance on the Manoka Island.

2. Materials and Methods
2.1 Study area
The present study was conducted at Douala (04°03′N 09°41′E), the main port and the largest industrial city of Cameroon, 260 km northwest of the capital city Yaoundé. The city sits on the Wouri river estuary in a degraded Atlantic rain forest. The climate is tropical with two main seasons, a dry season from November to February and a rainy season from March to October.

However, the city sees rainfall throughout the year with an average of 3,600 mm rainfall per year [10]. The climate is typically warm and humid with an average annual temperature of 27.0 °C and an average humidity of 85% [11]. The city and its surrounding area had an estimated population that surpassed 3 000 000 inhabitants. Two surrounded districts of Douala located in the wetlands of the estuary have been selected for the study, Manoka and Youpwè (Figure 1). Manoka (03°47' N; 09°37' E) is an island in the south of the estuary where fishing and agriculture are main activities. Youpwè (04°00' N; 09°42' E) in the left bank of Wouri river is a landing hub for fresh fish from Manoka island and other places, and the main fish market along the Douala bay. Houses in Manoka are usually built with provisory materials while Youpwè displays high proportion of houses with cement blocks.

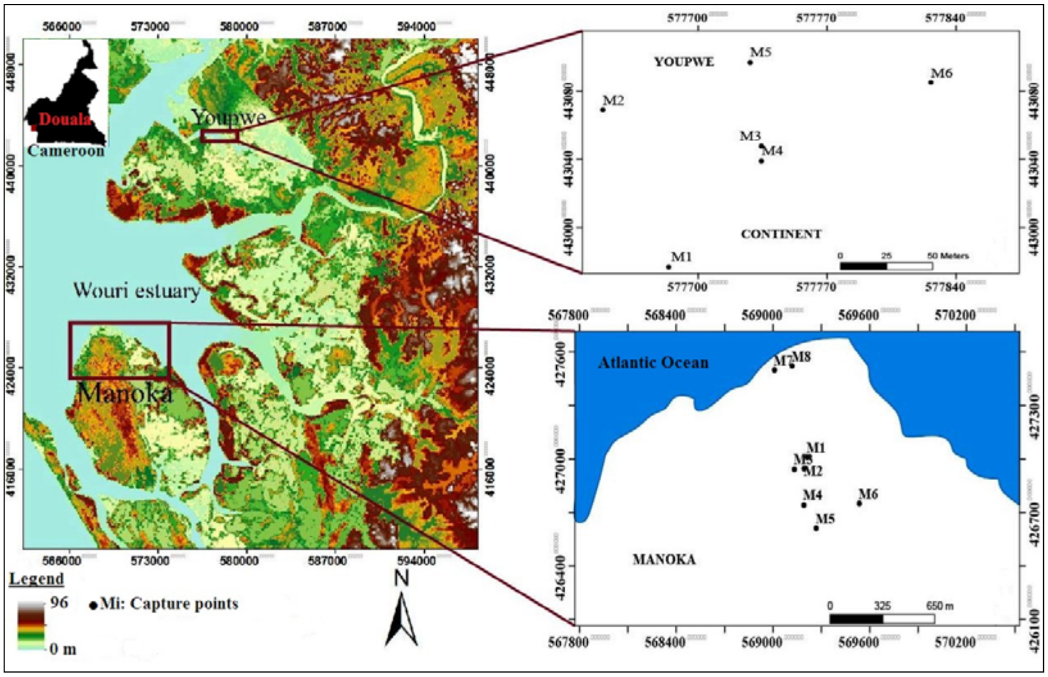


Fig 1: Manoka and Youpwè map showing capture points.

2.2 Adult mosquito collections and field processing

Mosquito collections were planned in December 2013 (dry season) and April 2014 (rainy season). Adult mosquitoes were sampled while landing on human volunteers from 6.00 pm to 6.00 am, simultaneously indoor and outdoor. The overall mosquito collections consisted of 48 man-nights in Manoka and 24 man-nights in Youpwè. Sampled mosquitoes were identified following morphological identification keys of Gillies & De Meillon [12] and Edwards [13] and ovaries of anopheline specimens checked for parity according to Detinova's method [14]. Legs and head-thorax of each *Anopheles* specimen was kept separately in an individual tube with silicagel at – 20 °C until the molecular identification of members of the *An. gambiae* complex and ELISA (enzyme linked immunosorbent assay) detection of *Plasmodium* parasite infection in laboratory.

2.3 Laboratory procedures

The ELISA technique was performed on head and thorax of each anopheline specimen to detect the *P. falciparum* parasite circumsporozoite protein following the method described by Burkot *et al*. [15], modified by Wirtz *et al* [16]. DNA extracts from legs of *An. gambiae* specimens were proceed for polymerase chain reaction (PCR) identification of sibling species belonging to the *An. gambiae* complex as described by Fanello *et al*. [17].

2.4 Data analyses

The following parameters were considered: i) species diversity expressed by the number of species recorded per location and per season, ii) the human biting rate (HBR) as the number of bites/person/night (b/p/n) is the product of number of mosquito collected and number of man-night, iii) the parity rate (PR) as the proportion of parous specimens over the total number of mosquitoes checked for parity, iv) the infection rate (IR) as the proportion of infected mosquitoes over the total number of mosquitoes checked for parasite infection, and v) the daily entomological inoculation rate (EIR) as the number of infected bites/person/night (ib/p/n) was the product of the infection rate and the biting rate. The monthly EIR was estimated by multiplying the daily EIR by an average of 30 days. The R software (version 2.15.2) [18] was used for statistical analyses at risk α = 0.05.

2.5 Ethical consideration and informed consent

This study was approved by the ethic committee of the University of Douala. The purpose of the study was clearly explained to the community with the help of headquarters and opinion leaders. The community members were informed that participation in the research was voluntary without penalty for the give up. The heads of each household was asked to sign the consent form for their household to participate in the study. The participants in the study were remunerated agreeing adults. At the first suspicion of malaria, volunteers were provided with the curative treatment recommended by the National Malaria Control Programme in Cameroon.

3. Results

3.1 Diversity of aggressive culicidae fauna

A total of 2,827 mosquitoes were collected during the two periods in Manoka (1,202) and Youpwè (1,625). The number of species recorded between 7 in December 2013 and 6 in April

2014 in Manoka, but was invariable (3 species) in Youpwè (Table 1). In Manoka, the mosquito fauna consist of at least 12 species belonging to genus *Anopheles* (81.1%, n=975), genus *Culex* (14.5%, n=174) and genus *Aedes* (4.4%, n=52). Among *An. gambiae* complex, two species were found, the most predominant *An. coluzzii* (98%, n = 192) and *An. melas* (2%, n =4). One specimen of *An. tenebrosus* was also found. The remaining mosquito fauna was represented by *Culex quinquefasciatus* (7.07%, n = 85), *Culex poicilipes* (0.67%, n = 8), *Culex gr decens* (0.5%, n = 6), *Culex duttoni* (1.58%, n = 19), *Aedes aegypti* (2.91%, n = 35), *Aedes albopictus* (0.42%, n = 5), *Aedes vexans* (0.83%, n = 10) and non-identified specimens (4,83%, n=58) because of their degraded state. In Youpwè, 3 mosquito species including *Culex quinquefasciatus* (77.9%, n = 1,265), *An. coluzzii* (21.4%, n = 348) and *Aedes aegypti* (0.7%, n = 12) were collected.

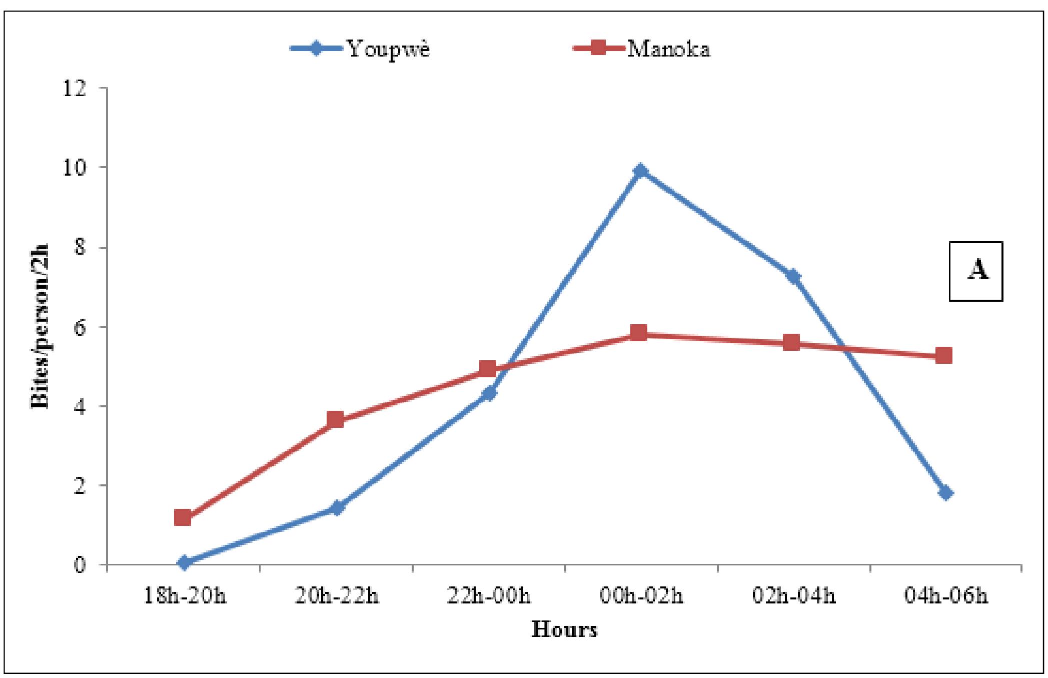
Table 1: Diversity of aggressive culicidae fauna in Manoka and Youpwè in December 2013 and April 2014

Species	Total		December 2013				April 2014			
			Manoka		Youpwè		Manoka		Youpwè	
	n	%	n	%	n	%	n	%	n	%
Anopheles gambiae s.l.	1,323	46.8	344	66.6	50	6.6	631	92	298	34.3
Anopheles tenebrosus	1	0.1	1	0.2	0	0.0	0	0.0	0	0.0
Aedes aegypti	47	1.6	10	1.9	1	0.2	25	3.6	11	1.3
Aedes albopictus	5	0.2	4	0.8	0	0.0	1	0.2	0	0.0
Aedes vexans	10	0.4	0	0.0	0	0.0	10	1.5	0	0.0
Aedes sp	2	0.1	2	0.4	0	0.0	0	0.0	0	0.0
Culex duttoni	19	0.6	19	3.7	0	0.0	0	0.0	0	0.0
Culex gr decens	6	0.2	6	1.2	0	0.0	0	0.0	0	0.0
Culex quinquefasciatus	1,350	47.7	74	14.4	704	93.2	11	1.6	561	64.4
Culex poicilipes	8	0.4	0	0.0	0	0.0	8	1.2	0	0.0
Culex sp	56	1.9	56	10.8	0	0.0	0	0.0	0	0.0
Total	2,827	100	516	100	755	100	686	100	870	100

n = effective; % = genus percentage

3.2 Biting habits of *An. coluzzii*

Anopheles coluzzii was aggressive in the study sites with an average man-bite rate of 20.29 b/p/n in Manoka and 14.50 in Youpwè. The difference in biting density between Manoka and Youpwè was significant ($P<0.001$). Night biting cycles of *An. coluzzii* (Figure 2) varied with the study site and did not vary with the season. In Youpwè, it was characterized by a peak between 12.00 pm to 02.00 am while in Manoka, it was characterized by a plateau around 12.00 pm until 06.00 am. In total, 75% of the bites of *An. coluzzii* in Manoka and 76% in Youpwè occurred after midnight. The biting behavior of *An. coluzzii* was similar in the two study sites. It was observed to bite mainly outdoor compared to indoor in both localities (table 2). In fact, the overall biting density of *An. coluzzii* was 13, 96 b/p/n indoor and 34.76 b/p/n outdoor in Manoka, and 4,75 b/p/n indoor and 24,83 b/p/n outdoor in Youpwè. In both localities, density of *An. coluzzii* was significantly higher outdoor than indoor ($P<0,001$).



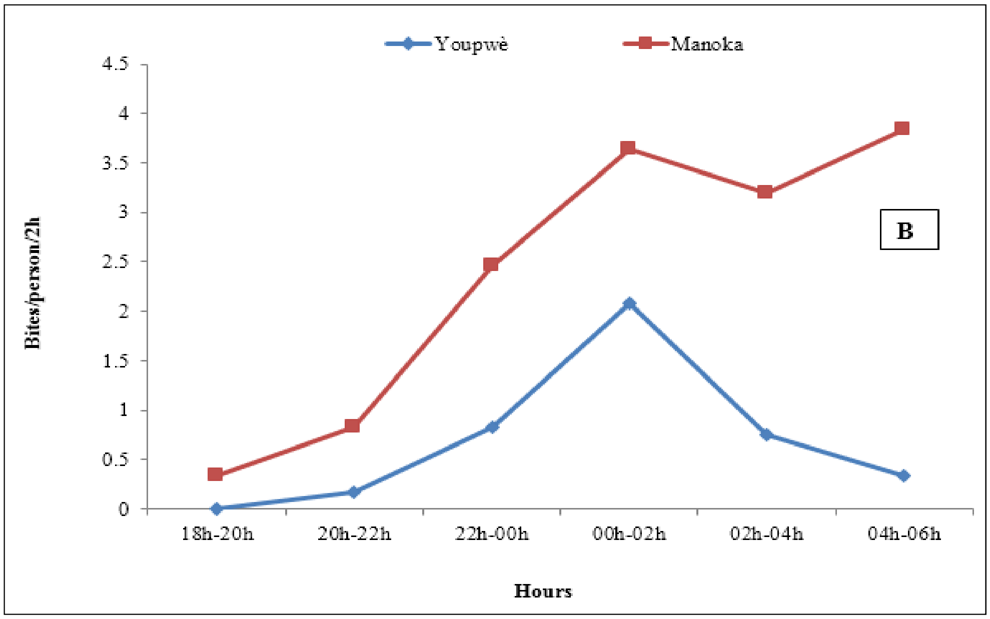


Fig 2: Biting cycle of *An. coluzzii* in Manoka and Youpwè in December 2013 (A) and April 2014 (B).

3.3 Parity rate

The average percentage of parous females were 39.7% (n = 934) in Manoka and 40.5% (n = 348) in Youpwè. In general, the difference in parity rate of *An. coluzzii* between Manoka and Youpwè was not significant (P = 0,799). However, parity rate was significantly higher during the dry season in Youpwè (P = 0.015).

3.4 Infectivity of *An. coluzzii* and malaria transmission

Entomological parameters of malaria transmission in Manoka and Youpwè are shown in table 2. The average of the infection rate of *An. coluzzii* was 2.58% in Manoka and 5.53% in Youpwè. The average of the Entomological Inoculation Rate was 18.32 ib/p/m in Manoka and 28.18 ib/p/m in Youpwè. Between Manoka and Youpwè, there was no significant difference in the infection rate (P=0.158) and EIR (P = 0,968). However, in both locations, the highest infection rate (4% in Manoka and 6.88% in Youpwè) and EIR (31.55 ib/p/m in Manoka and 51.25 ib/p/m in Youpwè) occured during rainy season in April (*P*<0.001).

Table 2: Entomological parameters of malaria transmission in Manoka and Youpwè in December 2013 and April 2014.

		December 2013					April 2014				
		N	**dHBR**	**IR**	**dEIR**	**mEIR**	**N**	**dHBR**	**IR**	**dEIR**	**mEIR**
Manoka	Indoors	142	11.83	0.00	0.00	0.0	193	16.08	1	0.16	4.8
	Outdoors	202	16.83	1.16	0.17	5.1	438	36.50	3	1.1	33
	Pooled	344	14.33	1.16	0.17	5.1	631	26.29	4	1.05	31.5
Youpwè	Indoors	11	1.83	9.1	0.17	5.1	46	7.67	0.36	0.03	0.9
	Outdoors	39	6.50	2.7	0.18	5.4	252	42.00	6.52	2.74	82.2
	Pooled	50	4.16	4.17	0.17	5.1	298	24.83	6.88	1.71	51.3

N = effective; dHBR = daily human biting rate; IR = infection rate; dEIR = daily entomological inoculation rate; mEIR = monthly entomological inoculation rate

4. Discussion

At Manoka and Youpwè, aggressive Culicidae fauna consists of three genera: *Anopheles*, *Culex* and *Aedes*. The frequency of the genus *Anopheles* is significantly higher in Manoka than in Youpwè, and *vice versa* for the frequency of *Culex*. The predominance of the genus *Anopheles* in Manoka could be due to the rural character of the island with natural water collections favorable for the development of *Anopheles* larvae. The predominance of culicinae in Youpwè could be due to uncontrolled urbanization characterized by unsafe and proliferation of waste water collections, which favors the development of the genus *Culex* larvae to the detriment of the *Anopheles* genus. These results are in agreement with those of Darriet *et al.* [19] in Burkina Faso and Antonio Nkondjio *et al.* [20] who observed a dominance of *Culex* in Douala. The presence of *An. coluzzii* in the urban area of Youpwè is in agreement with Gimmoneau *et al.* [21] and Kamdem *et al.* [22]. The observed *Anopheles* diversity in both localities was low. In fact, only three species were collected on the quarantine known in Cameroon [23]. This low *Anopheles* diversity could be due to the salty character of breeding sites. Moreover, Manoka has another time made object of oil exploitation. Only *An. coluzii* has capabilities to adapt to such conditions [24]. The predominance of this species to the detriment of *An. gambiae* which is absent is due to its tolerance to salinity. This is consistent with previous works in Cameroon that highlight the prevalence of this species in the coastal region [25, 26]. *An. melas* were only collected in island area. This observation confirms that of Antonio Nkondjio *et al.* [27] showing that this species is present only in the coast. In fact, *An. melas* is a species of halophilous natural environments of the West African coastal zones i.e. mangroves.

The aggressivity of *Anopheles* is significantly higher in Manoka than in Youpwè; that may be due to the fact that rural areas are more favorable to the proliferation of *Anopheles* [28]. Night biting cycles of *An. coluzzii* observed in Youpwè is in agreement with previous studies in Cameroon [2, 20]. The one observed in Manoka, different from that observed on the island of Bioko by Cano *et al*. [29], would be in connection with the population's habits. In fact, the population mainly practise fishing during the night and are back in the second half of the night. In this moment, the host is available in significant quantity for the vector. In both localities, an inhabitant receives the majority of bites during the second half of the night according to the observations made in other studies [4, 20]. Whatever the ecological zone, the aggressivity of *Anopheles* was significantly higher at the beginning of the rainy season than during the dry season. Rainfall favors the larval development by creating the breeding sites and thus supporting the production of adults, but also supporting the biting activity and the reproduction of adult because of optimum temperature and hygrometry [28]. *An. coluzzii* is exophagic in both localities. This trend exophagy is an adaptation of the vector to the habits of human populations. In fact, in both localities, human populations are mainly fishermen. Preparatory activities for fishing are largely held by night, involving a late human presence outside the houses. This favors the exophagic behavior of *Anopheles* which is also subjected to the repulsive effect of nets (long lasting insecticidal nets) present in 95% of households. The exophagic behavior of *An. coluzzii* in both localities leads to associate other vector control skills to the use of nets like cleansing of the environment.

The level of malaria transmission is similar between the two localities. Our findings contradict those of Akogbeto *et al* [28] who reported that malaria transmission is lower in urban areas than in rural areas in Benin. In reality, high anopheles number in Manoka is offset by the high level of sporozoitic index in Youpwè to balance the transmission in both localities. The level of malaria transmission in Youpwè is however relatively high compared to other reports from similar urban areas [20, 28, 30]. *An. melas* were not found infected with *P. falciparum*. This confirms earlier observations made in Cameroon where this species has never been found infected [27]. However, this species has been found weakly infected with *P. falciparum* in the coastal areas of Benin by Akogbeto and Romano [31] who reported a close relationship between *An. melas* and animals which, according to them, is the fundamental reason for its low infectivity.

The parity rate of *An. coluzzii* was higher during the dry season. This observation can be explained by the rarity of the larval breeding sites during this season. In fact, the rarity of the larval breeding sites during this season force mosquito to retain their eggs in the ovaries while waiting for the ideal conditions [32]. Thus, this rarity of egg-laying justifies a growing old population. However, the repetitive egg-laying during the rainy season, according to the abundance of larval breeding sites enables the emergence of young mosquitoes in great number.

5. Conclusion

Anopheles coluzzii is the malaria vector on the Manoka Island and Youpwè mainland area of Douala. These findings are of great importance for the malaria vector surveillance and for targeting malaria elimination in the Manoka Island. The exophagous behaviour of *An. Coluzzii* leads to associate other vector control skills to the use of nets like cleansing of the environment including larviciding and the destruction of the larval breeding sites around habitations.

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7. References

1. Minsanté. Plan Stratégique National de lutte contre le Paludisme 2011-2015, 82.
2. Antonio-Nkondjio C, Awono AP, 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; 39:350-355.
3. Fontenille D, Cohuet A, Awono AP, Antonio NC, Wondji C, Kengne P *et al*. Systématique et biologie des anophèles vecteurs de plasmodiums en Afrique, Données récentes. Méd. Trop. 2003; 63(3):247-253.
4. 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; 6:135-138.
5. Cavalié P, Mouchet J. La campagne expérimentale d'éradication du paludisme dans le nord de la République du Cameroun, 1961.
6. Manga L, Toto JC, Carnevale P. Malaria vectors and transmission in an area deforested for a new international airport in southern Cameroon. Ann. Soc. belge Méd. Trop. 1995; 75:43-49.
7. Njan Nloga A, Robert V, Toto JC, Carnevale P. Anopheles moucheti, vecteur principal du paludisme au Sud Cameroun. Bull. Liais. Doc. OCEAC. 1993; 26(2):63-67.
8. Robert V, Le Goff G, Toto JC, Mulder L, Fondjo E, Manga L *et al*. Anthropophilic mosquitoes and malaria transmission at Edea, Cameroon. Trop. Med. Parasitol: (GTZ). 1993; 44(1):14-18.
9. Manga L, Bouchite B, Toto JC, Froment A. La faune anophélienne et la transmission du paludisme dans une zone de transition forêt/savane au centre du Cameroun. *Bull Soc Path Exo* 1997; 90(2):128-130.
10. World Meteorological Organization. "World Weather Information Service - Douala". Retrieved December 6, 2012.
11. Weatherbase. Historical Weather for Douala, Cameroon. Retrieved December 6, 2012.
12. Gillies MT, De Meillon B. The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region), 1968.
13. Edwards FW. Mosquitoes of the ethiopian region. III. Culicine adults and pupae. *London Birt Mus Nat Hst* 1941; 499.
14. Detinova TS. Méthodes à appliquer pour classer par groupe d'âge les Diptères présentant une importance médicale. O.M.S., Génève, série des Monographies 1963; 47:220.
15. Burkot TR, Williams JL, Schneider I. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am. J Trop. Med. Hyg.1984; 33(5):783-788.
16. Wirtz RA, Burkot TR, Graves PM. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. J Med. Entomol. 1987; 24(4):433-437.
17. Fanello C, Santolamazza F, Della Torre A. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR/RFLP. Med. Vet. Entomol 2002; 16(4):461-464.

18. Development R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2011; ISBN 3-900051-07-0, URL http://www.R-project.org/.
19. Darriet F, Robert V, Carnevale P. Nouvelles perspectives de lutte contre *Culex quinquefasciatus* dans la ville de Bobo-Dioulasso (Burkina Faso). Congrès « L'Eau, la Ville et le Développement », 1986, 9-11.
20. Antonio-Nkonjio C, Talom BD, Tagne FR, Fossog TB, Ndo C, Lehman GL *et al*. High mosquito burden and malaria transmission in a district of the city of Douala, Cameroon. BMC Infectious Diseases 2012; 12:275-282.
21. Gimonneau G, Pombi M, Choisy M, Morand S, Dabiré RK, Simard F. Larval habitat segregation between the molecular forms of the mosquito *Anopheles gambiae* in a rice field area of Burkina Faso, West Africa. Med Vet Entomol. 2012; 26:9-17.
22. Kamdem C, Tene Fossog B, Simard F, Etouna J, Kengne P, Bousses P *et al*. Anthropogenic habitat disturbance and ecological divergence between incipient species of the malaria mosquito *Anopheles gambiae*. PLoS One 2012; 7(6):e39453.
23. Hervy JP, Le Goff G, Geoffroy B, Herve JP, Manga L, Brunhes J. Les anophèles de la région afro-tropicale. Orstom editions, Coll. Didactiques, CD-Rom, 1998.
24. Akogbéto M. Etude des aspects épidémiologiques du paludisme côtier lagunaire au Bénin. Thèse de doctorat ès-sciences, Université de Paris XI, France, 1992.
25. Wondji C, Simard F, Petrarca V, Etang J, Santolamazza F, Della Torre A *et al*. Species and populations of the *Anopheles gambiae* complex in Cameroon with special emphasis on chromosomal and molecular forms of *Anopheles gambiae* ss. J Med. Entomol. 2005; 42(6):998-1005.
26. Slotman MA, Reimer LJ, Thiemann T, Dolo G, Fondjo E, Lanzaro GC. Reduced recombination rate and genetic differentiation between the M and S forms of *Anopheles gambiae* ss. Genetics 2006; 174(4):2081-2093.
27. Antonio-Nkondjio C, Kerah CH, Simard F, Awono-Ambene P, Chouaibou M, Tchuinkam T *et al*. Complexity of the malaria vectorial system in Cameroon: contribution of secondary vectors to malaria transmission. J Med. Entomol. 2006; 43(6):1215-1221.
28. Akogbeto M, Chippaux JP, Coluzzi M. Le paludisme urbain côtier à Cotonou (République du Bénin). Étude entomologique. Revue d'Epidémiologie et Santé Publique 1992; 40:233-239.
29. Cano J, Berzosa PJ, Roche J, Rubio JM, Moyano E, Guerra-Neira A *et al*. Malaria Vectors in the Bioko Island (Equatorial Guinea): Estimation of Vector Dynamics and Transmission Intensities. J Med. Entomol 2004; 41(2):158-161.
30. Manga L, Robert V, Messi J, Desfontaine M, Carnevale P. Le paludisme urbain à Yaoundé, Cameroun: 1. étude entomologique dans deux quartiers centraux. Mémoires de la Société Royale d'Entomologie de Belgique, 1992; 35:155-162.
31. Akogbéto M, Romano R. Infectivity of *Anopheles melas* vis-à-vis *Plasmodium falciparum* in the coastal lagoon area of Benin. Bulletin de la Société de Pathologie Exotique 1999; 92(1):57-61.
32. Holtein M. Biologie d'*Anopheles gambiae*: Recherches en Afrique occidentale française, O.M.S. Editions, Genève Suisse, 1952, 176.