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Comparative study of Culicidae biodiversity of Manoka island and Youpwe mainland area, Littoral, Cameroon

Abdou Talipouo[1], Patrick Ntonga Akono[1], Darus Tagne[2], Arthur Mbida Mbida[*1], Josiane Etang[3,4], Roméo Tchoffo Fobasso[1], Wolfgang Ekoko[1], Jerome Binyang[1], Alain Dongmo[1]

[1]*Department of Biology of Animal Organisms, University of Douala, Douala, Cameroon*

[2]*Department of Animal Biology and Physiology, University of Yaoundé I, Yaoundé, Cameroon*

[3]*Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon*

[4] *Department of Biological Sciences, University of Douala, Douala, Cameroon*

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Abstract

A comparative study of the Culicidae biodiversity was carried out on the island of Manoka and Youpwe mainland area, in the Wouri river estuary in Cameroon. This study aimed to identify the mosquito species in the two localities and to compare their abundances for a good planning of the mosquito control on Manoka island. Mosquito surveys were conducted during the dry and rainy seasons. Two methods made it possible simultaneously to sample the larval populations by dipping and aggressive fauna by the human landing catches. Collected mosquitoes were morphologically identified using determination keys and those belonging to *Anopheles gambiae* complex by molecular biology using the Polymerase Chain Reaction-Fragment Lengt Polymorphism. We compared mosquito abundance and diversity across the two ecological zones. A total of 7,425 mosquitoes belonging to 12 species were identified, 11 species at Manoka and 9 species at Youpwe. Mosquito's abundances varied with ecological site. At Manoka, 66.83% (n=2,441) of the collected mosquitoes was the Anophelinae whereas in Youpwe, the subfamily of Culicinae was dominant with 80.10% (n=3023) of the collected mosquitoes. However, the crossing of the two profiles of diversity does not enable us to say that a community is more varied than the other. The presence of *Anopheles moucheti* at Youpwe causes a risk of importation of this species at Manoka. These results bring significant knowledge which could contribute to the development of a judicious mosquito control strategy on the island of Manoka.

* **Corresponding Author:** Arthur Mbida Mbida ✉ mbidajean@yahoo.fr

Introduction

The Wouri river estuary represents a vast wetland favorable to the proliferation of a much diversified flora and fauna among which exist many mosquito species (Maire and Aubin, 1976). The ecology and ethology of mosquito were described in many studies (Service, 1993 ; Manga *et al*., 1997 ; Fontenille *et al*., 2003 ; Kamdem *et al*., 2012). In Sub-Saharan Africa, mosquitoes are among the most widespread insects where they are responsible for significant harmful effects due to the bite which they inflict during their blood meals. Some mosquito species are biological vectors of diseases like those of *the Anopheles* genus accused in the transmission of malaria, *the Culex* genus accused in the transmission of filariosis and *the Aedes* genus in the transmission of arboviroses. Among the diseases transmitted by mosquito, malaria remains the most dangerous (Gratz, 1999).The World Health Organization (OMS, 2014) estimates at 198 million the number of malaria cases and 587 000 deaths of which respectively 80% and 90% occured in Sub-Saharan Africa. In Cameroon, morbidity due to malaria represents 41% in the 10 Regions (Minsanté, 2011).The transmission of the diseases is mainly assured by six anopheline species whose distribution is based on the eco-climatic factors (Fontenille and Simard, 2004), thus determining variable levels of transmission according to the vectors, their vectorial and competence capacities. Then, it results an epidemiologic diversity with three major facies: soudano sahelian in the septentrional part, savanna in the area of Adamaoua and equatorial in the areas of the South. Moreover, some particular biotopes confer to the disease the specific epidemiologic facies such as dam, the urban, coastal, rice and insular zones (MARA, 1999). However, very little is known on the insular facies in Cameroon. The insular zones because of their enclosure would be favorable to the implementation of an experimental strategy to eradicate the vectors. The island of Manoka have approximately 40 000 inhabitants and does commercial activities with the city of Douala. Youpwe 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 situation would complicate the mosquito eradication on the island. The knowledge of the level of importation of the mosquito is significant for the development of a strategy of vectors control and the reduction of the mosquito harmful effects on the Manoka island. The identification of the vectors implied in the transmission of diseases in the two localities and their abundance is one of the preconditions for any action of vectors control (Fontenille *et al*., 2003).The present study aimed to identify the mosquito species on Manoka island and Youpwe mainland area and to compare their abundances for a good planning of the mosquito control on the island.

Materials and methods

Study site

The present study was carried out in Douala (0403'N; 0941'E), the economic capital of Cameroon. The climate is a typical mode of monsoon, with a long succession of rainy days due to the oceanic environment (4,067 mm) (Suchel, 1987).

The climate is typically warm and humid with an average annual temperature of 27°C and an average relative humidity of 85 % (Weatherbase, 2012; WMO, 2012). The hydrographic network consists of a principal river, Wouri, surrounded by Moungo, Dibamba and Sanaga rivers.

The city sits on the Wouri river estuary in a degraded Atlantic rain forest (Letouzey, 1985).The study was undertaken in two different ecological sites, the island of Manoka (0347'N; 0939'E) and the Youpwe (0400'N;0942'E) mainland area (Fig.1). Manoka, is characterized by a rural environment and houses are usually built with provisory materials.

The hydrographic network consists of small temporary rivers of which most known are "Polongo", "Mishimizon" and "petit plateau" (Emassi, 2013). There are also many brooks, the sources, the ponds and the swamp which constitute potential mosquito breeding sites.

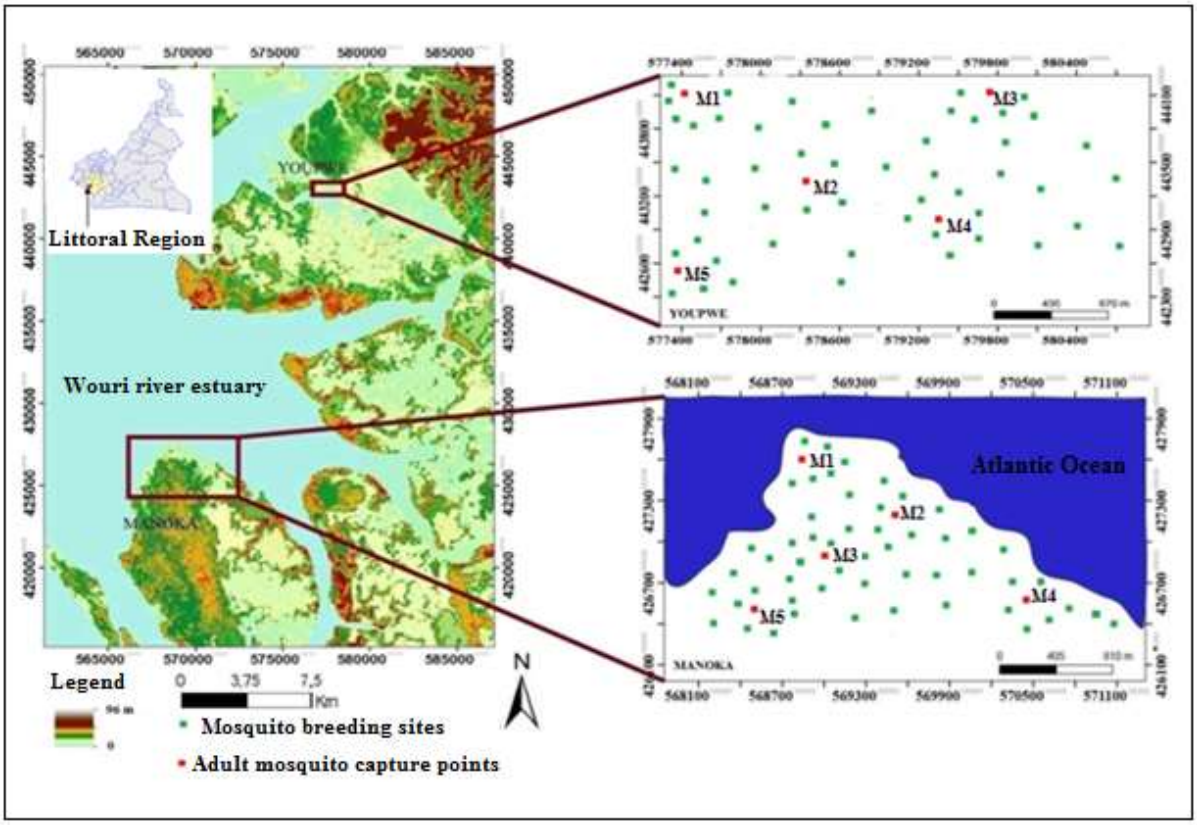

Fig. 1. Study site.

The vegetation mainly consists of mangrove swamp and is dominated by the species *Rhizophora mangle*, *Rhizophora racemosa* and *Avicenia* sp. Youpwe is located in the District of Douala 2nd where the dugouts embark for Manoka. The natural environment is of urban type, the houses are usually built with cement blocks. There are many collections of water (ponds, gutters, river and so on) which constitute potential mosquito breeding sites and the vegetation mainly consists of mangrove swamp.

Sampling procedures

Sampling was carried out in a series of three entomological investigations in December 2013 during the dry season and in April and August 2014 during the rainy season. To diversify the specimens of collected mosquitoes, sampling consisted of the collection of the both mosquito pre imaginal stages and adults populations.

Collection and rearing of the mosquito pre imaginal populations

The sampling of the mosquito pre imaginal populations was done in the larval breeding sites. Sampling consisted to browse the study sites and inspect all water collections in search of pre imaginal stages of mosquitoes. The pre imaginal stages were collected by`` dipping '' method (Service, 1993) which consists in plunging, in several places of the larval breeding site in a random way, a ladle or a container of a known capacity. The larvae and the pupae collected in each breeding sites were brought to the insectarium for rearing and identification of the species. The mosquito larvae were nourished with the TétraMin® Baby. The relative humidity varied between 70 % and 80 % and the temperature between 25°C and 30°C.

Adults mosquitoes collections

Adults mosquitoes were sampled while landing on human volunteers from 6.00 pm to 6.00 am, simultaneously indoor and outdoor, during two consecutive nights.

Identification of the species

The adults mosquitoes resulting from the larval collections and those collected on humans volunteers were identified according to the morphological

criteria using the determination keys (Holstein, 1949 ; Gillies and De Meillon, 1968 ; Gillies and Coetzee, 1987 ; Jupp, 1996). The specimens belonging to the *An. gambiae* complex were identified by molecular biology according to the "Polymerase Chain Reaction-Restriction Fragment Lengt Polymorphism" technique (Fanello *et al.*, 2002).

Data analysis

The biodiversity was estimated by the specific index of richness Hr=S-1 of Pavoine (Pavoine, 2005), where S is the number of species in the sample to be evaluated. Thus, a sample including only one species has a null diversity. The relative abundance of each species was calculated by the $Pi=n_i/N$ formula, where n_i is the effective of the species of rank i and N the total effective (Barbault, 1992).The specific diversity was estimated using the diversity index of Shannon-Weaver (a) and Simpson (b) (Barbault, 1992). The degree of similarity of the mosquitoes communities was estimated by the Sorensen index (Is = (2c X 100)/(a+b)) where a= number of species of site 1, b = number of species of site 2 and c = number of common species to both sites; and the profile of diversity of Renyi (c) (Kindt *et al.*, 2006).The equitability of Piélou (d) enabled us to define the equitable distribution of effective between the species in presence.

a) $$\hat{H}' = -\sum_{i=1}^{S}\left[\left(\frac{n_i}{n}\right) * \mathrm{Log}_2\left(\frac{n_i}{n}\right)\right]$$

b) $$\hat{\lambda} = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{n(n-1)}$$

c) $$H_\alpha = \frac{\ln\left(\sum_{i=1}^{S} p_i^\alpha\right)}{1-\alpha} \quad (\alpha = 0; 0.25; 0.5; 1; 2; 4; 8; \infty)$$

d) $$E_1 = J' = \frac{H'}{\ln(S)} = \frac{H'}{H_{max}} = \frac{\ln(N_1)}{\ln(N_0)} = \frac{Log_2 N_1}{Log_2 N_0}$$

The PAST version 2.10 Software enabled us to calculate the diversity index and the comparison of the species abundances between Manoka and Youpwe was performed using the SPSS version 20.0 software. The threshold of significativity was fixed at a probability $\alpha=0.05$ and the differences were considered significant for $p < 0.05$.

Results

Specific richness of Culicidae fauna at Manoka and Youpwè

A total of 12 mosquito species were identified during this study, 11 species at Manoka, 9 species at Youpwe and 8 common species to both localities (table 1). All these species belonged to two subfamilies, the Anophelinae and the Culicinae, and to three genera: *Anopheles* Meigen, 1818, *Culex* Linné, 1758 and *Aedes* Meigen, 1818.

The subfamily of Culicinae was richest with 8 species among which *the Culex* genus was dominant with 5 species. *A. melas, A. tenebrosus* and *A. vexans* were present only at Manoka while *A. moucheti* was present only at Youpwe. The greatest number of identified species at Youpwe came from the larval collections.

Abundance of Culicidae at Manoka and Youpwe

A total of 7,425 mosquitoes, of which 3,652 in Manoka and 3,773 in Youpwe were collected in the 2 sites (table 2). The subfamily of Culicinae was the most represented with 57.02% (n= 4,234) of total abundance. There was no significant difference between the genera *Culex* (44.40%, n=3,297) and *Anopheles* (42.98%, n=3,191), while the genus *Aedes* (12.62%, n=937) was the least represented. The mosquito's abundances varied according to the ecological zone. At Manoka, 66.83% (n=2,441) of the collected mosquitoes belonged to the subfamily of Anophelinae whereas at Youpwe, the subfamily of Culicinae was dominant with 80.10% (n=3,023) of the collected mosquitoes. At Manoka, the genus *Anopheles* was the most represented with 66.83% (n=2,441) of the total abundance, followed by the genera *Culex* (20.56%, n=751) and *Aedes* (12.61%, n=460).

Table 1. Culicidae fauna richness at Manoka and Youpwe.

Sampling methods	Genera collected	Species collected	Manoka	Youpwe	Total
Dipping		*An. coluzzii*	1	1	1
	Anopheles	*An. moucheti*	0	1	1
	Culex	*Cx. quinquefasciatus*	1	1	1
		Cx. poecilipes	1	1	1
		Cx. Decens	1	1	1
		Cx.duttoni	1	1	1
		Cx. Simpsoni	1	1	1
	Aedes	*Ae. Aegypti*	1	1	1
		Ae. albopictus	1	1	1
		Ae. Vexans	1	0	1
		S_1	9	9	10
Human Landing Catches	*Anopheles*	*An. coluzzii*	1	1	1
		An. melas	1	0	1
		An. tenebrosus	1	0	1
	Culex	*Cx. quinquefasciatus*	1	1	1
		Cx. Poecilipes	1	0	1
		Cx. Duttoni	1	0	1
		Cx. Decens	1	0	1
	Aedes	*Ae. Aegypti*	1	1	1
		Ae. albopictus	1	0	1
		Ae. Vexans	1	0	1
		S_2	10	3	10
		S_1+S_2	11	9	12

1=Presence; 0=Absence; S_1= Number of species identified by larval collection; S_2= Number of species identified by Human Landing Catches.

At Youpwe, the Culicidae fauna was dominated by the genus *Culex* with 67.45%, n=2,546) of the collected mosquitoes, followed by the genera *Anopheles* (19.9%, n=750) and *Aedes* (12.65%, n=477).

Diversity of Culicidae fauna at Manoka and Youpwe

The diversity index of Shannon-Wiever (H ' = 1.22 at Manoka and H' =1. 31 at Youpwe) and of Simpson (0.54 at Manoka and 0.62 at Youpwe), are near in both localities (Table 3).

The equitability index of Piélou of the two sites (J ' = 0.51 at Manoka and J ' = 0.59 at Youpwe) are near, which translates a similar homogeneity of the two communities.

Table 2. Abundance and relative abundance of Culicidae fauna at Manoka and Youpwe.

Species	Total		Manoka		Youpwe		P
	n	%	n	%	n	%	
Anopheles coluzzii	3,125	42.09	2,390	65.44	735	19.50	0.008
Anopheles melas	50	0.67	50	1.36	0	0.00	<0.001
Anopheles moucheti	15	0.20	0	0.00	15	0.40	0.017
Anopheles tenebrosus	1	0.01	1	0.03	0	0.00	0.191
Aedes aegypti	813	10.95	346	9.47	467	12.38	0.023
Aedes albopictus	86	1.16	76	2.10	10	0.27	0.006
Aedes vexans	38	0.51	38	1.04	0	0.00	<0.001
Culex duttoni	141	1.90	86	2.35	55	1.46	0.843
Culex decens	147	1.98	66	1.81	81	2.15	0.224
Culex quinquefasciatus	2,666	35.90	518	14.18	2,148	56.90	<0.001
Culex poicilipes	188	2.53	17	0.47	171	4.53	0.032
Culex simpsoni	155	2.10	64	1.75	91	2.41	0.030
Total	7,425	100	3,652	100	3,773	100	

The value of the index of Sorensen (Is = 80%) shows that the communities of Culicidae of the two sites are very similar. In addition, the mosquito profile of diversity at Manoka is dominating when the values of α are ≤ 2 (Fig. 2).However, the crossing of the two profiles of diversity does not enable us to say that a community is more varied than the other.

Discussion

Specific richness of Culicidae fauna at Manoka and Youpwè

The use of two sampling methods such as that was carried out in this study makes it possible to sample a greater number of mosquito species than when they are used individually. This shows the advantage of diversifying the sampling methods to increase the chances to have a better representation of fauna that we want to study. A total of 12 mosquito species belonging to three genera (*Anopheles*, *Culex* and *Aedes*) were sampled during this study, which indicates that mosquito fauna in Manoka and Youpwe is diversified. Except *An. moucheti*, these species were already identified in littoral-Cameroon (Rageau and Adam, 1952 ; Fontenille et Toto, 2001 ; Nchoutpouen, 2003). The presence of *An. moucheti* at Youpwe causes a risk of importation of this species at Manoka where it will be able to accentuate malaria transmission.

Table 3. Diversity index of Culicidae fauna at Manoka and Youpwe.

Locality	Abundance	Number of species	Shannon	Simpson	Piélou
Manoka	3,652	11	1.22 (1.18-1.25)	0.54 (0.52-0.56)	0.51 (0.49-0.54)
Youpwe	3,773	9	1.31 (1.27-1.34)	0.62 (0.60-0.63)	0.59 (0.58-0.61)
Analyse			Close diversity	Close diversity	Similarity of communities

he mosquito were collected in diversified breeding sites (ponds, containers, tires, river, well, gutters, etc) presenting or not signs of pollution. This situation confirms the capacity of the mosquito larvae to develop in various types of habitat in littoral zone of Cameroon. A recent study carried in Benin (Agbanrin *et al*., 2015) puts forward this capacity of mosquito to be developed in any type of habitat in the littoral part of the country. The proliferation of mosquito in this zone increases risks for the local human populations to contract the diseases such as malaria, the yellow fever and the bancroftian filariasis of which they are vectors.

Abundance of Culicidae at Manoka and Youpwe

In the urban zone of Youpwe, the *Culex* genus dominates mosquito fauna and *Cx. quinquefasciatus* is the most represented species. In rural zone of Manoka, the *Anopheles* genus is prevalent and *An. coluzzii* is the most represented species. Similar results were reported in littoral-Cameroon (Mbida *et al*., 2016b) and Morocco (Kbibch *et al*., 2009 ; Hadji *et al*., 2013). In addition, it was underlined the prevalence of the *Culex* genus in a study undertaken in another urban district of Douala (Antonio-Nkondjio *et al*., 2012). In fact, the not planned urbanization and the bad management of waste of the households contribute to the creation of many collections of polluted water favorable to the proliferation of the *Culex* larvae which adapt better to this type of environment than the *Anopheles* larvae, as reported in many studies (Darriet *et al*., 2003 ; Amarasinghe *et al*., 2011 ; Agbanrin *et al*., 2015).

The predominance of *the Anopheles* genus in Manoka would result from the rural character of the environment, relatively little polluted by domestic and industrial waste. This genus develops better in the natural environments in rural zone (Kamdem *et al*., 2012 ; Mbida *et al*., 2016a). *An. coluzzii* is the only member of the *An. gambiae* complex sampled in Youpwe. In Manoka, this species is always predominant, followed by An.*melas* which is very rare. The predominance of *An. coluzzii*, with the detriment of *An. gambiae*, a close species which was rare would be the fact of its greater tolerance to salinity and pollution. This result is in agreement with those obtained by Etang *et al.*, (2016) which

identified *An. coluzzii* like major species of the *An gambiae* complex in the Wouri river estuary. This result is also similar to those of former studies in Cameroon which underlines the predominance of *An. coluzzii* in the littoral zone of country (Wondji *et al.*, 2005 ; Slotman *et al.*, 2006). In addition, *An. melas* was collected only in the insular zone of Manoka. This result is in agreement with that of Antonio-Nkondjio *et al.* (2006) who underlined the presence of this species on the Atlantic coast of Cameroon.

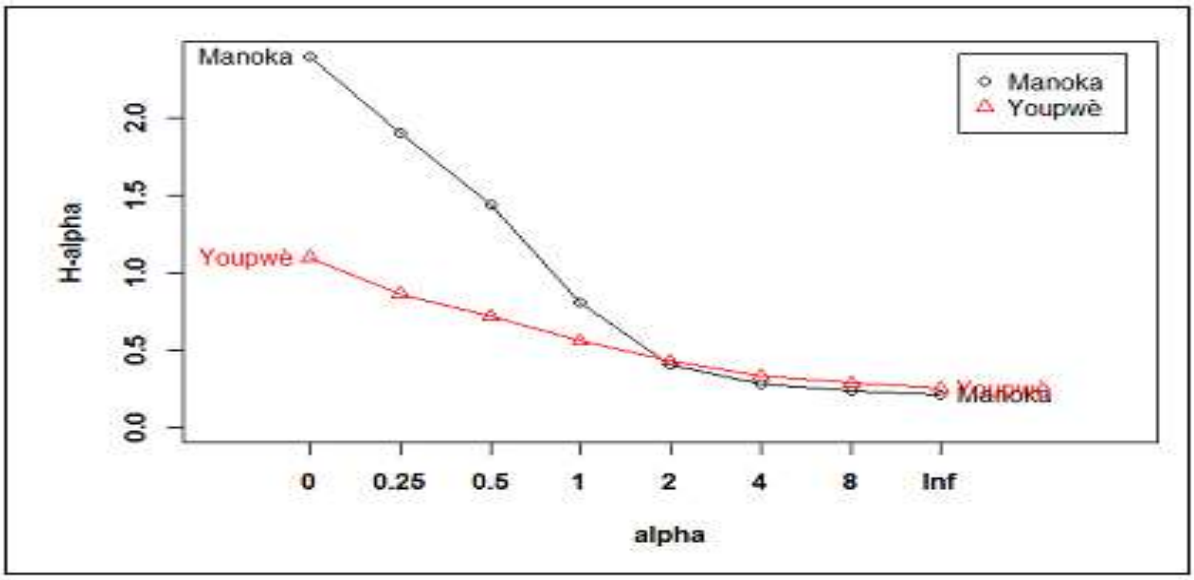

Fig. 2. Diversity profiles between Manoka and Youpwe based on H-alpha series of Rényi.

Diversity of Culicidae fauna at Manoka and Youpwe

The various ecological index calculated in the two study sites are rather close. In addition, the similarity of the mosquito communities observed in the two sites suggests their ecological resemblance. In addition, the mosquito profiles of diversity at Manoka and Youpwe, traced using the H-alpha series of Rényi, cross.

This crossing translates the absence of a relation order between these profiles of diversity and consequently, we cannot say that one of these communities is diversified than the other (Tothmeresz, 1995).

The water collections used as mosquito breeding sites can lodge other invertebrates and micro-organisms. This situation involves interspecific relations like the competition and the predation (Juliano, 1998 ; Juliano *et al.*, 2004). So the success of the mosquito larvae in these biotopes will be dependent on their aptitudes in these various relations (Lounibos *et al.*, 2002).

Conclusion

The Culicidae fauna on Manoka island and that of Youpwe mainland area are rather, rich and diversified, constituted by the genera *Anopheles*, *Culex* and *Aedes* whose proliferation represents an epidemiological risk of transmission of the diseases of which they are vectors.

The presence of *An. moucheti* at Youpwe must be taken into account in vector control strategy on Manoka island.

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