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Diversity and spatiotemporal distribution of mosquito species in Ngaoundere, Cameroon

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Abstract
Knowing diversity and distribution of mosquitoes, vectors of numerous drastic diseases in a particular locality is as important key for development a sustainable strategy to control efficaciously these insects and the diseases they can transmit. In that context, mosquito population survey was conducted from September to November 2012 in three localities including Dang, Onaref and Sabongari III in Ngaoundere. Mosquito larvae were collected twice in a month from gutters, lakes and rivers and reared until their emergence into adults and were kept in the Eppendorf tube containing silica gel for identification. In results, a total of 1619 mosquitoes collected from the different breeding sites of Ngaoundere and 10 mosquito species grouped in 6 genera were identified. Culicinae sub-family (90.11%) was the most represented and included *Culex quinquefasciatus* (56.39%), *Eretmapodite chrysogaster* (27.67%), *Aedes aegypti* (0.25%), *Coquilletidia maculipennis* (2.66%), *Culex annuliotris* (0.25%), *Culex pipiens* (0.19%), *Culex tigripes* (2.10%) and *Mansonia africana* (0.62%). The sub-family Anophelinae (9.89%) rate was low and comprised *Anopheles gambiae* (8.96%) and *An. funestus* (0.93%). The locality of Dang was the richest in mosquito proportion (51.30%) and species (8 species). *Cx. quinquefasciatus* was the most abundant in the three localities. Mosquitoes were highly collected from the gutters (49.80%) and lake (10 species) was the richest in mosquito species. During the sampling period, a high mosquitoes collection was registered in October (44.20%) compared to September (29.50%) and November (26.30%). From these results, Ngaoundere is well diversified in mosquito species, especially with the presence of the malarial vectors *Anopheles* species and communities should mostly focused on mosquito vector control measures especially during September-November period.

Keywords: Mosquito species, diversity, distribution, breeding sites, localities, Ngaoundere

1. Introduction
Culicidae commonly known as mosquitoes constitute a class of insect potentially involved in the transmission of pathogens to humans and animals. Both males and females mosquito adults feed on fruit juices and plant nectars, but females moreover feed on human or animal blood for egg maturation. From that behavioral habit as blood-sucking insects, they transmit several pathogen agents such as diverse arboviruses (encephalitis, dengue and yellow fevers, etc.,), protozoan (malaria) and lymphatic filariasis (bancroftian diseases) parasites, etc., to humans [1, 2]. Worldwide, 4 main mosquito genera including *Anopheles, Aedes, Culex* and *Mansonia* are the most implicated in the diseases transmission of which the first 3 genera are the most commonly encountered in Africa [3]. In the world, malaria caused by *Plasmodium* spp and transmitted through the females of *Anopheles* spp remains the most dreadful disease causing million cases and thousand deaths [4]. The worldwide incidence several arbovirus infections including yellow fever, chikungunya dengue fever, etc., in which *Aedes* spp are acting as potential vectors are increasing drastically in the last two decades [5]. Worldwide, *Culex* spp are involved in the transmission of lymphatic filariasis, with 120 million cases reported and 30% of these infected people are found in Africa [6].
To ameliorate qualities of public health, environment and to control outbreaks of mosquito-borne diseases in the communities, mosquito control measures become essential since numerous mosquito species act as super vectors of deathful diseases leading a serious destabilization of the developments of the sub-Saharan countries [7]. Besides, some mosquito species constitute an intolerable biting nuisance that disturb human outdoor leisure festivities

[8]. However, several strategies and measures were put in place to tackle whether the parasites or the vectors of these diseases.

Nowadays, the world is facing the problem of biodiversity loss caused by climate change, population expansion and the disappearance of species and their habitats caused mostly by human activities. That situation has created changes in diversity and dynamic distribution of the living species and during the recent years has led to the emergence of new mosquito-borne diseases like Zika and the resurgence of the past-known insect-transmitted diseases [9]. Moreover, that can also affect the synergistic role play by pathogens, arthropod vectors, the environment and host species, causing a high risk of public health and livestock pathogens transmission [10]. Hence, knowing the diversity and spatiotemporal variation of Culicidae fauna in relation with the characteristics of their habitats would be necessary for the development a suitable, effective and sustainable mosquito vector control measures. Therefore, many previous studies reported significant variations in diversity and density of mosquito population in different breeding sites [10-14]. In this context, the purpose of the present study was to evaluate the diversity and spatiotemporal distribution of mosquito species in three localities of Ngaoundere during the high prevalence of mosquito-borne diseases period ranging from September to November 2012 in Cameroon.

2. Materials and Methods

2.1 Study site

The collection of mosquito was carried out from September to November 2012 in three localities of Ngaoundere (figure 1). Indeed, Ngaoundere is county town of Adamaoua region with hilly relief surrounded with mountains. Its climate belongs to soudano-sahelian type with two seasons: rainy (April-October) and dry (November-March), with an average rainfall of 1479 mm. Annual average temperature fluctuates around 22 °C with relative humidity range of 64.1-67.6%. Vegetation is an arborous and shrub savannah of *Daniellia oliveri* and *Lophira lanceolate* plant species. Mosquito larvae were sampled in three localities including Dang, Sabongari III and Onaref in which potential mosquito breeding sites were previously identified.

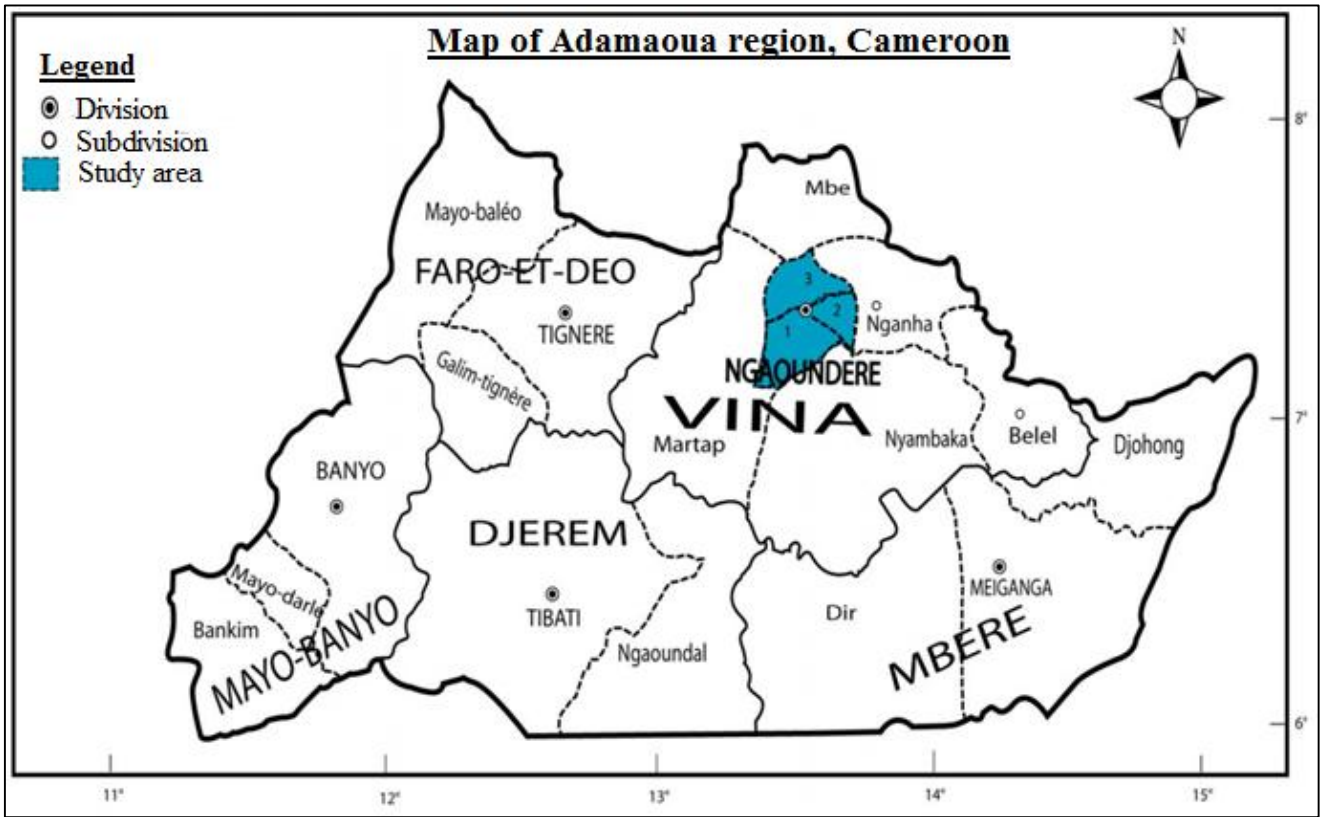

Fig 1: Map of Adamaoua region showing study site

2.2 Sampling of mosquito larvae and rearing

Dipping method described by Saotoing *et al.* [13] was followed for mosquito larvae sampling in temporary and permanent mosquito breeding sites such as lakes, gutters and rivers in three localities including Dang, Sabongari and Onaref of Ngaoundere. These potential mosquito breeding sites included gutters, rivers and lakes found in the three localities targeted (Figure 2). These three localities were targeted in this present study based on the availability of diverse breeding sites and the high prevalence of malaria reported from these localities. In each breeding site, 4 zones were targeted and with entomological ladle, 10 times dipping were realized in each zone. Larvae collected in each mosquito breeding site were transferred into plastic bottles containing each larvae breeding site water and transported to the insectarium of the laboratory of applied Zoology, Faculty of Science, University of Ngaoundere, Cameroon. In the insectarium, larvae were separated in sub-family according to their position in the surface of water and reared in trays containing each larvae breeding site water according standard protocol of WHO. Mosquito larvae were feed with the mixture of crayfish and biscuit in 1:3 ratio and water was renewed every 2 days to avoid larvae suffocation caused by decaying mosquitoes' food. At the pupal stage, mosquitoes were transferred into the

cages for emergence and the emerged mosquito adults were feed with 10% of sugar solution for 3 days. After that period, the emerged mosquito adults were killed with a commercial insecticide spray (Rambo) and preserved in Eppendorf tubes containing silica gel previously labeled, ready for identification.

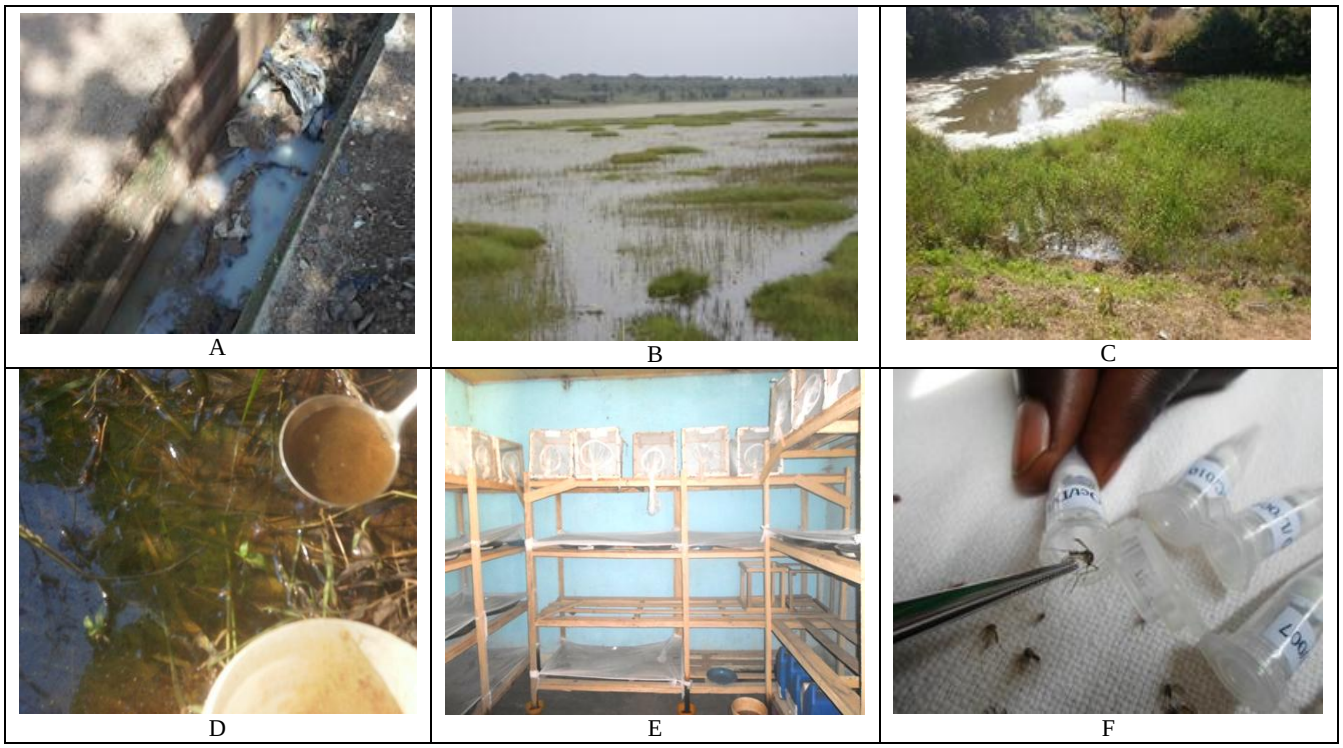

Fig 2: Different mosquito breeding sites (A=gutter, B=lake and C=river), mosquito larvae sampling (C), rearing of mosquitoes in the insectarium (D), and conditioning of mosquito adults for identification (E).

2.3 Identification of adult mosquito species

Identification of mosquito adults species obtained from larvae rearing and those captured at adult stage was carried out at the WHO/National Arbovirus and Vector Research Centre of Enugu, Enugu state, Nigeria in March 2013. Mosquito species were identified under binocular magnifying microscope (Figure 3) following identification keys performed by [15, 16, 17].

Fig 3: Mosquito adults stored in the Eppendorf tubes for identification (A) and identification process of adult mosquito species using binocular magnifying microscope (B).

2.4 Statistical Analyses

Data obtained from this present investigation were submitted to descriptive analyses to determine frequencies of mosquito diversity and abundance using SPSS version 16.0 software. Chi-square (χ^2) test of Pearson was employed to carry out relationship between mosquito species and localities, breeding sites as well as mosquito sampling period using the same software.

3. Results

3.1 Characteristics of mosquito breeding sites sampled

In this present study, three types of mosquito breeding sites including gutters, lakes and rivers were selected. In the three different mosquito breeding sites, the average temperatures were 19.6, 23.0 and 21.2 °C during the months of September, October and November, respectively. The rainfalls registered were 276.4 mm in September, 148.2 mm in October and 2 mm in November. Turbidity of the breeding site waters

measured according to the index (I) ranging from 1= clearly to 5= highly turbid. During the sampling period gutters' water was highly turbid (I=5), lakes' water was less turbid (I=3) and rivers' water was lightly turbid (I=2).

3.2 Diversity of mosquito species in Ngaoundere

Table 1 presents the diversity of mosquito species collected in Ngaoundere from September to November 2012. During the sampling period of three months, a total of 1619 mosquito species were collected and identified. After identification, 10 mosquito species belonging to 6 genera and subdivided in two subfamilies were listed. The sub-family of Culicinae representing 90.11% of the total insects collected listed 8 species regrouped in 5 genera and majorly represented by *Culex quinquefasciatus* (56.39%) followed by *Eretmapodites chrysogaster* (27.67%) while other species such as *Aedes aegypti* (0.25%), *Coquilletidia maculipennis* (2.66%), *Culex annuliotris* (0.25%), *Culex pipiens* (0.19%), *Culex tigripes* (2.10%) and *Mansonia africana* (0.62%) were fairly represented. Anophelinae sub-family represented 9.89% of overall mosquitoes captured in this present study and registered two species including *Anopheles gambiae* (8.96%) and *An. funestus* (0.93%).

Table 1: Diversity of mosquito species collected in three districts of Ngaoundere town from September to November 2012.

Sub-family	Genus	Mosquito species	No. of mosquito collected	%
Anophelinae	*Anopheles*	*An. gambiae*	145	8.96
		An. funestus	15	0.93
Culicinae	*Aedes*	*Ae. aegypti*	4	0.25
	Coquilletidia	*Coquilletidia maculipennis*	43	2.66
	Culex	*Culex quinquefasciatus*	913	56.39
		Culex annuliotris	4	0.25
		Culex pipiens	3	0.19
		Culex tigripes	34	2.10
	Eretmapodites	*Eretmapodites chrysogaster*	448	27.67
	Mansonia	*Mansonia africana*	10	0.62
		Total	1619	100

3.3 Spatial variation of mosquito species in Ngaoundere

Table 2 presents the special distribution of mosquito species collected in different breeding sites of Dang, Onaref and Sabongari III of the Ngaoundere from September to November 2012. Generally, the number mosquito species significantly (χ^2=461.38, df=18, P<0.001) varied with localities sampled. The locality of Dang with 8 mosquito species identified was the richest species compared to Onaref (6 species) and Sabongari III (6 species).

In the three localities sampled, *Cx. quinquefasciatus* was the most represented with 361(39.50%) in Dang, 163(17.90%) in Onaref and 389(42.60%) in Sabongari III. *Er. chrysogaster* were also found abundantly in Dang (n=333(74.30%)) mosquito breeding sites compared to Onaref (n=34(7.60%)) and Sabongari III (n=81(18.10%)) localities. The malarial vector *An. gambiae*, were also highly represented in Dang (82(56.60%)) compared to Onaref (n=49(33.80%)) and Sabongari III (n=14(9.70%)), while *An. funestus* (n=12(80%)) was found in low proportion only in Dang and Sabongari III (n=3(20%)) localities. The mosquito species *Ae. aegypti* (n=4), *Cx. pipiens* (n=3) and *Cx. tigripes* (n=34) were found only in Dang mosquito breeding sites while *Cx. annuliotris* was collected only in Onaref (n=3) and Sabongari III (n=1) breeding sites. *Ma. africana* was weakly represented in the three localities with 2, 1, 7 mosquito larvae collected respectively in Dang, Onaref and Sabongari III breeding sites.

Table 2: Distribution of mosquito species collected in different breeding sites of Dang, Onaref and Sabongari III of the Ngaoundere from September to November 2012.

Mosquito species	Localities			Total n(%)	Chi-square test
	Dang n(%)	Onaref n(%)	Sabongari III n(%)		
Ae. aegypti	4(100%)	0(0%)	0(0%)	4(100%)	χ^2=461.38 df=18 P<0.001
An. funestus	12(80%)	0(0%)	3(20%)	15(100%)	
An. gambiae	82(56.60%)	49(33.80%)	14(9.70%)	145(100%)	
Cq. maculipennis	0(0%)	43(100%)	0(0%)	43(100%)	
Cx. quinquefasciatus	361(39.50%)	163(17.90%)	389(42.60%)	913(100%)	
Cx. annuliotris	0(0%)	3(75%)	1(25%)	4(100%)	
Cx. pipiens	3(100%)	0(0%)	0(0%)	3(100%)	
Cx. tigripes	34(100%)	0(0%)	0(0%)	34(100%)	
Er. chrysogaster	333(74.30%)	34(7.60%)	81(18.10%)	448(100%)	
Ma. africana	2(20%)	1(10%)	7(70%)	10(100%)	
Total	831(51.30%)	293(18.10%)	495(30.60%)	1619 (100%)	
Number of species	8	6	6	10	

The spatial variation of mosquito species within the different mosquito breeding sites of Ngaoundere is presented in table 3. Globally, the mosquito species proportion significantly (χ^2=513.35, df=18, P<0.001) varied with the different mosquito breeding sites sampled in the localities of Dang, Onaref and Sabongari III of Ngaoundere. In general, mosquito species were abundantly collected from Gutter (n=806 (49.80%) followed by Lake (n=549(33.9%)) and River (264(16.30%)). In the Gutter breeding site, the mosquito population is dominated by *Cx. quinauefasciatus* (618) and

Er. chrysogaster (n=177). In that breeding site (gutter), mosquito species *An. gambiae* (n=3), *An. funestus* (n=2), *Cx. annuliotris* (n=1), *Cx. tigripes* (n=4) and *Cx. pipiens* (n=1) were found at low proportion while *Ae. aegypti*, *Cq. maculipennis* and *Ma. africana* were absent. In the lake breeding site, all mosquito species identified were present in which, *Er. chrysogaster* (n=235) and *Cx. quinquefasciatus* (n=129) were the most abundant. In that breeding site (Lake), *An. gambiae* (n=90), *Cq. maculipennis* (n=39) and *Cx. tigripes* (n=28) were fairly found while *Ma. africana* (n=10), *An. funestus* (n=9) *Ae. aegypti* (n=4), *Cx. annuliotris* (n=3), *Cx. pipiens* (n=2) were found in low proportion. In the river, *Cx. quinquefasciatus* (n=166) was the most represented followed by *An. gambiae* (n=52), *Er. chrysogaster* (n=36), *An. funestus* (n=4), *Cq. maculipennis* (n=4) and *Cx. tigripes* (n=2).

Table 3: Distribution of mosquito species collected in different mosquito breeding sites in Ngaoundere

Mosquito species	Breeding sites			Total n(%)	Chi-square test
	Gutter n(%)	Lake n(%)	River n(%)		
Ae. aegypti	0(0%)	4(100%)	0(0%)	4(100%)	χ^2=513.35 df=18 P<0.001
An. funestus	2(13.30%)	9(60%)	4(26.70%)	15(100%)	
An. gambiae	3(2.10%)	90(62.10%)	52(35.90%)	145(100%)	
Cq. maculipennis	0(0%)	39(90.70%)	4(9.30%)	43(100%)	
Cx. quinquefasciatus	618(67.70%)	129(14.10%)	166(18.20%)	913(100%)	
Cx. annuliotris	1(25%)	3(75%)	0(0%)	4(100%)	
Cx. pipiens	1(33.30%)	2(66.70%)	0(0%)	3(100%)	
Cx. tigripes	4(11.80%)	28(82.40%)	2(5.90%)	34(100%)	
Er. chrysogaster	177(39.50%)	235(52.50%)	36(8%)	448(100%)	
Ma. africana	0(0%)	10(100%)	0(0%)	10(100%)	
Total	806(49.80%)	549(33.9%)	264(16.30%)	1619(100%)	
Number of species	7	10	6	10	

3.4 Temporal distribution of mosquito species in Ngaoundere

Monthly variation of mosquito species population collected from September to November 2012 in Ngaoundere is presented in table 4. Globally, the population of mosquito species collected from the three localities of Ngaoundere varied significantly (χ^2=104.88, df=18, P<0.001) from one month to another. In general, mosquito species were abundantly collected in October (n=715(44.20%)) compared to the two other months with 478 mosquitoes collected in September and 426 mosquitoes sampled in November. The mosquito species *Cx. quinquefasciatus* was the most abundant in the 3 different months of sampling in proportion of 23.70, 42.30 and 34.10% in September, October and November, respectively. Moderate proportions of 38.60, 46.90 and 14.50% of *Er. Chrysogaster* were registered respectively in September, October and November in the three locality of Ngaoundere. The malarial vector An. gambiae was also fairly collected and mostly in the month of October (n=75) compared to the months of September (47) and November (n=25). The mosquito species *Ae. aegypti* (n=4), *An. funestus* (n=15), *Cq. Maculipennis* (n=43), *Cx. tigripes* (n=34) and *Ma. Africana* (n=10) were found at low proportion and distributed alongside the 3 sampling months studied. *Cx. pipiens* was collected only in October (n=1) and November (n=2) while *Cx. annuliotris* was sampled only in the month of October (n=4).

Table 4: Monthly variation of mosquito species population collected from September to November 2012 in Ngaoundere.

Mosquito species	Months			Total n(%)	Chi-square test
	Sep n(%)	Oct n(%)	Nov n(%)		
Ae. aegypti	1(25%)	2(50%)	1(25%)	4(100%)	χ^2=104.88 df=18 P<0.001
An. funestus	10(66.70%)	1(6.70%)	4(26.70%)	15(100%)	
An. gambiae	47(32.40%)	73(50.30%)	25(17.20%)	145(100%)	
Cq. maculipennis	13(30.20%)	24(55.80%)	6(14%)	43(100%)	
Cx. quinquefasciatus	216(23.70%)	386(42.30%)	311(34.10%)	913(100%)	
Cx. annuliotris	0(0%)	4(100%)	0(0%)	4(100%)	
Cx. pipiens	0(0%)	1(33.30%)	2(66.70%)	3(100%)	
Cx. tigripes	14(41.20%)	9(26.50%)	11(32.40%)	34(100%)	
Er. chrysogaster	173(38.60%)	210(46.90%)	65(14.50%)	448(100%)	
Ma. africana	4(40%)	5(50%)	1(10%)	10(100%)	
Total	478(29.50%)	715(44.20%)	426(26.30%)	1619(100%)	
Number of species	8	10	9	10	

4. Discussion

Results from this present investigation showed that the different mosquito breeding sites of Ngaoundere abound in, a large diversity of mosquito species belonging to the sub-families of Anophelinae (*Anopheles* genus) and Culicinae (*Aedes*, *Culex*, *Mansonia*, *Coquilletidia* and *Eretmapodites* genera) comprising a total of 10 mosquito species identified. In the same way, study conducted by Ganser and Wisely [10] in the mixed-grass prairie habitat of the Smoky Hills of Kansas recorded a total of 11 species, belonging to *Aedes*, *Anopheles*, and *Culex* genera in which 98% of the collected mosquito species included *Ae. nigromaculis*, *Ae. sollicitans*, *Ae.*

taeniorhynchus, *Cx. salinarius*, and *Cx. tarsalis*. From Yaounde in the Centre region of Cameroon, 11 species including *Anopheles funestus* Giles (36.22%), *An. gambiae* (15, 09%), *An. nili* (12.72%), *An. ziemanni* (3.83%), *Aedes albopictus* (8.66%), *Ae. aegypti* (3.81%), *Cx. poicilipes* (10.86%), *Cx. tigripes* (4.58%), *Cx. zombaensis* (3.21%), *Cx. duttoni* (0.69%) and *Cx. perfuscus* (0.33%) were identified [11]. In three Malaysian malaria endemic areas, 36 mosquito species distributed in 6 genera were also identified in which the predominant mosquito species included *An. maculatus* (31.0%), *Armigeres flavus* (11.3%), *Armigeres annulitarsis* (11.0%), *Cx. vishnui* (9.6%) and *Ae. albopictus* (7.0%) [12]. Rural environment, vegetation in the lakes and agriculture practiced around the sampled localities of the study site (Ngaoundere) might create a microclimate favorable to the development and proliferation of these different mosquito species identified.

In this present study, *Culex* spp was the most abundant compared to the other mosquito genera and especially *Cx. quinquefasciatus* was the most represented. From 3251 mosquito species collected at larval stage in different breeding sites of Yaounde, 95% (n=3088) belonged to the genus of *Culex* and *Cx. quinquefasciatus* (79.4%) was the most abundant compared to the other mosquito species identified [8]. Similarly, from Maroua town in the Far North region of Cameroon, Saotoing *et al*. [13] identified five mosquito species in which *Cx. quinquefasciatus* (53.33%) and *Ae. aegypti* (32.84%) were the most abundant compared to *Cx. tigripes* (09.45%), *An. gambiae* (03.70%) and *An. funestus* (0.66%) mosquito species. Besides, study carried out in Minna, North central Nigeria revealed a high density of *Culex* spp (47.74%) and *Aedes* spp (53.54%) compared to the low rate of *Anopheles* species (0.87%) registered in that zone [14]. Mosquito species sampled in the extensive wetland of Camarge in the southeast of France using carbon hydroxide as bait in CDC light traps led to the collection of 16 mosquito different mosquito species in which, 98.7% of the all Culicidae captured were majorly *Culex* spp and some *Ae. caspius* and *An. hyrcanus* species [18]. The high population of *Culex* spp registered in this present study compared to other mosquito species might be due to their ability to breed in any type of temporary or permanent stagnant water collections since they are found in diverse breeding sited such as pools, puddles, opened latrines, drains, septic tanks, gutters, organically polluted waters etc, [19]. Moreover, the presence of mountains and hills surrounding the three localities sampled in this current study might also explain the high density of that mosquito species by creating a microclimate favorable to development to that particular mosquito species.

Lakes and gutters inspected in this present study were the breeding sites in which a high population of mosquitoes was noticed and were significantly abundant in mosquito species. Similar observations were reported by Ntonga *et al*. [11] in which a majority of the mosquito species identified were collected from lakes and up to 9 species were collected from that breeding site. Similar findings were also reported in the survey conducted in Maroua by Saotoing *et al*. [13] and in which, gutters constituted a breeding sites where the majority of mosquito species were collected. This might be due to the high pollution with an important level of organic matters in the lake and gutter waters and might constituted a suitable environment for mosquito species proliferation and mostly for *Culex* spp.

The three consecutive months of survey targeted in this present study represent the last period marking the end of rainy season in the studied area and October was found to be the month in which the density of mosquito larvae in their breeding sites is high. In the same way, Saotoing *et al*. [13] reported a high density of mosquitoes collected in the rainy season in Maroua and the significant density (n=437) was registered in the month of October 2010. In the Republic of Rwanda, *Anopheles* species were highly collected in September-October period from almost all the localities targeted in the study conducted by Hakizimana *et al*. [20]. That might be explained by the fact that in many tropical and subtropical zones, the period September-November represents the transition time between rainy and dry season in which a large range of mosquito breeding sites are naturally put in place because of the scarcity of rainfall. The may be also due to the climatic parameters suitable to the rapid development of mosquitoes since temperature has increased while rainfall has decreased during that particular period.

5. Conclusion

In Ngaoundere, Culicidae fauna was well diversified and significantly varied with localities, breeding sites and collection time period. From the 1619 mosquitoes collected from the different breeding sites in Dang, Onaref and Sabongari III, 10 mosquito species grouped in 6 genera were identified. Culicinae sub-family (90.11% of total collection) was the most abundant compared to Anophelinae sub-family (9.89%). Up to half (51.30%) of total mosquitoes were collected in Dang locality and *Cx. quinquefasciatus* is the most abundant in the three localities sampled. Mosquitoes were highly collected from the gutters (49.80%) and lakes (10 species) were the richest breeding sites in mosquito species. Mosquitoes were highly collected in October (44.20%) compared to September (29.50%) and November (26.30%). Therefore, Ngaoundere is largely diversified in mosquito species and especially with the presence of the malarial vectors *Anopheles* species able to maintain the rate of malaria transmission in the communities. Thus, there is an urgent need to sensitize and encourage population to apply different mosquito control measures especially during September-November period.

6. Competing Interest

The authors declare that they have no competing interests.

7. Acknowledgements

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