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Efficacy of Trapping Methods in the Collection of *Eretmapodites* (Diptera: Culicidae) Mosquitoes in an Afrotropical Rainforest Region, South western Cameroon

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Abstract

Very little data exist on the biology of an afrotropical rainforest mosquito *Eretmapodites* (*Er.*) in a world undergoing dramatic changes due to deforestation. The aim was to assess the efficacy of different trapping methods in the collection of *Er.* mosquito in forested area.

This was a longitudinal study involving collection of mosquitoes for over two years. Multiple collection methods (grouped into two categories), were used; i) net baited and un-baited traps to collect adults, ii) techniques that target immature stages subsequently reared to adults. All males were identified by genitalia dissection. Five thousand seven hundred and four mosquitoes representing 11 genera among which 2,334 *Er.* were identified. Mosquito abundance was highest in the net traps (n = 1276 (56.4%)) and sweep nets (n = 393(17.4%)) respectively. The abundance was highest in green colored net traps (435(34.09%)) with significant value of $\chi 2$= 40.000, $P < 0.001$ and in pigeons baited traps (473 (37.06%)) with significant value of $\chi 2$= 42.000, P = 0.003. The diversity ranges from H′ = 2.65; DS = 0.84; SR = 24; ACE = 24.77 in sweep net to H′ = 0; DS = 0; SR = 1; ACE = 1 in rock pool among males mosquitoes. While for females, H = 1.14; DS = 0.71; SR = 5; ACE = 5.16, in sweep net to H = 0; DS = 0; SR = 1; ACE = 1 in rock pool, tarpaulin, resting cage.

Net traps, bamboo pot, and sweep netting are efficient in collecting high abundance of forest mosquitoes in the Talanagaye rainforest.

Key words: *Eretmapodites*, Talangaye rainforest, trapping method, mosquito, species abundance

Mosquitoes are among the most important group of arthropods affecting the health of humans, livestock, and wild animals. Because of their role as vectors of viruses (arboviruses), filarial worms (helminths), and protozoa (Zélé et al. 2014), the control tool for mosquitoes used by the population is long-lasting insecticide nets (LLINs (Minsanté 2017)). However, the rapid expansion of insecticide resistance in vector populations endangered the effectiveness of this tool (Russell et al. 2011, Govella and Ferguson 2012). In addition, the rate at which deforestation is alarming in Africa may lead to loss of species like *Eretmapodites* which are not well documented, if studies like this are not carried out. Knowing that *Er.* are principally forest mosquitoes, their bionomics as in any other vector can also be considered to limit the effectiveness of vector control tools (Bamou et al. 2018). Thus, the necessity for this study.

Before this study, 35 *Eretmapodites* species have been described in Cameroon and, none has been implicated in human malaria transmission particularly in the forest region even though laboratory transmission of Yellow fever and *Plasmodium gallinaceum* has been reported. Of the 3,586 formally recognized mosquito species (Harbach 2013), about 150 species, largely confined to the genera *Anopheles*, *Aedeni* (traditional classification), and *Culex*, are vectors of pathogens. The Abundance and behaviors of these mosquitoes are markedly affected by aquatic and ambient environmental conditions, and host preference (Becker et al. 2010).

Human landing catches (HLC) is the main sampling technique used to assess mosquito bionomics and malaria transmission patterns (Awono-Ambene et al. 2018). Although this technique is commonly used across sub-Saharan Africa (De Castro et al. 2007) provides a good estimation of mosquito biting behavior or of transmission patterns (Mboera 2005), the method is subjected to a certain number of limits. First, it is labor intensive; collectors have to remain alert all night long when collecting mosquitoes. Secondly, collectors have different attractiveness to mosquito (Tusting et al. 2013) and also different skill in mosquito collection (Antonio-Nkondjio et al. 2018). All these discordances can introduce sampling bias when it comes to the evaluation of the efficacy of trapping methods and control interventions (Maliti et al. 2015). Thirdly, HLC exposes collectors to risk of infection by parasites or arboviruses, and poses ethical issues/problems.

Moreover, it is still not well-known whether the use of other sampling techniques such as noninsecticide baited net traps, sweep netting, and other collection methods designed for immature stages (bamboo put, snail shells), could provide better information on the bionomic of local mosquito species.

The immature stages of mosquitoes develop in multiple temporary and permanent aquatic environments (Carlson et al. 2015), that include large bodies (such as groundwater pools, vernal pools, edges of lakes, dams, and river eddies and edges) and smaller bodies (such as leaf axils, tree-holes, rock-holes, crab-holes, bamboo internodes, bromeliad, and other plant leaf and flower bracts, fallen fruit husks and leaves, dead snail shells, and pitcher plants (Soghigian et al. 2017)). In addition, immature stages of some species develop in artificial containers holding water, such as tins, bottles, tires, and plastic wrappings (Tusting et al. 2013).

Several studies across the continent have reported the high sensitivity of Center for Disease and Control miniature Light trap (CDC-LT) for collecting host-seeking mosquitoes particularly when the traps are placed close to a person sleeping under a bed net (Sikaala et al. 2013). Also, Window exit trap has been used in different settings across the continent which in our study could not be applied (since we were sampling out in the forest undergoing active deforestation) though it is particularly appropriate for studying resting behaviors, and blood-feeding preference of mosquitoes (Sikulu et al. 2009). However, CDC traps used in this study in forested areas under the canopy were unproductive.

A few species of *Eretmapodites* are predators of other mosquitoes and other aquatic invertebrates (Sevice, 1990). Ambient conditions such as temperature and humidity affect especially the longevity of mosquitoes (Johnson et al. 2017, Barreaux et al. 2018, Holmes and Benoit 2019) and the mosquito genus *Eretmapodites* would be no exception to being affected by the environment. This is why a study of this nature should be carried out before the forest which is their natural habitat is completely cut down.

Eretmapodites species, Culicinea subfamily, and tribe Aedini, (Harbach 2013) are all confined to the Afrotropical region. *Eretmapodites* are considered most closely related to *Armigeres* morphologically (Edwards 1932, Reinert et al. 2009, Wilkerson et al. 2015, Soghigain et al. 2017) and behaviorally (Haddow 1956). Most species are confined to rainforests in Central and West Africa, but a few have adapted to exist in riverine and small enclaves of thicker vegetation in the savanna regions of the Afrotropics. Most species are considered opportunistic mammophilic blood-feeders (Service 1990) and some species are capable of autogeny (Mcintosh et al. 1973). Currently, the *Eretemapodites* species are assembled into groups based on species that share similar basic male genitalia features and females within groups are mostly indistinguishable (Service 1990). Male species within groups can only be distinguished by careful examination of the genitalia. The females and males can be identified to group level by differences in scaling patterns on the scutum and color and setal ornamentation of the third, fourth and fifth hind tarsi.

Studies on *Eretampodites* are quite sparse with unfortunately very little knowledge about their biology. However, there are reports incriminating multiple *Eretmapodites* species as minor vectors of arboviruses (Service 1990, Epelboin et al. 2017, Braack et al. 2018). More specifically, few viruses like Chikungunya, Rift Valley Fever, Semliki forest virus, and an unidentified virus MTMP 13 have been isolated from *Eretmapodites* species (Hartberg and Gerberg 1971). Several mosquito species have been incriminated as vectors of avian malaria parasites (Njabo et al. 2011, Zele et al. 2014, Schmid et al. 2017), but so far, no *Eretmapodites* species. However, *Eretmapodites quinquevittatus* Theobald can be experimentally infected with *Plasmodium gallinaceum* Brumpt (Hartberg and Gerberg 1971). *Eretmapodites* are often noticeably one of the most common mosquitoes biting humans during the day in forests in West Africa (personal observations AJC). This fact combined with their opportunistic blood-feeding habits could result in *Eretmapodites* serving as significant bridge vectors of diseases and very important in natural pathogen cycles in forests. Warranting an efficient trapping and control method. Multiple arboviruses occur in forests where *Eretmapodites* reside (Braack et al. 2018), and efficient methods to capture *Eretmapodites* for pathogen isolation and surveillance would be useful to build predictive models of disease emergence in humans and wildlife, in natural and more human disturbed environments. Deforestation which is a major threat to biodiversity (Ribeiro et al. 2012, Lutomiah et al. 2013) also has implications in increasing risks to transmission of certain diseases such as human malaria (Kutz et al. 2005) and viruses affecting livestock and wildlife.

Conversely, deforestation might result decline and elimination of pathogens cycling among forest-dwelling hosts and vectors. The study was conducted to assess whether the use of techniques like net traps of different colors, bait, and larval collection methods for sampling mosquitoes are efficient in collecting mosquitoes, and can provide further information to better understand the bionomic of mosquito population in the Talangaye rainforest, in the southwest region of Cameroon, before it is completely cut down for palm oil production.

Materials and Methods

Study Area

This study was carried out in the Talangaye forest, Nguti subdivision in the South-West Region of Cameroon within a 3-km radius of the GPS coordinates of 5°.175428" N and 9°.455888" E. (Fig. 1). The village nearest to our collecting sites is Manyamene. This is a rainforest corridor of approximately 2,000 hectares located between four protected areas in Cameroon: Korup National Park, Bayang

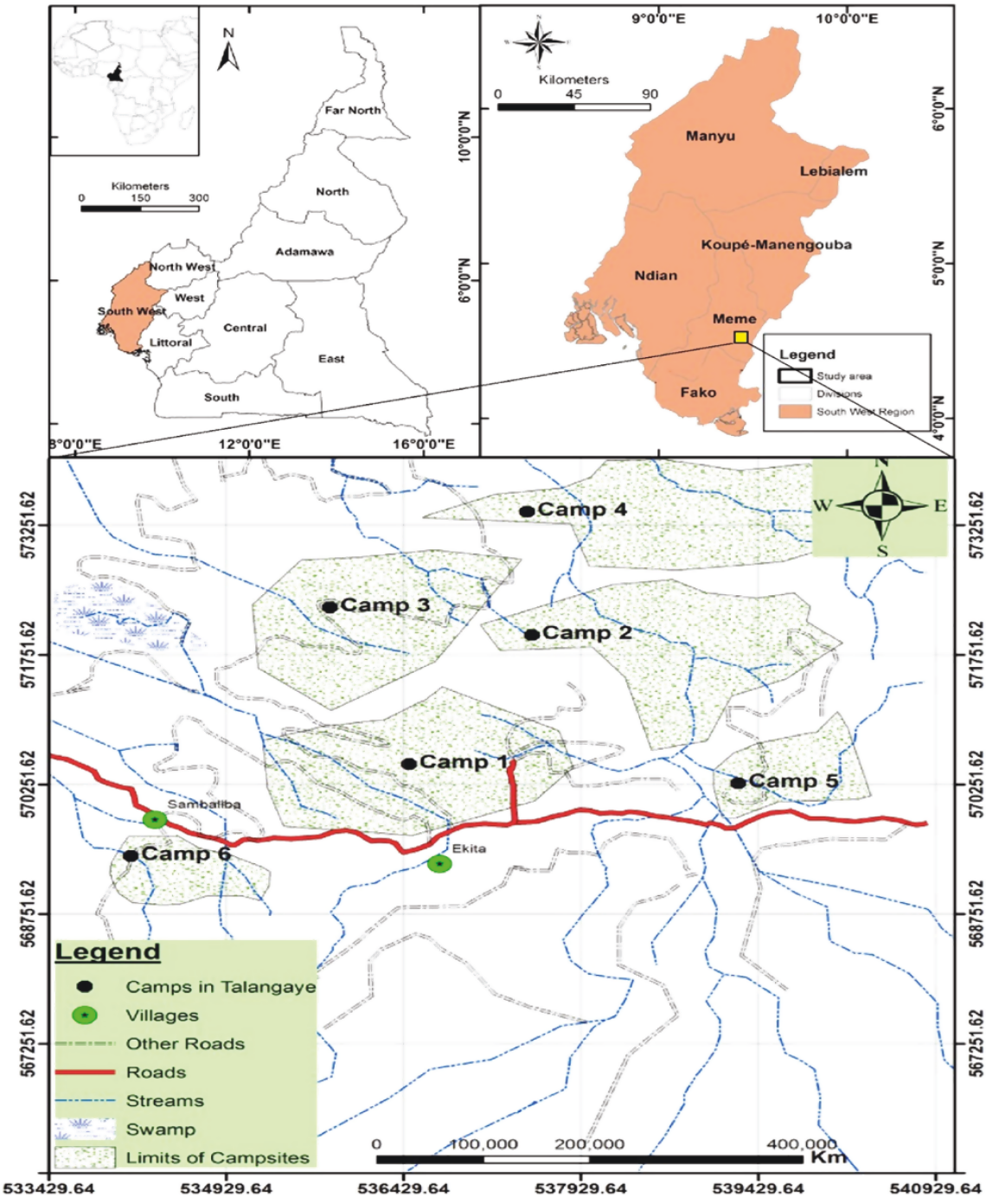

Fig. 1. Study sites in the Talangaye rainforest.

Mbo Wildlife Sanctuary, Rumpi Hills Forest Reserves, and the Bakossi Mountain Reserves. Before 2018, this hilly rainforest corridor was covered with lowland broad-leaf Mahogany and Sapele hardwood timber trees, dense understory of broad-leaf plants. The study area experiences a long rainy season from mid-March to mid-October, and a short dry season for the remainder of the year. The relative humidity was 97% during the wet seasons and temperatures ranged from 19–25°C, measured using data-loggers (HOBO-U23 Pro. Version 2, Onset Computer Corporation, Bourne, MA) positioned in the shade 1 m above the ground. In the forest, six campsites (Camp 1 to 6) that were earmarked for logging and deforestation were sampled (Fig. 1). This was a longitudinal survey in which the specific areas or sites were surveyed after every three months (January, April, July, and October), spending at least 21 d per trip in the field for collection of mosquitoes for two years.

Mosquito Capture

Mosquitoes were captured in areas where the forest had been earmarked for either logging and/or all the vegetation is to be cut down. Samples were collected before cutting down of trees. Multiple collecting methods were used. The collection methods were grouped into two categories. 1) Techniques that targeted

adult mosquitoes such as; i) 4 modified miniature CDC light trap (Sudia and Chamberlain 1962) baited with a sugar-yeast mixture (Smallegange et al. 2010) to release CO2. This was operated from sunset to sunrise. The use of this trap was discontinued after the first year of field trip due to unproductivity (no mosquito captured). The number of trap days for the CDC light traps was (4 CDC traps x 21 d x 4 times x 1 yr) 336; ii) 5 net traps (of different colors) baited with pigeons (Feral pigeon (*Columba livia*)) and domestic fowls (*Gallus gallus*) that were held in cages 30.5 cm above the ground on tables. The number of trap days for the baited-net traps was (5 traps x 21 d x 4 times x 2 yr) 840; iii) 5 un-baited net traps of different colors with number of trap days equal 840; iv) 2 resting cages constructed out of local forest sticks and branches and wrapped with brown paper or black plastic, based on the dimensions of the red box shelters of Goodwin (1942). The number of trap days for the resting cage was (2 traps x 21 d x 4 time x 2 yr) 336; v) Sweep netting through vegetation from ground to five feet above ground was done by 5 person for four hours each day (two hours in the morning from 8:00 am to 10:00 am and two hours in the afternoon from 3:00 pm to 5:00 pm). The number of trap days was (5 x 21 x 4 x 2) 840 trap/d; vi) Resting on inside walls of the processing tent at the campsites. The precessing tent trap days was (1 x 21 d x 4 time x 2 yr) 168 d. The bottoms of the net traps, both bird-baited and un-baited, were always folded upwards at the bottom so mosquitoes could access the net traps all day and night for the entire period in the field. Mosquitoes were cleared from the net traps and resting boxes every day from 6:00 to 7:00 am, 12:00 to 1:00 pm, and 5:00 and 6:00 pm.

2) Techniques that captured immature stages and reared from larvae to adults were: i) collection in rock pools, ii) snail shells, iii) discarded cocoa pods, iv) coconut seed pods, v) old tarpauline, vi) cooking pot holding water on the ground and from river and steam eddies, vii) 5 bamboo pots attached to tree trunks three feet above ground. The number of trap days for the bamboo pots was (5 traps x 21 d x 4 time x 2 yr) 840 d, and viii) 2 plastic cups placed on the ground and filled with water from a nearby stream and thereafter with rainfall with corresponding trap days of (2 traps x 21 d x 4 times x 2 yr) 336 d. The positions of all the net traps were changed in 7 d interval so that we could measure the impact of position of trap.

Mosquito Identification and Preservation

Eretmapodites were identified to group, and whenever possible, to species, using a dissecting microscope following taxonomic key described in Service (1990). Most males were preserved individually in a 1-ml tube with silica gel to keep them dry. In most instances the silica gel stayed dry enough to prevent fungal growth despite the very humid conditions in the field. All tubes were labeled with corresponding codes indicating date of collection, method, and species. Females captured as adult flying mosquitoes and identified were pooled in batches of 1 to 20. They were preserved in 95% ethanol for DNA extraction and avian malaria assays. Avian malaria prevalence is presented elsewhere (unpublished data). All ethanol tubes were labeled with corresponding codes indicating date of collection, method, and species. Larvae collected in bamboo pots and other sites were removed and reared to adults in plastic containers and provided a small quantity of finely ground TetraMin Tropical Flakes (Melle, Germany) for food. A small quantity of crushed dried local forest vegetation was also added as a source of natural organic food matter to improve rearing success. Larvae of most species unfortunately died, indicating that conditions used for immature stages were not appropriate, and much still needs to be learned about their nutritional requirements. Females reared from larval were preserved in silica tubes for morphological examination.

Upon return from the field, all males and some females were pinned for identification and held in plastic containers (voucher specimen) kept in a refrigerator at 4°C to avoid them from being destroyed by fungi as the laboratory was not air-conditioned for many hours and was humid. For species identification, the genitalia of all males were dissected, softened, and cleared for 12 h in Specimen Clearing Fluid (Bioquip Products Inc., Rancho Dominguez, CA). This was followed by passing them through two washes of 100% ethanol before mounting on microscope slides in Polyvinyl alcohol (PVA) Mounting Medium (Bioquip Products Inc.). Digital images of the genitalia, hindlegs, and scutum of specimens were taken using an OMAX A3518OU attached to a Nikon SMZ800 dissecting microscope, and an Amscope MU2003-Bl attached to a Nikon E600 compound microscope with phase contrast optics. Images were processed and archived using Amscope Capture software version 3.7 (Amscope, Irvine, CA). Digital images were compared to drawings and descriptions for *Eretmapodites* in Service (1990), and to descriptions of species described since the review by Service (1990) in da Cunha Ramos and Ribeiro (1990, 1992) and d Cunha Ramos et al. (1992). Spelling of *Eretmapodites* species names followed that of Harbach (2018).

Ethical Considerations

Access to these forest sites was obtained from Sithe Global-Sustainable Oils Cameroon (SG-SOC), an affiliate of Herakles Farms. The approval for the use of animals (fowls and pigeons) for this research was obtained from the University of Buea Institute of Animal Care and Used Committee (IACUC) with authorization number protocol # UB-AP_2015_004#.

Statistical Analysis

Statistical analyses was performed using the software R (ver. 3.4.1). Mosquito abundance was determined per trap type. Shannon-Wiener (H) and Simpson's (DS) indices were used to determine the diversity of mosquitoes among trap types. Species richness (number of species) was determined per trap type. To estimate the number of rare and undetected species and add them to the observed richness, two estimators of the 'true' number of species in each site, Chao1 and ACE (Abundance-base Coverage Estimator), were calculated. ANOVA was used to test for significant differences in diversity indices, and richness among trap types. Chi-square was used to compare mosquito abundance, in trap type, colors, and baits.

Table 1. Composition of mosquito fauna at genus level following two years of sampling

Mosquito genera	Number (%)
Aedes	716 (12.55)
Culex	2,339 (41.01)
Eretmapodites	2,334 (40.91)
Malaya	07 (0.12)
Ficalbia	04 (0.07)
Uranotaenia	223 (3.91)
Hodgesia	13 (0.23)
Culiseta	7 (0.12)
Toxorhynchites	2 (0.04)
Mimmomyia	07(0.12
Anopheles	55 (0.96)
Total	5,704 (100)

Table 2. Abundance of *Eretmapodites* collected and reared from various traps and water bodies *N*(%)

Males	Bamboo pot	Cocoa pod	Coconut shell	Cooking pot	Dead leaves	Net trap	Plastic cup	Rock Pool	Snail shell	Sweep net	Tarpaulin	Resting cage	Total
Er. mahaffyi	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	1(.2)
Er. pauliani	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(2.3)	0(0)	0(0)	3(.5)
Er. spp 1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	1(.2)
Er. brenguesi	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(1.6)	0(0)	0(0)	2(.3)
Er. spp 2	0(0)	0(0)	0(0)	0(0)	0(0)	2(1.6)	0(0)	0(0)	0(0)	5(3.9)	0(0)	0(0)	7(1.2)
Er. chrysogaster	52(77.6)	36(87.8)	11(92)	3(75)	11(19)	68(53.5)	33(75)	10(100)	19(39.6)	30(23.3)	52(98.1)	0(0)	325(54)
Er. ferrarai	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	9(6.8)	0(0)	0(0)	9(1.5)
Er. forcipulatus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	20(41.7)	6(4.6)	0(0)	0(0)	26(3.8)
Er. germaini	0(0)	0(0)	0(0)	0(0)	1(1.7)	5(3.9)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	6(1.1)
Er. gilletti	1(1.44)	0(0)	0(0)	0(0)	2(3.5)	7(5.5)	0(0)	0(0)	0(0)	11(8.5)	0(0)	0(0)	21(3.4)
Er. grahami	2(2.98)	0(0)	1(8)	0(0)	28(48.3)	5(3.9)	1(2.3)	0(0)	1(2)	6(4.6)	0(0)	0(0)	44(7.7)
Er. hamoni	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	1(.2)
Er. intermedius	0(0)	0(0)	0(0)	0(0)	2(3.5)	5(3.9)	1(2.3)	0(0)	0(0)	5(3.9)	0(0)	0(0)	13(2.2)
Er. jani	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)	0(0)	0(0)	1(.2)
Er. leucopus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2)	0(0)	0(0)	0(0)	1(.2)
Er. spp 3	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(3.9)	0(0)	0(0)	5(.8)
Er. oidipodeios	5(7.5)	0(0)	0(0)	0(0)	0(0)	7(5.5)	6(13.6)	0(0)	2(4.3)	21(16.3)	0(0)	0(0)	41(7.8)
Er plioleucus	0(0)	0(0)	0(0)	0(0)	0(0)	7(5.5)	0(0)	0(0)	0(0)	5(3.9)	0(0)	0(0)	12(2)
Er. plioleucus plioleucus	2(2.98)	0(0)	0(0)	0(0)	0(0)	6(4.7)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	9(1.5)
Er. productus	0(0)	1(2.4)	0(0)	1(25)	12(20.6)	4(3.3)	0(0)	0(0)	0(0)	5(3.9)	0(0)	0(0)	23(3.9)
Er. quinquevittatus	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	1(.2)
Er. rickenbachi	0(0)	0(0)	0(0)	0(0)	0(0)	7(5.5)	0(0)	0(0)	0(0)	6(4.6)	0(0)	0(0)	13(2.2)
Er. salauni	0(0)	0(0)	0(0)	0(0)	0(0)	2(1.6)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	3(.5)
Er. semisimplicipes	5(7.5)	4(9.8)	0(0)	0(0)	1(1.7)	0(0)	3(6.8)	0(0)	0(0)	0(0)	1(1.9)	0(0)	14(2.4)
Er. spp 4	0(0)	0(0)	0(0)	0(0)	1(1.7)	1(.8)	0(0)	0(0)	4(8.4)	3(2.3)	0(0)	0(0)	9(1.5)
Er. spp 5	0(0)	0(0)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	0(0)	1(.8)	0(0)	0(0)	2(.3)
Total Males	**67**	**41**	**12**	**4**	**58**	**127**	**44**	**10**	**48**	**129**	**53**	**0(0)**	**593**
n(%)	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**0(0)**	**(100)**
Er. chrysogaster group	43(86)	65(98.5)	20(90.9)	2(100)	42(73.6)	669(58.2)	7(70)	7(100)	7(53.8)	141(53.2)	23(100)	5(100)	1,031(61.8)
Er. inornatus group	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(7.7)	4(1.3)	0(0)	0(0)	5(.3)
Er. leucopus group	0(0)	0(0)	0(0)	0(0)	0(0)	1(.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(.1)
Er. oidipodeios group	4(8)	0(0)	0(0)	0(0)	3(5.3)	25(2.2)	3(30)	0(0)	5(38.5)	22(9)	0(0)	0(0)	62(3.7)
Er. plioleucus group	3(6)	0(0)	2(9.1)	0(0)	0(0)	446(38.8)	0(0)	0(0)	0(0)	90(34)	0(0)	0(0)	541(32.4)
Er. productus group	0(0)	1(1.5)	0(0)	0(0)	12(21.1)	8(.7)	0(0)	0(0)	0(0)	7(2.5)	0(0)	0(0)	28(1.7)
Total female *N*	**50**	**66**	**22**	**2**	**57**	**1,149**	**10**	**7**	**13**	**264**	**23**	**5**	**1,668**
(%)	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**	**(100)**
Ratio of male: female	M = 67, F = 50	M = 41, F = 66	M = 13, F = 22	M = 4 F = 2	M = 58, F = 57	M = 127, F = 1,149	M = 44, F = 10	M = 10, F = 7	M = 48, F = 13	M = 129 F = 264	M = 53, F = 23	M = 0, F = 5	M = 593 F = 1,668

M = Total number of male mosquitoes in different trap type. F= Total number of female mosquitoes in different trap type. Females were not identified to species due to their morphological similarities

*** 73 mosquitoes collected from the processing tent were excluded from the analysis ***Trap type was significantly associated with mosquito catch ($\chi^2 = 2{,}168.644$, $P < 0.001$)

Results

Characteristics of the Mosquito Population

A total of 5,704 mosquitoes were collected, representing 11 genera (Table 1) among which 2,334 were *Eretmapodites* consisting of 1,735 (74.34%) females and 599 (25.66%) males.

A summary of the *Eretmapodites* mosquito species and groups collected in the various trap types and water bodies is represented in Table 2. There were a few males that had genitalia different from all known described species, and for convenience, we named them *Er. spp* 1 through to 5 and are likely undescribed species.

Most *Eretmapodites* adults were collected in net traps (n = 1,276 (56.4%)) and via sweep netting through forest ground vegetation (n = 393 (17.4 %)), while the fewest were collected in the walk-in resting cages (n = 05(0.2%)). Most larvae were collected in bamboo pots followed by cocoa pods. No larvae were found in tree holes. There was a significant association between trap types and mosquito species abundance (χ^2 = 2168.644, $P < 0.001$) when compared (Table 2).

Abundance of *Eretmapodites* Mosquitoes With Respect to Bait Type and Trap Color

Net traps of various colors were used to collect mosquitoes based on their attractiveness to available color. This however provided an opportunity to assess attractiveness of different colors (Table 3) to mosquito. Net trap colors green (n = 435(34.09%)), blue (n =344(29.96%)), and white (n = 295(23.12%)), had higher catch counts than pink (n = 198(15.52%)) and black (n = 4(0.31%)). Trap color was significantly associated with mosquito abundance (χ^2 = 42.000, P = 0.003). Three kinds of baits were used to attract mosquitoes (Table 3) in the net traps. Chicken (377 (29.55%)) Carbon dioxide (CO_2 (314 (24.61%)), and pigeons (473 (37.06%). The observed mosquito abundance was significantly associated (χ^2 = 40.000, $P < 0.001$) with baits used in the net traps (Table 3).

Diversity of Mosquitoes With Respect to Trap Type, Colour, and Bait

The diversity of mosquitoes in the various trap types ranged from H' = 2.65; DS = 0.84 (sweep net) to H' = 0.0; DS = 0.0 (lowest diversity in the rock pool, and resting cage) among the males species identified. The diversity among female groups ranged from H' = 1.14; DS = 88 (highest diversity captured via sweep netting) to H = 0.0; DS = 0.0 (lowest diversity captured in the rock pool, tarpaulin, resting cage and cooking pots (Table 4)).

Despite lower numbers collected, the blue-colored net traps attracted the highest diversity of male species (H = 2.09; DS = 0.81) whereas the diversity of female species groups was (H' = 0.851; DS = 0.52) highest in the blue net trap. The lowest diversity of females groups collected was (H' = 0; DS = 0) in the resting box. Male mosquito Species diversity was significant associated (P = 0.0223) with net trap color (Table 5). Female group attractiveness of odors released from birds, humans (sleeping tents and processing tent), CO_2 from sugar yeast mixture revealed some differences with interestingly highest diversity in un-baited net traps and other structures with no specific odors (H' = 0.853; DS = 0.53 and H = 1.56; DS = 0.75 for males and female groups respectively). There was no difference in diversity of female groups captured between the domestic fowls and pigeons with values of H' = 0.741; DS = 0.46 and H' = 0.791; DS = 0.49 respectively (Table 5).

Sweep netting captured the highest male *Eretmapodites* species richness (SR = 24; ACE = 24.78; Chao1 = 60.38) and close the highest for females *Eretampodites* groups (SR = 5; ACE = 5.16; Chao1 = 7. 24.) Among the baited traps, richness was highest for males in pigeon baited traps (SR = 12; ACE = 15.73; Chao1 = 16.12) and for female groups (SR = 5; ACE = 6.66; Chao1 = 6.0). High species richness was also captured in blue net traps for males (SR = 13; ACE = 16.13; Chao1 = 19) and female species groups (SR = 5; ACE = 5.5; Chao1 = 5.5).

Discussion

This study assessed whether the use of techniques like net traps of different colors, baits, and larval collection methods for sampling mosquitoes are efficient in collecting mosquitoes and can provide further information to better understand the bionomic of *Er.* mosquito population in the Talangaye rainforest, in the southwest region of Cameroon. Before the Talangaye forest is completely cut down for palm oil plantation. It is the first study to look at the population of *Er*. mosquito Cameroon in an area undergoing deforestation.

A high species abundance of *Er*. was found in the Talangaye rainforest. This was in line with previous reports by Service (19900) and Edwards (1941), who reported that the genus is confined in Afrotropical region. Most *Eretmapodites* species are restricted to equatorial African forests, however, how widespread each species is within the forests, that extend from Nigeria east to Uganda and from Nigeria south to northern Angola, is not known due to restricted sampling efforts within the forests. High *Er.* catches were recorded in net traps compared to the other collection methods. Though sweep net recorded second-highest catch, the sweep net technique was during the day and sampling was done every day at least for hours during the entire study period. It sole applicability in control of mosquito is not feasible since it cannot be performed at night to collect night flying adults. Secondly, the efficiency of this technique depends on the sampling efforts and experience of field collectors.

The CDC light traps was not productive outdoors in our study area (was discontinued after first year of sampling) maybe because of the influence of winds or because they also attract a high number of other insects which by flying around the trap could divert mosquitoes from being trapped. These findings were in line with that of Bamou et al. (2020) who reported less performance of CDC-light trap in outdoors collection.

The employment of collection techniques such as bamboo pots, cocoa pods, coconut shell, snail shells, collection from dead leaves on the ground, plastic cups, and rock pools, that targeted immature stages of mosquitoes enabled us to get a glimpse of *Eretmapodites* oviposition, blood-feeding, and resting behaviors. *Er.* was noted chasing humans to bite early in the morning from 6:00 am to 8:00 am and in the evening from 5:00 pm. They were mostly found resting under tree leaves. This is in line with report by Service (1990) *Er.* was caught chasing human in the Bwamba district. Amongst techniques that targeted immature stages of mosquitoes, bamboo pots recorded the highest collection of *Er. Eretmapodites chrysogaster* was the only species reared from larvae originating from all natural water bodies and water sources set out by us in the forest. *Er. chrysogaster* larvae are predacious and were therefore the species most likely to survive to adult emergence. Due to high mortality of *Eretmapodites immatures* and not knowing if mortalities of some species were higher than others we did calculate species diversity and richness of larval collections. *Eretmapodites productus* adults reared from larvae was the next most common. It was surprising that *Eretmapodites* often laid eggs in bamboo pots, and no *Eretmapodites* were collected in any natural tree or rot holes examined. Bamboo was introduced from Asia and planted in Africa by humans for use as building material and is now invading

forests in Equatorial Africa. A few adults were reared from coconut shells that had been brought in as food and dropped on the ground by forest hunters and workers clearing the forests. In Talangaye rainforest it was noted that many *Eretmapodites* species are quite nonspecific where they lay their eggs. This was because we were able to rear adults of the same species from larvae in bamboo pots, in water from fallen banana and leaves of other tree species on the forest floor and snail shells. However, some species such as *Eretmapodites forcipulatus, Eretmapodites jani,* and *Eretmapodites leucopus* were reared from larvae collected only in snail shells. Water-filled snail shells were those from the common giant African snail (for example *Eretmapodites penicillatus*, *Eretmapodites silvestris subs. conchobius*, *Eretmapodites quinquevittatus* are reported to specialize in breeding in snail shell). Mostly only species of the *chrysogaster* group laid eggs in artificial containers (plastic cups, cooking pot, and tarpaulin) that included; *Eretmapodites chrysogaster, Eretmapodites grahami, Eretmapodites intermedius,* and *Eretmapodites semisimplicipes*. *Er. productus* males were reared from the cooking pots and male *Eretmapodites oidipodeios* and female *oidipodeios* group were reared from plastic cups. It is important to note that a few species such as *Er. chrysogaster, Er. subsimplicipes, Er. quinquevittatus,* and *Er. silvestris* are more widespread. They occupied different breeding site and inhabited mountain and riverine forests enclaves in savanna regions (Edwards 1941, Service 1990 and personnel observations by AJC).

During the application of nine breeding sites collection techniques different *Er.* species were recorded. Their diversity was far lower compared to those recorded using net traps and sweep net techniques. Net trap baited with pigeons recorded a high number of *Er.* which fed on different hosts (domestic fowls, pigeon, some were caught biting humans) in the forest. Approximately one-third of *Eretampodites* mosquitoes collected in the forest were collected in pigeon and chicken baited net traps respectively. Few Males *Eretmapodites* also collected could have been attracted to hosts to mate with ornithophily females which possibly explains why males were collected in bird baited traps since male mosquito do not suck blood. It was equally noted that none of the females collected in bird baited (chicken and pigeon) net traps were blood-fed, even though bait was significantly associated ($P < 0.05$) to *Eretmapodites* mosquito abundance.

Collection of a few *Eretmapodites* (73) in the field processing and sleeping tents suggests that the *Eretmapodites* mosquito species were also attracted to $C0_2$ and/or odors emitted from humans. Anthropophilic blood feeding has been reported for multiple *Eretmpoadites* species (Edwards 1941, Service (1990)) in Afrotropical forests. Only a few species of the *chrysogaster* group blood-fed on us at any time during daylight hours.

The presence of carbon dioxide generated from mixture of yeast and sugar, in the net traps also attracted a variety of *Eretmapodites*

Table 3: Abundance of mosquitoes with respect to bait and trap colour

Bait	Abundance *N*(%)	*P*- value
Chicken	377 (29.55)	
Carbon dioxide (CO_2)	314 (24.61)	
Pigeon	473 (37.06)	**$\chi^2 = 40.000$,**
No bait	112 (8.78)	
Total	1276 (100)	***P < 0.001***
Color black	04 (0.31)	$\chi^2 = 42.000$,
Color blue	344 (29.96)	$P = 0.003$
Color green	435 (34.09)	
Color pink	198 (15.52)	
Color white	295 (23.12)	
Total	1276 (100)	

Table 4. Diversity of mosquitoes with respect to trap type

		Diversity metric										
Trap types	Males	H'	SR	DS	ACE	Chao1	Females group	H	SR	DS	ACE	Chao1
Cooking pot		0.56	2	0.81	2.00	2.0		0.0	1	0.0	1.00	1.0
Cocoa nut-shell		0.29	2	0.41	2.00	2.0		0.31	2	0.44	2.00	2.0
Bamboo pots		0.86	6	0.48	8.75	9.0		0.50	3	0.46	4.33	4.5
Snail shell		1.31	7	0.67	9.87	10		0.90	3	0.82	4.33	4.5
Cocoa pods		0.43	3	0.39	4.00	4.0		0.08	2	0.03	2.00	2.0
Net traps		1.84	13	0.72	16.24	16.16		0.82	5	0.51	5.07	5.05
Sweep nets		2.65	24	0.84	24.77	60.38		1.14	5	0.71	5.16	7.24
Resting cage		0.0	0	0.0	0.00	0.0		0.0	1	0.0	1.00	1.0
Dead leaves		1.44	8	0.69	8.93	11.6		0.71	3	0.65	3.34	4.35
tarpaulin		0.09	2	0.03	2.00	2.0		0.0	1	0.0	1.00	1.0
Rockpool		0.0	1	0.0	1.00	1.0		0.0	1	0.0	1.00	1.0
Plastic cup		0.90	6	0.51	7.12	10		0.61	2	0.88	2.37	3.3
Bait												
Chicken		1.564	8	0.75	7.86	8.0		0.741	5	0.46	6.20	6
Carbon dioxide		2.101	12	0.85	11.8	13.25		0.833	4	0.60	4.22	4
pigeon		1.909	12	0.75	15.73	16.12		0.791	5	0.49	6.66	6
no bait		1.560	8	0.75	39.00	13		0.853	5	0.53	4.00	4
Color												
Resting box		0.0	1	0.0	1.00	1.0		0.0	1	0.0	1.00	1.0
Color blue		2.087	13	0.81	16.13	19.0		0.851	5	0.52	5.25	5.5
Color green		2.040	11	0.85	11.15	11.12		0.812	5	0.50	5.25	5.0
Color pink		2.056	11	0.86	10.58	10.50		0.799	4	0.58	4.00	4.0
Color white		1.44	9	0.65	10.80	16.0		0.733	3	0.66	4.50	4.0

Table 5. Comparison of diversity indices among males and females species in different traps type, baited traps, and color

ANOVA	means	Mean diff	SE of diff	95% CI	SS	DF	MS	F (DFn, DFd)	*P* value
Color									
Females	0.6390	0.8856	0.2444	0.2072 to	3.116	4	0.7791	F (4, 4) = 5.220	*P* = 0.0692
Males	1.525			1.564	1.961	1	1.961	F (1, 4) = 13.14	*P* = 0.0223
Residual		0.5971				4	0.1493		
Baits									
Males	1.857	1.031	0.1677	0.3095 to 1.752	1.594	3	1.594	F (1, 3) = 37.80	*P* = 0.0254
Females	0.8257				0.0609	1	0.03405	F (3, 3) = 0.8072	*P* = 0.5533
Residual		0.08436				3	0.04218		
Trap types									
Females	0.4609	0.4309	0.1453	0.1071 to	7.788	11	0.7788	F(11,11) = 6.703	P= 0.0029
Males	0.8918			0.7548	1.021	1	1.021	F(1,11)= 8.790	P= 0.0142
Residual	1.162					11	0.1162		

mosquito species. Carbon dioxide is an effective long-distance attractant and the fact that none blood-fed on birds but did occasionally blood feed on us, especially during the day suggests that most *Eretmapodites* species are mammal blood feeders.

Different net trap colors (green, blue, white, and pink) showed variation in the abundance of *Eretmapodites* mosquito species. The proportions of mosquito collected in each trap of the respectively colors above indicated that *Er.* showed preference to particular colors as net trap green had the highest catch of *Er.* mosquito while net trap black the least. There was a significant difference ($P < 0.05$) in net trap of different colors in attracting mosquito in the forest environment.

Colored traps possibly attract mosquitoes only through visual stimuli and this could explain the high efficiency of this sampling technique especially when baits or some chemical stimuli are attached to it.

Among collection methods that targeted the immature stages, the least mosquito diversities were recorded in rock pools, tarpaulin, and coconut shell while the highest in dead leaves, snail shells and bamboo pots; among the male mosquitoes reared. Among the female groups reared, the least diversities were recorded in rock pools, tarpaulin, and abandoned cooking pots (Muangangi et al. 2009). The highest diversities were recorded in snail shells and dead leaves. The used of bamboo pots, snail shells and collections of immature stage on dead leaves proved to be efficient in collecting different (diverse) population of species of *Er*. mosquito. On the order hand, the highest species richness, diversities of adult flying males and females were recorded in sweep net and net traps. The sweep net techniques, which involved searching and capturing of mosquito understory at their resting site, is a technique that, its efficiency depends largely on the effort of collectors and their skills. Yet sweep net provided interesting findings by indicating that many mosquitoes rest under leaves of trees and dark areas after feeding and to get blood meal digested in the forest.

Arboviruses are known to lurk in African forest regions with multiple species of *Aedes* and *Culex* species serving as vectors of RVFV (Eiden et al. 2014), dengue fever, yellow fever, and other hemorrhagic fevers (Mayi et al. 2019). *Eretmapodite*s which is principally a forest mosquito have been incriminated as minor arboviral vectors as reported by many authors (Rickenbach et al. 1976; Service 1990; Epelboin et al. 2017; Braack et al. 2018). But concerted efforts to more clearly elucidate their roles in disease transmission can be better done by using efficient collecting/trapping methods such as net traps. Collection of adults from resting boxes proved largely unsuccessful and based on the successes of sweep netting, suggest that *Eretmapodites* prefer resting in vegetation rather than in dark environments. The fact that many mosquitoes enter net traps stresses the need to incorporate it in the control arsenal, and additional tools such as larval collection in order to increase the chance of reducing transmission of mosquito-borne disease. Especially as most of the species were collected resting in forest floor vegetation and in net traps baited and un-baited with birds and carbon dioxide.

The Carbon dioxide net baited trap and the blue color trap recorded a high diversity of *Er.* mosquito in the forest area. This information (Nchoutpouen et al. 2019) likely suggests high attractiveness provided by the stimuli and net color. The efficacy of traps could be affected by variation in trap design, and behavior of both mosquitoes and bait type. These trapping techniques which are simple to implement could be used as a routine monitoring tool for vector surveillance in poor resource communities yet the design still need to be improved to increase its efficiency.

Conclusion

Net traps are very efficient collection methods of mosquitoes in the Talangaye rainforest. Using both the net traps and larval collection methods simultaneously is productive in elucidating more succinctly the vectors abundance and species diversity. Relying only on the traditional human landing catches and CDC traps will not serve as nearly as effective methods of capture of *Eretmapodites* in African forests.

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Authors' Contributions

AJC, RS, TT and ADN planned the study design. FDF, MMPA, KC, KKB, AEF, CD, and JF-C performed field activities, laboratory work and analyzed the data. FDF, RS and ADN drafted the manuscript. AJC, KKB, FDF, KC identified the mosquitoes. AJC, RS and ADN provided substantial improvement of the manuscript. All authors approved the final version of the manuscript.

References Cited

Antonio-Nkondjio, C., N. N. Sandjo, P. Awono-Ambene, and C. S. Wondji. **2018**. Implementing a larviciding efficacy or effectiveness control intervention against malaria vectors: key parameters for success. *Parasit Vectors*. 11: 57.

Awono-Ambene, P. H., J. Etang, C. Antonio-Nkondjio, C. Ndo, W. E. Eyisap, M. C. Piameu, E. S. Mandeng, R. L. Mbakop, J. C. Toto, S. Patchoke, *et al*. **2018**. The bionomics of the malaria vector *Anopheles rufipes* Gough, 1910 and its susceptibility to deltamethrin insecticide in North Cameroon. *Parasit Vectors*. 11: 253.

Bamou, R., L. R. Mbakop, E. Kopya, C. Ndo, P. AwonoAmbene, T. Tchuinkam, M. K. Rono, J. Mwangangi and C. Antonio-Nkondjio. **2018**. Changes in malaria vector bionomics and transmission patterns in the equatorial forest region of Cameroon between 2000 and 2017. *Parasit Vectors*. 11: 464.

Bamou, R., E. Kopya, L. Djamouko-Djonkam, P. Awono-Ambene, T. Tchuinkam, F. Njiokou, and C. Antonio-Nkondjio. **2020**. Assessment of the anophilinae blood seeking bionomic and pyrethroids resistance of local malaria vectors in forest region of Southern Cameroon. *JEZS*. 8: 1054–1062.

Barreaux, A. M., C. M. Stone, P. Barreaux, and J. C. Koella. **2018**. The relationship between size and longevity of the malaria vector *Anopheles gambiae* (s.s.) depends on the larval environment. *Parasit. Vectors*. 11: 485.

Becker, N., D. Petric, M. Zgomba, C. Boase, M.B. Madon, C. Dahl, and A. Kaiser. **2010**. *Mosquitoes and Their Control*. 2nd ed. Springer Verlag, Berlin, Germany, pp. 608.

Braack, L., A. P. de Almeida, A. J. Cornel, R. M. Swanepoel, and C. de Jager. **2018**. Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasit. Vectors*. 11: 29.

Carlson, J. S., W. Erika, T. F. Rebecca, S. Sarah, A. T. Lisa, R. N. M. Sehgal, C. M. Barker, and A. J. Cornel. **2015**. Identifying avian malaria vectors: sampling methods influence outcom. *Parasites & Vectors*. 8: 365.

De Castro, M., K. Kannady, B. Singer, D. Mtasiwa, H. Mshinda, M. Tanner, Y. Geissbuhler, S. Lindsay, U. Fillinger, G. Killeen. **2007**. Reduction in malaria prevalence in Dar es Salaam, Tanzania following vector control with microbial larvicides. *PLoS Med*. 2007.

Edwards, F. W. **1932**. Diptera Fam. Culicidae. *Genera Insect*. 194: 1–258.

Edwards, F.W. **1941**. *Mosquitoes of the Ethiopian Region III. Culicine adults and pupae*. Printed by order of the Trustees, British Museum (Natural History), London; viii: 499pp.

Eiden, M., A. Vina-Rodriguez, B. O. El Mamy, K. Isselmou, U. Ziegler, D. Höper, S. Jäckel, A. Balkema-Buschmann, H. Unger, B. Doumbia, *et al*. **2014**. Ngari virus in goats during Rift Valley fever outbreak, Mauritania. *Emerg. Infect. Dis*. 20: 2174–2176.

Epelboin, Y., S. Talaga, L. Epelboin and I. Dusfour. **2017**. Zika virus: an updated review of the competent or naturally infected mosquitoes. *PLOS Negl. Trop. Dis*. November 16, **2017**. doi:10.1371/journal.pntd.0005933.

Goodwin, M. H. **1942**. Studies on artificial resting places of *Anopheles quadrimaculatus* say. *J. M. Natn. Malar. Soc*. 1: 93–99.

Govella, N. J., and H. Ferguson. **2012**. Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination. *Front. Physiol*. 3: 199.

Haddow, A. J. **1956**. Observations on the biting-habits of African mosquitoes in the genus *Eretmapodites* Theobald. *Bull. Entomol. Res*. 46: 761661–761772.

Harbach, R. E. **2013**. Mosquito Taxonomic Inventory, http://mosquito-inventory.info/, Accessed on 16 August 2021.

Harbach, R. E. **2018**. Mosquito Taxonomic Inventory, http://mosquito-taxonomic-inventoryinfo/, Accessed 15 May 2018.

Hartberg, W. K., and E. J. Gerberg. **1971**. Laboratory colonization of *Aedes simpsoni* (Theobald) and *Eretmapodites quinquevittatus* Theobald. *Bull. World Health Organ*. 45: 850–852.

Holmes, C. J., and J. B. Benoit. **2019**. Biological adaptations associated with dehydration in mosquitoes. *Insects*. 10(11): 375. doi:10.3390/insects10110375

Johnson, T. L., Y. Haque, A. J. Monaghan, L. Eisen, M. B. Hahn, M. H. Hayden, H. M. Savage, J. McAllister, J. P. Mutebi, and R. J. Eisen. **2017**. Modeling the environment suitability for *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in the contiguous United States. *J. Med. Entomol*. 54: 1605–1614.

Kutz, S. J., E. P. Hoberg, L. Polley, and E. J. Jenkins. **2005**. Global warming is changing the dynamics of Arctic host–parasite systems. *Proc. R. Soc. Biol*. 272: 2571–2576.

Lutomiah, J., J. Bast, J. Clark, J. Richardson, S. Yalwala, D. Oullo, J. Mutisya, F. Mulwa, L. Musila, S. Khamadi, *et al*. **2013**. Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya: public health implications. *J. Vector Ecol*. 38: 134–142.

Maliti, D. V., N. J. Govella, G. F. Killeen, N. Mirzai, P. C. D. Johnson, K. Kreppel, H. M. Ferguson. **2015**. Development and evaluation of mosquito-electrocuting traps as alternatives to the human landing catch technique for sampling host-seeking malaria vectors. *Malar. J*. 14: 502.

Mayi, M. P. A., D. F. Forfuet, C. Kowo, T. Tchuinkam, K. Brisco, D. A. Anong, S. Ravinder, and A. J. Cornel. **2019**. Impact of deforestation on the abundance, diversity, and richness of *Culex* mosquitoes in the southwest Cameroon tropical rainforest. *J. Vector Ecol*. 44: 271–281.

Mboera, L. **2005**. Sampling techniques for adult Afrotropical malaria vectors and their reliability in the estimation of entomological inoculation rate. *Tanzan. Health Res. Bull*. 7: 117–124.

McIntosh, B. M., P. G. Jupp, D. Anderson, and D. B. Dickenson. **1973**. Rift valley Fever 2. Attempts to transmit virus with seven species of mosquito. J. S. African. *J. S. Afr. Vet. Assoc*. 44: 57–60.

Minsanté. **2017**. *Enquete post camapgne sur l'utilisation des moustiquaires imprégnées à longue durée d'action 2016/2017*, 120.

Muangangi J. M., E. J. Maturi, and C. M. Mbogo. **2009**. Seasonal mosquito larval abundance and composition in Kibwezi lower Eastern Kenya. *J. Vector Borne Dis*. 46: 66–71.

Nchoutpouen, E., A. Talipouo, B. Djiappi-Tchamen, L. DjamoukoDjonkam, E. Kopya, C. S. Ngadjeu, P. Doumbe-Belisse, P. Awono-Ambene, C. S. Wondji, and C. Antonio-Nkondjio. **2019**. *Culex* species diversity, susceptibility to insecticides and role as potential vector of Lymphatic fariasis in the city of Yaoundé, Cameroon. *PLoS Negl.Trop. Dis*. 13: 1–16.

Njabo, K. Y., A. J. Cornel, C. Bonneaud, E. Toffelmier, R. N. M. Sehgal, and G.Valkiūnas. **2011**. Nonspecific patterns of vector, host and avian malaria parasite associations in a central African rainforest. *Mol. Ecol*. 20: 1049–1061.

da Ramos, C., and H. Ribeiro. **1990**. Research on the mosquitoes of Angola (*Diptera:Culicidae*) XX- *Eretmapodites mortiauxi*. A new species of the *Leucopus* group. *Bol. Soc. Entomol*. 115: 137–144.

da Ramos, C., and H. Ribeiro. **1992**. Research on the mosquitoes of Angola (*Diptera:Culicidae*) XXI—description of *Eretmapodites angolensis* sp.nov. and *Eretmapodites dundo* sp.nov, of the «*oedipodeios*» group. *Garc. Orta Sér. Zool. (for 1990)*. 17: 31–33.

da Ramos, C., H. Ribeiro, and H. de Barros Machado. **1992**. Research on the mosquitoes of Angola (*Diptera:Culicidae*) XIX- *Eretmapodites tendeiro*, a new species of the «*plioleucus* group». *Garc. Orta Sér. Zool. Lisboa (for 1989)*. 16: 133–136.

Reinert, J. F., R. E. Harbach, and I. J. Kitching. **2009**. Phylogeny and classification of tribe Aedini *(Diptera: Culicidae)*. *Zool. J. Linn. Soc*. 157: 700–794.

Ribeiro, A. F., P. R. Urbinatti, A. M. Ribeiro de Castro Duarte, M. Bicudo de Paula, D. Mendes Pereira, L. F. Mucci, A. Fernandes, M. H. S. Homem de Mello, M. Otávio de Matos Júnior, R. Correa de Oliveira, *et al*. **2012**. Mosquitoes in degraded and preserved areas of the Atlantic Forest and potential for vector-borne disease risk in the municipality of São Paulo, Brazil. *J. Vector Ecol*. 37: 316–324.

Rickenbach, A., G. Le Gonidec, and P. Ravisse. **1976**. L'incidence des arbovirus isolés des moustiques dans une région forestiére du sud Cameroun, la région de Yaoundé. *Bull. Soc. Pathol. Exot.* 69: 372–381.

Russell, T., N. Govella, S. Azizi, C. Drakeley, S. Kachur, G. Killeen. **2011**. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar. J.* 1: 80.

Schmid, S., A. Dinkel, U. Mackenstedt, M. L. Tantely, F. J. Randrianambinintsoa, S. Boyer, and F. Woog. **2017**. Avian malaria on Madagascar: bird hosts and putative vector mosquitoes of different *Plasmodium* lineages. *Parasit. Vectors*. 10: 1–7.

Service, M. W. **1990**. *Handbook to the Afrotropical toxorynchitinae and culicine mosquitoes, excepting Aedes and Culex*. British Museum (Natural History) London, United Kingdom, pp. 207.

Sikaala, C. H., G. F. Killeen, J. Chanda, D. Chinula, J. M. Miller, T. L. Russell, A. Seyoum. **2013**. Evaluation of alternative mosquito sampling methods for malaria vectors in Lowland SouthEast Zambia. *Parasit Vectors*. 6: 1.

Sikulu, M., N. J. Govella, S. B. Ogoma, J. Mpangile, S. H. Kambi, K. Kannady, P. C. Chaki, W. R. Mukabana, G. F. Killeen. **2009**. Comparative evaluation of the Ifakara tent trap-B, the standardized resting boxes and the human landing catch for sampling malaria vectors and other mosquitoes in urban Dar es Salaam, Tanzania. *Malar. J.* 8: 1–9.

Smallegange, R. C., W. H. Schmeid, J. Van Karel, N. O. Verhulst, J. Spitzen, W. R. Mukabana, and W. Takken. **2010**. Sugar-fermented yeast as an organic source of Carbon dioxide to attract the malaria *An. gambiae*. *Malar. J.* 292.

Soghigian, J., T. G. Andeadis, and T. P. Livdahl. **2017**. From ground pools to treeholes: convergent evolution of habitat and phenotype in *Aedes* mosquitoes. *BioMed Centr. Evol. Biol.* 17: 2262.

Sudia, W. D., and R. W. Chamberlain. **1962**. Battery operated light trap, an improved model for sampling mosquito population. *J. Am. Mosq. Control Assoc.* 4: 536–538.

Tusting, L. S., J. Thwing, D. Sinclair, U. Fillinger, J. Gimnig, K. E. Bonner, C. Bottomley, S. W. Lindsay. **2013**. Mosquito larval source management for controlling malaria. *Cochrane Database Syst. Rev*. 2013: 8.

Wilkerson, R. C., Y. -M. Linton, D. M. Fonseca, T. R. Schultz, D. C. Price, and D. A. Strickman. **2015**. Making mosquito taxonomy useful: as table classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. *PLoS One*. 10: e0133602.

Zélé, F., J.Vézilier, G. L'Ambert, A. Nicot, S. Gandon, A. Rivero, and O. Duron. **2014**. Dynamics of prevalence and diversity of avian malaria infections in wild *Culex pipiens* mosquitoes: the effects of *Wolbachia*, filarial nematodes and insecticide resistance. *Parasit Vectors*. 7: 1–16.