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# A cross-sectional study of Simulium damnosum s.l. breeding sites and species distribution in sudan savanna, mixed savanna-forest and rainforest regions in Cameroon

**Franklin Ayisi** ( franklinayisi@yahoo.com )
University of Ghana, African Regional Postgraduate Programme in Insect Science (ARPPIS)
**Naniogué Sedou**
WHO/ESPEN Laboratory, Ouagadougou
**Stephanie Kouahou Dieunang**
National Onchocerciasis Control Programme, Ministry of Public Health, Yaounde
**Florent Yaya**
National Onchocerciasis Control Programme, Ministry of Public Health, Yaounde
**Edmond François Tchago**
National Onchocerciasis Control Programme, Ministry of Public Health, Yaounde
**Cosmas Ejong Ndellejong**
National Onchocerciasis Control Programme, Ministry of Public Health, Yaounde
**Benjamin Biholong**
National Onchocerciasis Control Programme, Ministry of Public Health, Yaounde
**Daniel Adjei Boakye**
University of Ghana Noguchi Memorial Institute for Medical Research https://orcid.org/0000-0001-6434-7298

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# Abstract

**Background:** The presence of breeding sites and distribution of species of *Simulium damnosum* s.l are critical in understanding the epidemiology of onchocerciasis and evaluating the impact of elimination interventions. Reports on breeding sites and species distribution of members of *S. damnosum* s.l in Cameroon are scarce and the few available date back to more than 3 decades. The aim of this study is to provide information on *S. damnosum* breeding sites across the rainy (RS) and dry (DS) seasons and the species composition in three different regions in Cameroon: Southwest (SW), Northwest (NW) and North (N).

**Methods:** A cross-sectional two-seasons study was carried out in three regions with different ecological characteristics (SW – rainforest; NW- mixed forest-Guinea savanna; N- Sudan savanna). Pre-control onchocerciasis endemicity, relief maps and historical entomological information were used to identify potential rivers for purposive sampling. Sampled larvae were fixed in Carnoy's solution, sorted and *S. damnosum* s.l larvae stored until identification by cytotaxonomy. Geographical coordinates of potential breeding sites were recorded to produce maps using ArcGIS, while SPSS used for Chi-square test to estimate difference between blackfly seasonal breeding rates.

**Results:** 237 potential breeding sites were sampled (RS=81; DS=156) and 72 were found positive for *S. damnosum* s.l. SW had the most positive sites [67 (RS=24; DS=43)], having significant difference in rate of breeding between the seasons ($p<0.05$). Among 68 sites visited in both seasons, 16(23.5%) were positive in one of two seasons, 14(20.6%) and 38(55.9%) respectively positive and negative in both seasons, and more sites positive in DS(11) than in RS(05). *Simulium damnosum* s.s and *S. sirbanum* were the main species in N while *S. squamosum* and *S. mengense* the predominant species in NW and SW. *S. soubrense* and *S. yahense* were uniquely recorded in SW.

**Conclusions:** A comprehensive mapping of breeding sites requires rainy and dry seasons sampling. Study also demonstrates *S. damnosum* s.l breeding site survey is achievable in forest as in savanna zones. Not all potential breeding sites are actual breeding sites. Observation of *S. soubrense* in SW indicates changes in species composition over time and could affect onchocerciasis epidemiology in this area.


# Background

Human Onchocerciasis, also called river blindness, is a parasitic infection caused by the filarial nematode Onchocerca volvulus (Leuckart) transmitted by females of blackflies of the genus Simulium (Diptera: Simuliidae) [1]. The most important vectors are members of the Simulium damnosum complex which have a wide range throughout Africa and the Middle East [2]. The flies breed in fast-flowing stretches of water, where juvenile stages could be found attached to submerged vegetation, rocks or other substrates.

*Simulium damnosum* s.l are the only known vectors of human onchocerciasis in West and Central Africa. The species of this complex vary in their biting cycle and population age structure [3], response to control measures, ecological adaptations, host preference as well as their ability to transmit the causative agent of human onchocerciasis [4-7]. Members of the *S. damnosum* complex have been categorized into forest and savanna species [8] depending on their predominant ecological preferences.

The transition from control to elimination of onchocerciasis brings along the necessity for reassessment of all previously excluded areas from onchocerciasis interventions during the control era, termed 'elimination mapping' [9, 10]. Positive findings from breeding sites assessments are important in defining target population for such mapping [9], giving that the presence of vector *Simulium* flies define the epidemiology of onchocerciasis [11]. In addition, breeding sites surveys constitute important first steps in entomological and epidemiological evaluations of interventions in the different stages of onchocerciasis elimination [10].

Among the entomological procedures in the fight against human onchocerciasis, breeding site surveys form the basic unmissable step as it is required in most of the phases of onchocerciasis control and elimination. It provides information on where different vector species occur and define communities exposed to different levels of onchocerciasis transmission. However, breeding sites surveys is often thought to be impractical or difficult to carry out, especially in forest zones [12]. This has served as a limitation to onchocerciasis control/elimination efforts in forest zones.

The presence and characteristics of breeding sites of *S. damnosum* s.l may change with seasons and over time either due to pressure from human action like deforestation [13], climatic changes [14], or due to other anthropogenic or natural causes. This might lead to changes in vector species distribution and consequently the transmission pattern of the disease. Therefore, it is important to regularly update the breeding sites and vector species distribution to detect early changes in the epidemiology of onchocerciasis.

Documentation of breeding sites and distribution of onchocerciasis vectors in Cameroon are scarce, with the available information more than 3 decades ago and mostly restricted to either a particular area, a locality or river basin of interest [15-17]. The present study was therefore carried out to provide an updated map of breeding sites and species distribution of blackflies in three administrative regions of Cameroon having different vegetation types – North (Sudan savanna), Northwest (mixed forest-savanna) and Southwest (rainforest). The study also demonstrates feasibility of carrying out breeding site surveys in forest zones similar to savanna zones.

# Methods

### Study Site

This study took place in three administrative regions of Cameroon, namely the Southwest (SW), Northwest (NW) and North (N) regions (Figure 1).

The Southwest region has a dense evergreen and montane vegetation and humid tropical rainforest (that is gradually being degraded for lumbering and agricultural activities). It has an equatorial climate with rainfall between 2000 and 4000 mm per year and a dense hydrographic network. Part of this region falls within the coastal maritime zone with coastal vegetation consisting of mangroves and tropical rain forest. The topography of the region is made of mountains, hills and valleys generating fast water currents. Coupled with the long rainy seasons (March to October), this ecology favors the establishment of permanent breeding sites requisite for continuous onchocerciasis transmission.

The Northwest region falls within the mountain range of Cameroon, characterized by relief of massifs and mountains. The once large forest area has been reduced to mostly grassland and bush savanna, partly because of deforestation for timber, fuelwood, and agriculture, with only few patches of forests left. The region has a tropical climate characterized by two main seasons; rainy season, which starts from March and ends in October, and dry season from November to February. Rainfall varies between 1700 and 3000mm annually.

The North region falls within the Sudano-Sahelian ecological zone of the country, having a dense hydrographic network of seasonal and permanent rivers (*mayos*). It has a short rainy season (July to October) and a long dry season (November to June), with unevenly spatial and temporal distributed mean annual rainfall of about 800 to 900mm [18].

### Sample collection

Sampling took place in the months of July and September 2012, and December 2012 to February 2013 (taking a break from 22 December 2012 to 10 January 2013). Potential breeding sites map was created using data from pre-control REMO (Rapid Epidemiological Mapping of Onchocerciasis) infection map and other historical onchocerciasis information, coupled with a detailed relief map of the study regions. Purposive sampling for *S. damnosum* s.l larvae was undertaken on the rivers showing potential breeding sites. *S. damnosum* s.l larvae were collected from trailing vegetation and other submerged substrates (grass, leaves, rocks, etc) in fast-running water stretches. The larvae were stored in Carnoy's solution (absolute ethanol and glacial acetic acid mixture of v/v 3:1) and kept cold in ice chest containing ice blocks until taken to the lab where they were sorted into various *Simulium* species and those of the *S. damnosum* s.l were stored in the refrigerator for later cytotaxonomic analysis. Larvae of the *S. damnosum* s.l. were identified by the presence of scales (spines) over the dorsal and lateral surfaces of the body and proleg and their characteristic dorsal tubercles [19].

### Cytotaxonomic identification of *S. damnosum* s.l. larvae

The collected larvae were processed for cytotaxonomic identification as described in [20] and identifications made based on the species diagnostic inversions described in [21-23].

### Data analysis

The SPSS statistical software version 25 was used to perform Chi-square test to estimate significance between blackfly breeding rate and seasonality.

# Results

### Breeding sites prospection

A total of 81 and 156 potential breeding sites were prospected during the rainy and dry seasons respectively for a period of 37 days (Rainy season = 15 days; dry season= 22 days). Seventy-two sites (30.4%) were found to be positive (contain larvae of the *S. damnosum* complex) in total (a site positive in the two seasons is counted twice). Non-*Simulium damnosum* s.l larvae were as well observed in some positive sites as well as in other sites that were negative for *S. damnosum* s.l. The number of *S. damnosum* s.l. breeding sites and number of prospection days, and the positive rates were as shown on table 1.

Breeding rates were higher in the dry season for the Southwest (74.4%) and the Northwest (40%) whereas it was higher in the rainy season for the North region (14.3%) (Table 1). A significant difference in breeding rates between the two seasons was observed for the Southwest Region (p=0.04) but not for the Northwest (p=0.17) and North (p=0.15) Regions and for the three regions combined (p=0.24).

Out of the 68 sites that were visited on both sampling seasons (once per season), 16 (23.5 %) were positive in one of the two seasons, while 14 (20.6 %) and 38(55.9%) sites were respectively positive and negative in both seasons. More of these sites sampled twice, and positive in one of two season, were positive in the dry season than in the rainy season for SW (DS=6; RS=1) and NW (DS=02; RS=00), while in the North region it was almost a tie (DS=3; RS=4 - Figure 2).

### Species distribution

Six different cytospecies were identified in this study. These include the forest species *S. squamosum*, *S. mengense*, *S. yahense* and *S. soubrense* Beffa form; and the savanna species *S. damnosum* s.s and *S. sirbanum*. The Southwest and Northwest Regions had predominantly forest species while the North Region had predominantly savanna species (Figure 3).

Five of the six species identified in this study occurred in the Southwest region making it the region with the highest species diversity (main species: *S. squamosum* and *S. mengense*; other species: *S. soubrense*, *S. yahense* and *S. damnosum* s.s), followed by the North region with three species (Main species: *S. damnosum* s.s and *S. sirbanum*; other species: *S. squamosum*) and then the Northwest region with two (Main species: *S. squamosum* and *S. mengense*) (Figure 3). *S. soubrense* Beffa form was mostly observed in the Yoke-Muyuka and the surrounding breeding sites in both seasons.

No marked changes in species distribution was observed in the breeding sites that were sampled in the two seasons. Some species were noted to be absent in one season but present in the other season at the same sites. We did not, however, observe any association between a particular season and the presence/absence of a species, except for the presence of the individual larvae that could not be placed simply as *S. sirbanum* or *S. damnosum* s.s that were only observed in rainy season samples of the North region (Additional file 1: Table S1) at a frequency of 0.146 (n=103).

# Discussions

### Breeding sites of *S. damnosum* s.l

Sampling of aquatic stages of members of the *S. damnosum* complex is important in mapping the breeding sites of the vectors. This is necessary for determining the distribution of the vectors, selection of vector collection sites and sentinel communities for entomological and epidemiological evaluations respectively. Further, it helps in identification of areas of possible onchocerciasis transmission and understanding the local epidemiology of the disease. Hence breeding site survey is a key step in the monitoring and evaluation of the different phases of onchocerciasis elimination [10]. The present study has mapped more *S. damnosum* s.l breeding sites and species distribution in three onchocerciasis endemic regions of Cameroon. It provides an updated and more comprehensive survey encompassing several regions and vegetation types. This survey did not show any challenges peculiar to any vegetation type contrary to reports of difficulty in searching for breeding sites in forest areas [12].

Breeding sites of *S. damnosum* s.l were more prevalent in the Southwest region than the Northwest and North regions. This is due to the perennial nature of the rivers in the Southwest region and the dense tropical rainforest in this region which provides continues nutrient-rich water for the development of *Simulium* larvae. Similar to earlier observations [16, 17] most rivers in the North region, except the perennial rivers, were reduced to trickles or completely dried out. Variations in seasonal breeding was observed in all three regions, therefore highlighting the importance of proper timing of breeding sites surveys and other entomological studies.

Generally, it is considered that the highest transmission period is in the rainy season when the water courses are all flowing. However, this is not always the case as was observed in the Southwest and Northwest regions, as there was increase in prevalence of positive breeding sites in the dry season compared to the rainy season. In fact, comparing the potential breeding sites that were visited in both seasons in these two regions, it was observed that all the rainy season positive breeding sites were again positive during the dry season, meanwhile several sites that were negative during the rainy season became positive during the dry season. Achukwi et al. [24] also observed higher biting during the dry season in the Vina du Sud in Cameroon indicating increased breeding during the dry season.

Higher breeding in the dry season compared to rainy season could be explained by the flooding of some of these rivers during the rainy season that lead to the sweeping away of potential larval supports (e.g vegetation, trapped fallen leaves, fallen tree stem, etc) by the high water current, and/ or the submergence of breeding sites by the overflooding turbid dirty waters [25].

On the other hand, during the dry season (or early dry season for the large but seasonal rivers in the North Region), the water levels drop exposing submerged rocky beds and creating rapids, and the waters are less turbid, therefore favouring breeding. Hence, seasonal changes in water level [1, 25], turbidity [25] and availability of rapids and submerged vegetation are important factors in the breeding and distribution of blackfly vectors.

### Distribution of *S. damnosum* s.l

The distribution of *S. damnosum* s.l species was according to the bio-climate of the regions with the North (Sudan savanna) region habouring the savanna species (*S. damnosum* s.s and *S. sirbanum*) and the Southwest (rainforest - *S. squamosum*, *S. mengense*, *S. soubrense* Beffa form, *S. yahense*, *S. damnosum* s.s) and Northwest (mixed forest-savanna - *S. squamosum* and *S. mengense*) regions habouring the forest species. The high diversity of species found in the Southwest region could be due to the abundant breeding sites that provide a wide range of favorable microhabitat conditions for the adaptation of the diverse blackfly species of different ecological preferences. This area has been suggested to be one of the centres of greatest diversity of the *S. damnosum* complex in Africa where the Mount Fako and the associated forests may be playing an important role in their evolution [26].

Various species of the *S. damnosum* complex present different transmission efficiencies [5] hence increase diversity may lead to complex epidemiology of onchocerciasis. For example, *S. squamosum and S. mengense* the major onchocerciasis vectors in the Southwest and Northwest regions have been shown to present bilateral blindness similar to what has previously been established for savanna vectors [27]. Generally, there were no seasonal changes in species composition in the different regions. However, at some breeding sites, different species were observed in different seasons. This could be due to seasonal dispersal of species or seasonal variation in physico-chemical composition of the rivers that do not favour survival of certain species.

This study has shown how widespread *S. mengense* is since its first description from the Menge River in the Southwest region [28]. Furthermore, contrary to previous studies [15, 23, 29] which only recorded *S. squamosum* in Muyuka/Yoke and surrounding breeding sites, this present study observed predominance of *S. soubrense* Beffa form in the area. This is the first report of *S. soubrense* Beffa form in Cameroon. Longitudinal studies would be required to determine whether these changes in cytospecies composition in the Yoke/Muyuka breeding site and its environs is a permanent or temporary one.

## Conclusion

Although not exhaustive, this study provides a comprehensive, updated, and representative breeding sites and species distribution in the Southwest, Northwest and North regions of Cameroon. The difference in positive breeding sites with respect to seasons emphasizes the importance of carrying out breeding sites surveys in both rainy and dry seasons. This study has also demonstrated the feasibility of breeding sites surveys, in both rainy and dry seasons, in a rainforest zone. Also, this study has shown that not all potential breeding sites are actual breeding sites. The report of *S. soubrense* in the Southwest region indicates changes in species composition over time and could affect the epidemiology of onchocerciasis in this area.

# Abbreviations

DS: Dry Season; RS: Rainy Season; REMO: (Rapid Epidemiological Mapping of Onchocerciasis; SW: Southwest region; NW: Northwest region; N: North region

# Declarations

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**Authors' contributions**

DAB was involved in all aspects of the research. FA contributed to design, acquisition of data, and writing of the manuscript. NS contributed to design and acquisition of data. SKD, FY, FT, BB, CEN contributed to acquisition of data. All authors read and approved the final manuscript.

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**Availability of data and materials:** The datasets analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** The graphical abstract contains images of some of the authors of this paper who have given their consent for publication.

**Competing interests:** The authors declare that they have no competing interests.

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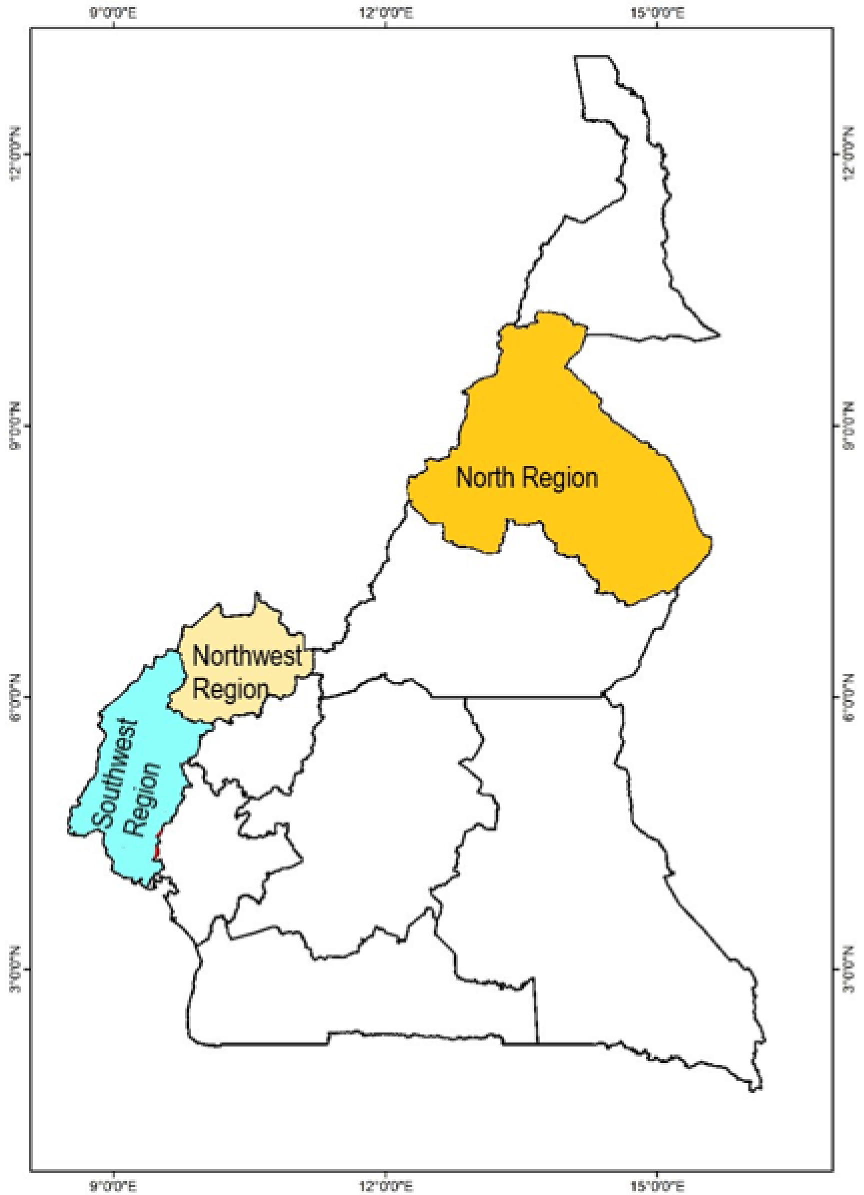
# Tables

Table 1: Number of prospected sites and prospection days per season and positive rates

| Region | Rainy season | | Dry season | | Positive rate (%) | |
|---|---|---|---|---|---|---|
| | Nº of prospected sites (positive sites) | Nº of days | Nº of prospected sites (positive sites) | Nº of days | Rainy | Dry |
| Southwest | 24(12) | 06 | 43(32) | 08 | 50 | 74.4 |
| Northwest | 15(03) | 03 | 35(14) | 05 | 20 | 40 |
| North | 42(06) | 06 | 78(05) | 09 | 14.3 | 6.4 |
| Total | 81(21) | 15 | 156(51) | 22 | 25.9 | 32.7 |

# Figures




## Figure 1

Map of Cameroon showing the three regions in this study

## Figure 2

Breeding sites maps showing sites that were sampled (a= Southwest region; b= Northwest region; c= North region).

## Figure 3

The study regions and the species of *S. damnosum* s.l identified for the two seasons. (Note: *S. da/si* is plotted on the map as *S. sirbanum*. The numbers attached to the symbols corresponds to the SN number on the Additional file 1: Table S1).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1TableS1.xlsx
- graphicalabstract2.png