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# Molecular detection of *Simulium damnosum S.l*, Vector of *Onchocerca volvulus* in Sanaga Maritime (Littoral-Cameroon)

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## ABSTRACT

**Aim:** The study was aimed to identify Simulium species of Mouanko using molecular tools.
**Method and Materials:** *Simulium* biting humans were aspirated using a sucking tube for molecular identification. Fifty female *Simulium* flies were caught using the mentioned technique.
**Results:** Molecular genotyping revealed that the anthropophilic *Simulium* black flies caught were of the *Simulium damnosums.l.* complex.
**Conclusion:** The presence of *Simulium damnosum s.l.* indicates the risk of ongoing onchocercosis transmission in the study area.

**Keywords:** *Simulium*, sucking tube, genotyping, Mouanko.

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## Introduction

Human onchocercosis remains a threat to the lives of individuals living in some rural communities of Cameroon. The WHO/APOC survey report of 2011 revealed >60% prevalence of onchocercosis in some foci of the Center 1, Littoral 2 and West region (Tekle et al., 2016). An entomological survey in the littoral zone of Cameroon reported the occurrence of *Simulium damnosum* Theobalt using morphological taxonomic keys (Kuete et al., 2014). Current data on *Simulium* of the Sanaga indicates their presence near households, farms and rivers nearer to human dwellings (Kuete et al., 2014), indicating the transmission risk of onchocercosis. There is need to confirm such anthropophilic species using molecular tools in order to design a sustainable vector control strategy.



## Materials and Methods

*Description of study area*

Fly collection was conducted in the littoral region precisely in Mouanko (Latitude 3° 38' 00''North and Longitude 9° 47' 00'' East). Mouanko is a coastal town in the Sanaga-Maritime division in the Littoral region of Cameroon. The capital is located on the north shore of the Sanaga, about 20 kilometers east of its mouth in the Gulf of Guinea. There are fast flowing rivers in the study area that serves as breeding sites for *Simulium* (Same-Ekobo, 1997).

*Morphological identification*

Flies were collected using sucking tubes from five volunteers who were willing to participate in the study. The sucking tube approach for the collection of *Simulium* from humans was conducted following Rauhöft et al., (2018) and flies were identified as *Simulium* using the key of Freeman and Meillon (1953).

*Molecular identification*

The collected flies were transferred in 70% ethanol, labelled and stored frozen at -20°C prior to DNA extraction. DNA was extracted using the Wizard® Genomic DNA purification kit (Promega™) (Sevidzem et al., 2020). Amplification of a portion of the mitochondrial 16S rRNA gene from the genomic DNA of flies was conducted following previously published protocol (Tang et al., 1996)

with modifications. Briefly, the PCR was conducted in a total volume of 25 µl, consisting of 13.8l RNase free water, 5µl 5x Buffer, 2µl $MgCL_2$, 1 µl each of the dNTPs, 0.5µl each of the primers, 0.2 Taq polymerase and 2µl genomic DNA. Amplification was conducted in a master cycler (Eppendorf®) with the following conditions: 95°C for 2mins followed by 35 cycles of 95°C for 30s, 53.0 for 30s , 72 °C for 1min with a final elongation of 72°C for 5mins. The pair of primers (16S F: 5′-CGCCTGTTTATCAAAAACAT-3′ and 16S R: 5′-CTCCGGTTTGAACTCAGATC-3′) used was from the published work of Hassan et al., (2015). The reaction signals were observed using the Midori-green stained 1.5% agarose gel with expected product size of 475bp (Hassan et al., 2015).

*Simulium DNA sequence analysis*

Samples were prepared for sequencing using the EZ-Seq User guide briefly, 5 µl template and 5 µl primer in each tube (1.5ml eppendorf tubes)/well. Sanger DNA sequencing was carried out. Sequences were aligned and blasted into the gene bank using Geneious version 10.2.3 and Evolutionary analyses were conducted in MEGA7using the Maximum Likelihood method.

## Results and Discussion

The result of gel electrophoresis was presented with samples having molecular weight of 475bp (Fig. 1). This product size has already been reported for *Simulium* species by Hassan et al., (2015).

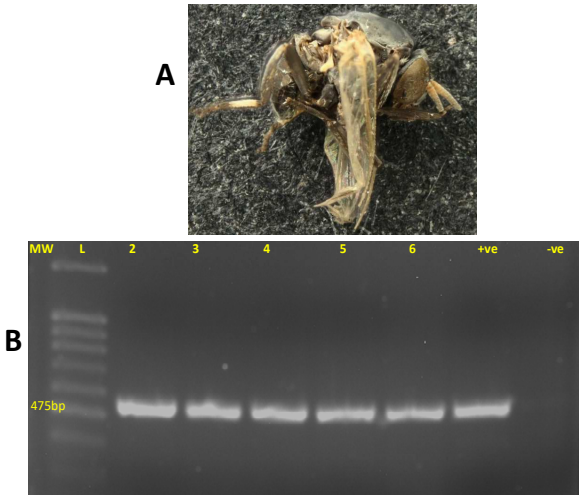


**Fig.1**. Gel electrophoresis showing the product sizes of *Simulium* samples. A) adult *Simulium*, B) Gel electrophoresis. MW molecular size, L Ladder, +ve: positive control, -ve: negative control. Lanes 2 to 6 represents *Simulium* samples.

The nucleotide sequences of flies from our study were compared with those in the genebank and it was noticed that they were identical to *Simulium damnosiums.l.* (Fig. 2).

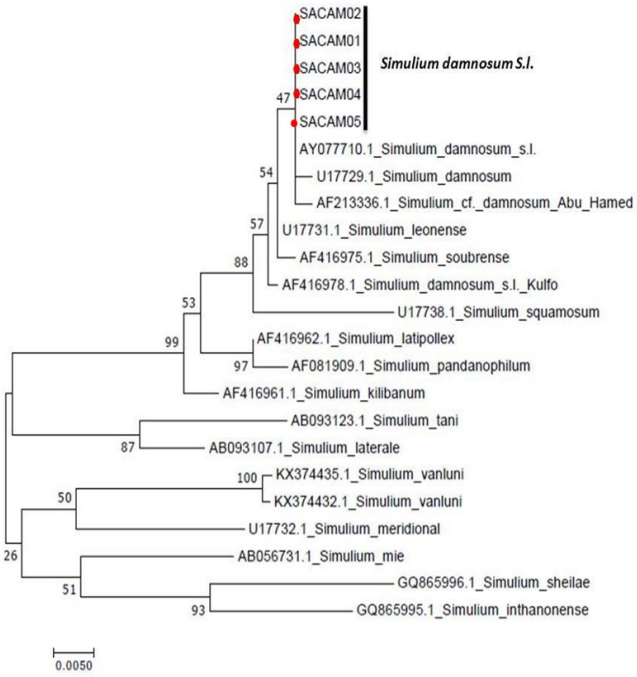


**Fig.2**. Phylogenetic tree of human biting *Simulium damnosum s.l.* (in red circles) from Sanaga region of Cameroon. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (1993). The tree with the highest log likelihood (-1310.25) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and Bio NJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 464 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016).

The presence of *Simulium* in Sanaga has already been signalled by Hougard et al., (1992) and Demanou et al., (2003). The *Simulium* black flies, collected in our study were individuals of the *Simulium damnosum s.l.* The flies were caught coming to take blood meal from humans at their homesteads. From the report of Kuete et al., (2014), high abundance of *Simulium* were observed around human dwellings, farms and rivers. The presence of anthropophilic *Simulium* black flies could be a

strong indication of the ongoing transmission of human onchocercosis in Sanaga. Apart from the occurrence of Simulium blackflies, Hiol et al., (2019) identified *Chrysops silacea* and *Chrysops dimidiata* which are vectors of Loa loa filarosis, indicating the potential transmission risk of this parasite in the community. The present study could be improved by increasing the sample number and sites as well as use different types of primers in order to enable the discrimination of the sibling species of the *Simulium damnosum* complex in Mouanko.

**Conclusions**

In conclusion, the presence of anthropophilic black flies of the *Simulium damnosum* s.l complex, indicated the risk of transmission of onchocercosis in the community of Mouanko in the Littoral region of Cameroon.

**Reference**

Demanou M, Enyong P, Pion SDS, Basáñez MG and Boussinesq M (2003). Experimental studies on the transmission of Onchocerca volvulus by its vector in the Sanaga valley (Cameroon): *Simulium squamosum* B. Intake of microfilariae and their migration to the haemocoel of the vector. Annals Trop. Med. Parasitol. 97: 381-402.

Freeman P and MeillonB (1953). Simuliidae of the Ethiopian Region. British Museum (National History) London. P 1-224.

Hassan KH,Shanna B,Kubofcik J, Nutman BT, Eberhard ML, Middleton K, Wakoli WJ, Ruedas G, Kimberly JN, Dubielzig R, De Lombaert M, Silverman B, Schorling JJ, Adler PH, Unnasch TR and Beeler ES (2015). Isolation of *Onchocercalupi*in Dogs and Black Flies, California, USA. Emerg. Infect. Dis. 21 (5), DOI: http://dx.doi.org/10.3201/eid2105.142011

Hiol V, Sieumeni AD, Mamoudou A, Sevidzem SL, Njan-Nloga AM and Nukenine EN (2019). Spatio-Temporal Dynamics of Glossinidae, Tabanidae and StomoxyidaeAround the Douala-EdeaWildlife Reserve in Cameroon. American J. Entomol. 3 (2) : 36-42.

Hougard JM , Escaffre H, Darriet F, Lochouarn L, Rivière F and Back C(1992). An episode of resistance to permethrin in larvae of *Simulium squamosum* (Diptera: Simuliidae) from Cameroon, after 3 1/2 years of control. J. American Mosq. Control Assoc. 8(2):184-6.

Kuete T, Nkoa T, Tassen F, Baleguel NP and Ekobo S (2014**)**. Biodiversity and ecology of Culicidae and Simulidae, probable vectors of infectious diseases of the Sanaga mid Valley, Cameroon: Influence of the Sanagariver. J. Parasitol. Vector Biol.6 (9): 142-150.

Kumar S, Stecher G and Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol., 33(7): 1870-4.

Rauhöft L, Sevidzem L andRenz A (2018). Developing new traps for catching blood-hungry *Simulium damnosum* in Cameroon. 28th Annual meeting of the German Society for Parasitol., 21-24th March 2018, p 291-292.

Same-Ekobo A. (1997). Santé Climat et environment au Cameroun. Edition Jutely-Sciences. P 329.

Sevidzem SL, Kong AB, Koumba AA, ZingaKoumba C, Mintsa-Nguema R and Mavoungou JF (2020). Molecular identification of Stomoxys and Musca (Diptera: Muscidae) of veterinary importance in the pasture area of Ngaoundere. ActaEntomol.Zool. 1(2): 33-36.

Tamura K and Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10(3): 512-26.

Tang J, Pruess K and Unnasch TR. (1996). Genotyping North American black flies using mitochondrial ribosomal RNA sequences. Can. J. Zool. 74:39–46.

Tekle AH, Zoure HG, Noma M, Boussinesq M, Coffeng LE, Stolk WA and Remme JH (2016). Progress towards onchocerciasis elimination in the participating countries of the African Programme for Onchocerciasis control: epidemiological evaluation results. Infect. Dis. Poverty. 5(1): 66.

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