



Research Article

Morphological studies of Female *Simulium damnosum* s.l. in Akamkpa Local Government Area, Cross River State, Nigeria

Cletus Inah Iboh^{1*} and Gabriel A. Arong²

^{1*}Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria.

²Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria

This study was carried out between February 2014 and January 2015 to investigate the morphology of *Simulium damnosum* s.l. populations along Kwa Falls and Rhoko river in Akamkpa Local Government Area of Cross River State, Nigeria, in relation to the strain of *Onchocerca volvulus* transmitted in the study area. Two fly boys working on shift of 6 hours captured *Simulium* flies settling on them for blood meals by inverting specimen vials over them. *Simulium* flies were preserved in 70% ethanol and transported to the Biological Science Laboratory of Cross River University of Technology, Calabar, for entomological studies. Of the 265 *Simulium* species studied, 29 species were identified as savanna-dwellers, 98 species as transition zone dwellers and 138 species as forest-dwellers. There was significant difference ($p < 0.001$) in the wing tufts colour but no significant difference ($p > 0.05$) between blackflies from these rivers. Mean thorax/antenna ratios of 2.4143, 1.9673, and 2.033 were recorded for savanna dwellers, transition zone dwellers and forest species respectively. Based on the morphology of *Simulium damnosum* s.l. studied, it could be inferred that there are likely four *Simulium* sibling species related to *Simulium sirbanum*, *S. squamosum*, *S. yahense* and *S. damnosum sensu stricto* in the study area.

Keywords: Forest, Kwa-Falls, Morphotaxonomy, Rhoko, *Simulium*, Savanna.

INTRODUCTION

Simulium damnosum sensu lato (s.l.) is a complex composed of sibling species which are medically important vectors of *Onchocerca volvulus*, the causative agent of human onchocerciasis or "river blindness", a debilitating human disease of tropical Africa, part of America and Yemen (Iboh and Braide, 1987). *Simulium* species are found from the arctic to tropical ecosystems, where they have profound economic effect on humans and animal production, and may reduce the fitness of wildlife (Adler *et al.*, 2004). About 1800 species of *Simulium* are recognized worldwide (Crosskey, 2002) and they often reach very high abundance, suggesting that their impact on wild animals in terrestrial landscape is significant (Wotton, 1988 in Okeke *et al.*, 2011). Oku *et al.* (2011) identified *Simulium* species among biting insects of evergreen Rhoko area in Akamkpa.

In West Africa, human onchocerciasis is transmitted exclusively by sibling species of the *S. damnosum* Theobald complex (Ikpeama *et al.* 2006). The nine sibling species of *S. damnosum* complex identified in West Africa include *S. damnosum sensu stricto*, *S. sirbanum*, *S. dieguerense*, *S. sanctipauli*, *S. soubrense*, *S. squamosum*, *S. yahense*, *S. leonense* and *S. konkorensis* (WHO, 1994).

***Corresponding Author:** Dr. Cletus Inah Iboh
Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria. Tel: +2348035825249; E-mail: clenaboh@yahoo.com

The first three species are regarded as savannah flies which transmit the savannah strain of *Onchocerca volvulus* while the remaining six belong to the forest group and transmit the forest strain of the disease of which the pathogenicity is more of skin disease with less blinding (Adeleke *et al.*, 2010). Due to changes in adult *Simulium* population structure in different ecozones and contributions of each sibling species to onchocerciasis epidemiology, there is apparent need for distinct identification of adult *S. damnosum* (Adeleke *et al.*, 2010). The characterization and differentiation of species are the most important practical functions of contemporary taxonomy. The ratio of the length of the thorax to the antenna is generally a very useful character in distinguishing savanna flies from the forest flies (Okeke *et al.*, 2011). Also the colours of the forecoxa, scutellar hairs, wing tuft (stem vein setae) and ninth abdominal tergite of each of the fly were usually observed and scored as pale, dark or intermediate according to standard keys (Kurtak *et al.*, 1981; Wilson *et al.*, 1993) to classify the fly into forest or savanna dwelling group.

A detailed morphological study of the six common sibling species of *S. damnosum* complex occurring in West Africa has been in progress since 1976. Its purpose was to establish morphotaxonomic characters which could provide a high degree of reliability to separate these species (Okeke *et al.*, 2011).

Various studies on onchocerciasis, adult *S. damnosum* complex and immature forms of black flies have been undertaken by several researchers in Nigeria (Ikpeama *et al.*, 2006; Oluwole *et al.*, 2009; Adeleke *et al.*, 2010; Post *et al.*, 2011; Okeke *et al.*, 2011; Osue *et al.*, 2013; Uzoigwe *et al.*, 2013). Although there are reported studies in Cross River State of Nigeria on the occurrence and distribution of *S. damnosum*, seasonal variation in human onchocerciasis and status of forest onchocerciasis (Iboh and Braide, 1987; Atting *et al.*, 2005, Opara *et al.*, 2005), there are no reported morphological studies of sibling species of *S. damnosum* in Akamkpa. In view of this, the present study was aimed at studying the morphology of adult female *S. damnosum* in relation to the strain of *Onchocera volvulus* transmitted in Akamkpa Local Government Area.

MATERIALS AND METHODS

Study area

The study was conducted in Akamkpa Local Government Area of Cross River State, Nigeria, situated between longitude 8° and 8° 45' East of the Greenwich Meridian and latitude 5° and 5° 45' North of the equator. Akamkpa Local Government Area is a rainy zone with a yearly average rainfall of about 360 millimetres. The relative humidity is between 89 percent and 93 percent with a yearly average temperature of about 31°C (FMAMA,

2015). The natural vegetation is dense tropical rainforest, which extends from Obudu through Akamkpa to Cameroon. The rainforest is one of the highest biodiversities in the world and harbours many species of insects of agricultural and public health importance. The local government is drained by a multitude of streams and rivers like Kwa falls and Rhoko river (our study sites) in an East-Westerly direction.

Sample collection

Female adult flies of *S. damnosum* s.l. were collected twice monthly near breeding sites on human baits at Kwa falls in Aningeje and Rhoko river in Iko Esai between February 2014 and January 2015. Catchers designated "fly boys" sitting on the banks of Kwa falls and Rhoko river with their legs and hands fully exposed and working on shift of 6 hours, captured all *Simulium* flies settling on them for blood meals by inverting specimen vials over them. Sampling was done fortnightly for a period of 12 hours within two consecutive days. The captured flies were pooled according to the hour of catch in each location before identification. *Simulium* flies were preserved in 70% ethanol and transported to Biological Science Laboratory of Cross River University of Technology, Calabar, for entomological studies.

Morphological identification of *Simulium damnosum* s.l.

Morphological study of two hundred and sixty-five (265) members of *S. damnosum* preserved in 70% ethanol was carried out in the Biological Science Laboratory of Cross River University of Technology, Calabar. The preserved blackflies were first rinsed with distilled water and fixed on microscope slides with glycerine. These slides were then viewed under Wild M5A microscope and the different parts were observed and measured using ocular and stage micrometer. All measurements were then transformed into millimetres as described by Usip *et al.*, (2003) and Okeke *et al.*, (2011), and the thorax/antenna (TZ/AZ) ratio obtained was used for identification and separation of *Simulium* sibling species into forest and savanna dwellers. *S. damnosum* species with thorax size/antenna size ratio below 2.0mm were classified as forest-dwellers and those with ratio above 2.0 mm were classified as savanna-dwellers. The wing-tufts, fore coxae, and antennal segments of each fly were examined for either pale or black coloration as well as the shape of antennae to be classified as savanna or forest dwelling species (Wilson *et al.*, 1993; Kurtak *et al.*, 1981). A fly having dark colour for any or all the morphological characters was grouped as forest species and one with pale colour in all the morphological characters as a savannah type. Flies that had half pale and half black wing tufts, and a mean thorax and antennal ratio of 1.9673 were grouped as transition zone dwellers.

Table 1. Colour of wing-tufts of *Simulium* species from Kwa Falls and Rhoko River

| LOCATION | Number of Flies Examined with Wing-Tufts Colour-Class | | | | | Total |
|-------------|---|------------------|--------------------------|----------------|-----------------|-------|
| | Pale | Black intermixed | Half pale and Half black | Most some pale | black All black | |
| Kwa Falls | 20 | 24 | 30 | 27 | 40 | 141 |
| Rhoko River | 9 | 20 | 24 | 39 | 32 | 124 |
| Total | 29 (10.94%) | 44 (16.6%) | 54 (20.38%) | 66 (24.91%) | 72 (27.17%) | 265 |

Table 2. Measurements of morphological characters of *Simulium* Sibling species collected from the study area.

| | SAVANNA DWELLER | | | TRANSITION ZONE DWELLER | | | FOREST DWELLER | | |
|------------|-----------------|-----------------|-----------------|-------------------------|-----------------|-----------------|----------------|-----------------|-----------------|
| | No of flies | Thorax (mm) | Antenna (mm) | No of flies | Thorax (mm) | Antenna (mm) | No of flies | Thorax (mm) | Antenna (mm) |
| | 12 | 0.94 | 0.39 | 54 | 0.84 | 0.36 | 46 | 0.78 | 0.39 |
| | 8 | 0.98 | 0.41 | 27 | 0.86 | 0.56 | 52 | 0.81 | 0.45 |
| | 9 | 0.88 | 0.36 | 17 | 0.85 | 0.37 | 40 | 0.87 | 0.40 |
| Mean | | 0.9324 ± 0.0913 | 0.3862 ± 0.1772 | | 0.8472 ± 0.0711 | 0.4168 ± 0.0976 | | 0.8174 ± 0.0645 | 0.4155 ± 0.0822 |
| Mean Ratio | 2.4143 | | | 2.033 | | | 1.9673 | | |

*mm =millimetre

Table 3. Morphological classification of female *Simulium* adults in the study area

| Month | SAVANNA-DWELLERS | TRANSITION ZONE DWELLERS | FOREST-DWELLERS | Total (%) |
|----------------|------------------|--------------------------|-----------------|-----------|
| February 2014 | 1 | 6 | 10 | 17 (6.4) |
| March 2014 | 2 | 7 | 16 | 25 (9.4) |
| April 2014 | 1 | 12 | 25 | 38 (14.3) |
| May 2014 | 0 | 7 | 12 | 19 (7.2) |
| June 2014 | 0 | 12 | 9 | 21 (7.9) |
| July 2014 | 2 | 3 | 4 | 9 (3.4) |
| August 2014 | 1 | 4 | 5 | 10 (3.8) |
| September 2014 | 2 | 9 | 11 | 22 (8.3) |
| October 2014 | 0 | 13 | 10 | 23 (8.7) |
| November 2014 | 8 | 12 | 14 | 34 (12.8) |
| December 2014 | 7 | 6 | 13 | 26 (9.8) |
| January 2015 | 5 | 7 | 9 | 21 (7.9) |
| Total | 29 | 98 | 138 | 265 (100) |

*Number in parenthesis = percentage

Statistical analysis

Chi-square test was used to determine the wing-tufts colour difference between *S. damnosum* species from Kwa Falls and Rhoko river.

RESULTS

Table 1 shows the colour of wing-tufts of *S. damnosum* complex collected from Kwa Falls and Rhoko river. Twenty-nine (29) female *Simulium* species had pale wing-tufts colour and were grouped in colour class 01. This constituted 10.94% of the total *Simulium* flies caught. Forty-four (44) *Simulium* species showed black intermixed wing-tufts hair and were categorized in colour-class 02. This gave a total catch of 16.6%. Fifty-four (54)

Simulium flies made up of 20.38% revealed half pale and half black wing-tufts hairs and therefore classified on colour-class 03. About 66 (24.91%) of *Simulium* flies had most of the wing-tufts hairs black and some pale, thus grouped in colour class 04. However, 72 (27.17%) *Simulium* flies were found to have black wing-tufts hairs and then grouped on colour-class 05. There was significant difference ($p < 0.001$) in the wing-tufts hair colour exhibited by *Simulium* species from Kwa Falls and Rhoko rivers. However, there was no significant difference ($p > 0.05$) in abundance between black-flies from Kwa Falls and Rhoko rivers.

Table 2 contains measurements of morphological characters of the thorax size (TZ) and antenna size (AZ) of *Simulium* species. The savanna dwellers of *Simulium* species (29) recorded thorax sizes (TZ) of 0.94 mm, 0.98

Table 4. Distribution of savanna and forest *Simulium* species from Kwa Falls and Rhoko River

| Type of species | Kwa Falls | Rhoko River | Total (%) |
|-----------------|-----------|-------------|-------------|
| Savanna-dweller | 20 | 9 | 29 (10.94) |
| Forest-dweller | 120 | 115 | 236 (89.06) |
| Total | 140 | 124 | 265 (100) |

*Number in parenthesis = percentage.

mm, 0.88 mm and antennal sizes of 0.39 mm, 0.41 mm, and 0.36 mm. These savanna dwellers revealed a mean thorax and antennal sizes of 0.9324 ± 0.0913 and 0.3862 ± 0.1772 respectively, with a mean ratio of 2.4143 (Table 2). The transition zone dwellers of 98 *Simulium* sibling species showed thorax sizes (TZ) of 0.84 mm, 0.86 mm, 0.85 mm and antennal sizes (AZ) of 0.36 mm, 0.56 mm and 0.37 mm. These transition zone dwellers had a mean thorax and antennal sizes of 0.8472 ± 0.0711 and 0.4168 ± 0.0976 respectively, with a mean ratio of 2.033. The forest dwellers of 138 *Simulium* sibling species had thorax sizes (TZ) of 0.78 mm, 0.81 mm, 0.87 mm and antennal sizes (AZ) of 0.39 mm, 0.45 mm and 0.40 mm. These forest dwellers showed a mean thorax and antennal sizes of 0.8174 ± 0.0645 and 0.3755 ± 0.0822 respectively, with a mean ratio of 1.9673.

Table 3 includes the classification of female adult *Simulium* species according to the month of collection. Of the 265 *Simulium* species collected, 29 *Simulium* species were identified as savanna-dwellers, 98 *Simulium* species as transition zone dwellers, and 138 *Simulium* species as forest-dwellers. The highest number of savanna species was collected during the months of November and January. The transition zone dwellers and forest dwellers were mostly collected in April and November.

Table 4 shows the morphological distribution of female *Simulium* adults from the study area. The fore coxae of the savanna dwellers of all *S. damnosum* were pale, whilst those of all *Simulium squamosum* were dark. These savanna species had a mean ratio above 2.0. The wing tufts of the forest species of all *Simulium sirbanum* were pale without markings whereas those of all *S. yahense* were pale with black spots. The forest species had a mean ratio of 1.9673.

DISCUSSION

A knowledge of the type of onchocerciasis prevalent in any bioclimatic zone is dependent on the taxonomy of the vectors and the parasite strain they vector. Although work has been done on cytotoxicity identification of *S. damnosum* complex (Dumbar, 1969; Dumbar and Vajime, 1972; Braide *et al.*, 1980; Mafuyai *et al.*, 1996; Onyewe *et al.*, 2007), more intensified work is needed on morphological identification of the adult

female blackflies, since this stage vectors the parasite into humans.

The results of this preliminary morphological study of *S. damnosum* complex in Akamkpa Local Government Area have identified and classified *Simulium* sibling species into both savanna and forest species. Based on their wing-tufts and fore coxae colours, thorax/antennal ratio and the colour and nature of antennae, 29 *Simulium* species were classified as savanna-dwellers and 236 as forest dwellers. The savanna-dwellers identified in the study area presented pale basal wing-tufts, pale fore coxae, pale antennae 1 and 4 segments and compressed segments 4 and 5. This finding confirmed the work of Post and Crosskey, 1985 and Wilson *et al.*, 1993; who posited that pale wing-tufts are almost always indicative of savanna cytospecies identity and likely that flies with such characters belong to the *S. sirbanum/S. damnosum* sensu-stricto (s. str). The transition zone dwellers showed intermixed wing tufts and were observed to be abundant at the beginning and end of the rainy season (Ikpeama *et al.*, 2006). The forest-dwellers presented morphological characters which categorized them in intermixed and forest dwellers.

It is a known fact that savanna-dwelling species of *S. damnosum* s. l. are usually found in the savanna zone and the forest-dwelling species of *S. damnosum* s. l. confined to the forest area (WHO, 1995). The abundance of forest-dwellers in this study was an expected outcome, because Kwa Falls and Rhoko River are situated in the tropical rainforest of Cross River State, Nigeria. This finding supported earlier observation that the distribution of *S. damnosum* complex is to a larger extent related to the bioclimatic zones, of which ecology played the greatest factor (Post and Crosskey, 1985; Adeleke *et al.*, 2010)

The high population of savanna-dwelling species caught in November and December 2014, confirmed the believe that savannah-dwellers of *Simulium damnosum* s.l. migrate southwards during the dry season in search of shelter, breeding sites and blood meals (Crosskey, 1990; Oluwole *et al.*, 2009). It is most likely that the north-east trade winds and deforestation for agricultural purposes also aided migration of savanna-dwelling *Simulium* flies into the south. These observations are in consonance with earlier reported findings (Onyenwe *et al.*, 2007; Oluwole *et al.*, 2009; Post *et al.*, 2011). However, the incursions of savanna-dwelling *S. damnosum* s.l. into the forest have significant public

health implications. This could be because savanna-dwellers have been known to be efficient vectors of the severe blinding savanna strain of *Onchocerca volvulus* and inefficient vector of the less blinding forest-strain known as onchodermatitis (Toe *et al.*, 1997).

The variation presented by wing-tufts colour of forest *S. damnosum* species in the study area indicated the unreliability of using wing-tufts as the main tool for separation of *Simulium* sibling species. For instance, in this study *Simulium sirbanum* was differentiated from *Simulium yahense* by having pale wing-tufts without markings, while that of *S. yahense* was pale with black spots. This result corroborates the work of Akpan *et al.* (2012). Although morphological identification of *Simulium damnosum* sibling species is not exhaustive compared to cytotaxonomy, it remains the fastest practical means of monitoring the movement of savanna species into southern Nigeria (Ibeh *et al.*, 2008). Information for a more realistic identification of *Simulium* sibling species transmitting *Onchocerca volvulus* in this study was obtained by the use of mean thorax/antenna ratio. The savanna-dwellers had mean thorax/antenna ratio of 2.4143 with morphological characteristics showing the presence of *Simulium damnosum* and *S. squamosum*. However, the presence of actual dark forest flies with mean thorax/antenna ratio between 1.9673 and 2.033 indicated the existence of *S. yahense*, and *S. sirbanum* in the study area. These findings were in line with the work of Post and Crosskey (1985), Mafuyai *et al.* (1996) and Adeleke *et al.* (2010).

From these findings, it could be inferred that four sibling species of *Simulium damnosum* identified and transmitting both the forest and savanna strains of *Onchocerca volvulus* in the study area include *S. damnosum sensu stricto*, *S. sirbanum*, *S. yahense*, and *S. squamosum*.

CONCLUSION

The morphological studies of *Simulium* sibling species revealed co-existence of both forest and savanna-dwelling species in a forest bioclimatic zone. The incursion of savanna-dwelling *Simulium* species into the forest is worrisome, because of the transmission of the severe blinding strain of *O. volvulus*. Although work has been done on cytotaxonomy identification of *Simulium damnosum* complex, more intensified work is needed on morphological identification of the adult female black-flies, since this stage vectors the parasite into humans. We also advocate cytotaxonomic identification of sibling species of *S. damnosum* in the study area, for rapid detection of changes in the epidemiology of the disease.

Competing Interest: This research was not funded by any organization and therefore no competing interest.

ACKNOWLEDGEMENT

We are grateful to the village heads and the entire communities of Aningeje and Iko Esai for their support during this study. We sincerely thank the fly boys who aided in the collection of *Simulium* flies at the breeding sites. The authors acknowledged the contributions of Prof. Axel P. Retana-Salazar, Dr. Evert Villanueva Sánchez, Prof. Upik Kesumawati Hadi, Samik Chowdhury, Ugwuanyi Ifeoma Kosisochukwu, Prof. Dipak Sharma and Dr. Nora B. Camino for donating their time, critical evaluation, constructive comments, and invaluable assistance toward the improvement of this very manuscript.

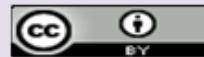
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Accepted 17 July, 2015

Citation: Iboh CI, Arong GA (2015). Morphological studies of Female *Simulium damnosum* s.l. in Akamkpa Local Government Area, Cross River State, Nigeria. *International Journal of Entomology and Nematology*, 2(1): 002-008.



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