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

Cletus Inah Iboh¹* and Gabriel A. Arong²

¹Department 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.


*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 onchocercosis (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 *Onchocerca 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 MSA 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 2.0 mm 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.

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>Number of Flies Examined with Wing-Tufts Colour-Class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pale</td>
</tr>
<tr>
<td>Kwa Falls</td>
<td>20</td>
</tr>
<tr>
<td>Rhoko River</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>29 (10.94%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>SAVANNA DWELLER</th>
<th>TRANSITION ZONE DWELLER</th>
<th>FOREST DWELLER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of flies</td>
<td>Thorax (mm)</td>
<td>Antenna (mm)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.94</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.98</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.88</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean</td>
<td>0.9324 ± 0.0913</td>
<td>0.3862 ± 0.1772</td>
<td>0.8472 ± 0.0711</td>
</tr>
<tr>
<td>Mean Ratio</td>
<td>2.4143</td>
<td>2.033</td>
<td>1.9673</td>
</tr>
</tbody>
</table>

*mm =millimetre

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

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

*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 mm and antenna sizes (AZ) of 0.39 mm and 0.41 mm.
Table 4. Distribution of savanna and forest Simulium species from Kwa Falls and Rhoko River

<table>
<thead>
<tr>
<th>Type of species</th>
<th>Kwa Falls</th>
<th>Rhoko River</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savanna-dweller</td>
<td>20</td>
<td>9</td>
<td>29 (10.94)</td>
</tr>
<tr>
<td>Forest-dweller</td>
<td>120</td>
<td>115</td>
<td>236 (89.06)</td>
</tr>
<tr>
<td>Total</td>
<td>140</td>
<td>124</td>
<td>265 (100)</td>
</tr>
</tbody>
</table>

*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. yaheense 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 cytotomy 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.s.). 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 belief 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 blackflies, 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.

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