Delineation of *Simulium Damnosum* Complex Composition in an Onchocerciasis Focus (Agba Village -Ebonyi State) with Reference to Forest and Savannah Types, Using Morphological Characteristics.

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Abstract
The study was carried out to determine the species composition of Simulium damnosum complex around Asu River, situated in Agba village, Ishielu, L.G.A of Ebonyi state, Nigeria; using morphological characteristics. A total of 91 flies were collected and observed for morphological characteristics; the thorax length/ antenna length ratio. Human baits were used for the collection. The flies were identified morphometrically using ocular microscope; and the body parts measured in millimetres using the ocular and stage micrometer. The thorax length/ antenna length ratios were determined according to published protocols. These were used to separate the flies into savannah and forest types. Any Simulium damnosum that had the thorax/antenna length ratio above 2.25 were identified as savannah dwellers, while those with TL/AL ratio of less than 2.25 were identified as forest dwellers. Of the 91 adult female black flies collected, 73 were forest dwellers representing (80.22%) of the sample population. The number identified as savannah dwellers was 18, representing (19.78%) of the entire sample population. The mean thorax length obtained for the forest and savannah dwellers were (0.8267±0.0404) and (0.9400±0.0529) respectively, while the mean antenna length were (0.4200±0.0346) for the forest dwellers and (0.3833±0.0306) for the savannah flies. The mean TL/AL ratios obtained in the study were 1.9683 and 2.4524 for the forest and savanna dwellers respectively. Sequel to the above, it can be concluded that the savannah and forest "types" of S. damnosum are coexisting

in the area as suggested by pockets of earlier epidemiological studies. This coexistence has a potential implication on the dynamics of transmission in the area.

Keywords: *Simulium damnosum complex, Onchocerciasis, Forest type, Savanna Type, Morphometrics, Agba Village.*

Introduction

Onchocerciasis is a chronic parasitic infection caused by the filarial nematode, *Onchocerca volvulus.* The disease is transmitted from one individual to another through the bites of the blackfly *Simulium damnosum* of the family Simulidae (Nwoke *et al.*, 1991). Onchocerciasis is basically a rural disease affecting communities sited along fast-flowing rivers with symptoms particularly irritating and disabling, often associated with long-term exposure to infection and this affects the social and economic activities of the inhabitants concerned (Edungbola and Asaolu, 1984). It is a chronic parasitic infection with public health and socioeconomic consequences of considerable magnitude in many sub-Saharan African countries (Moyou-Somo *et al.*, 1993; Opara *et al.*, 2005). In most of these countries, it constitutes a public health problem and a serious obstacle to socio-economic development (Etya'ale, 2001). It is one of the tropical diseases classified as orphan or neglected disease and is the world's second leading infectious disease (Thylefors, 1995; Pion *et al*., 2002).

The disease is endemic to Africa, tropical America and the Yemen but 99% of cases occur in Africa, and in Africa 95% is transmitted by *Simulium damnosum sensu lato* which is responsible for 250,000 cases of blindness and the loss of nearly one million Disability Adjusted Life Years (DALYs) every year (WHO 1995). As well as this direct effect on human health there is some evidence that Onchocerciasis may be associated with epilepsy, stunted growth, and increased susceptibility to malaria and reduced efficacy of vaccinations (Basanez *et al.*, 2006).

In Nigeria, Onchocerciasis is widespread and a cause of blindness in most rural communities. Of all the countries of the world, Nigeria has the largest number of persons with onchocerciasis accounting for about a third of the global prevalence with about 40 million at risk of infection (Edungbola, 1991). In Nigeria, *Onchocerca volvulus* is transmitted primarily by the *Simulium damnosum* complex (Opara *et al.*, 2008). The prevalence of human onchocerciasis was shown to be directly related to the abundance of this blackfly *Simulium damnosum* complex (Opara *et al.*, 2005).

In West and East Africa, the predominant species is *Simulium damnosum* complex, which coexist with other species such as *S. sirbanum, S. sanctipauli* and *S. soubrense* (Atting *et al.*, 2005). *Simulium neavei* is a much less important vector which is responsible for transmission in Congo, Zaire and Uganda (Service, 2004). Cytotaxonomic studies of the *S. damnosum* complex from different parts of Nigeria have revealed the presence of the five cytospecies, these include: *S. damnosum sensu stricto, S. sirbanum, S. squamosum, S. yahense* and *S. soubrense* (Atting *et al.*, 2005). There are two strains of the *Onchocerca* species, distinguishable at the DNA level by the polymerase chain reaction (PCR). One strain is usually found in the savannah regions of West Africa and the Americas, while the other strain is commonly found in the rain forest areas (Ogunrinade, *et al*., 1999. Roberts and Janovy, 2000). An understanding of the transmission of onchocerciasis as in other forms of filariasis is important in the knowledge of how vector capacity, vector abundance, survival rate, feeding habit and behaviour influence the level of infection and disease in susceptible human population. The knowledge of vectorial capacity would be of immense value in formulating the most appropriate control strategies in a given

locality (Opara *et al.*, 2008). The parasite has been targeted for global elimination by the world health organization using Community Directed Treatment with the drug Ivermectin (CDTI) (WHO, 1994). Annual treatment of communities can eliminate the parasite (Awadzie *et al*., 1985, Molyneux and Davies, 1997, Mustapha, *et al*., 2005) and progress towards elimination is routinely assessed by both epidemiological and entomological parameters WHO – recommended strategy for onchocerciasis elimination requires the delimitation of transmission zones where parasites form single population which is more or less homogenous within the transmission zones (Davies, 1993; Boatin *et al*.,1998; Kennedy *et al.*, 2003). Migration of parasites can occur through human or vector migration, and the most important is thought to be vector migration, (Walsh, 1990; and Davies, 1994) "for example, Onchocerciasis transmission is continuing in the eastern part of Kogi state in spite of continuing CDTI, and it is possible that this is the result of immigration of infective flies from surrounding areas".

In West Africa, studies have shown that human onchocerciasis is transmitted exclusively by sibling species of the *S. damnosum Theobald* complex (Ikpeama *et al.*, 2006), Nine sibling species of *S.damnosum* are recognized in West Africa .these include *S.damnosum sensu stricto, S. sirbanum, S. dieguerense, S.sanctipauli, S.soubrense, S.squamosum, S.yahense, S.leonense* and *S.konkorense* (WHO, 1994). Of these *S.damnosum s.l, S. sirbanum* and *S.diegrense* are regarded as savannah flies which transmit the savannah strain of *Onchocera volvulus,* while the rest are regarded s 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*).* Given that, the adult *Simulium* population structure has been found to exhibit changes in different ecozones and that the role of each sibling species to onchocerciasis epidemiology varies, (Adeleke *et al.,* 2010*),* the need therefore for distinct identification of adult *S. damnosum* complex cannot be over emphasized this is because, the characterization and differentiation of species are the most important practical functions of contemporary taxonomy. The thorax to the antenna length ratio is generally used in distinguishing savannah flies from the forest flies (Okeke *et al.*, 2011). Other important characters for distinguishing between these two types of flies are colours of the fore coxae, scuteller hairs, wing tuft (stem vein setae) and ninth abdominal tergite (Kurtak *et al*., 1981, Wilson *et al.*, 1993). Remarkable research activities have been in progress in the area of morphometrics of the common sibling species of *S. damnosum* occurring in West Africa since 1976. The purpose was to establish morphotaxonomic characters which would provide a high degree of reliability to separate these species (Okeke *et al*., 2011). Adult and immature forms of *S. damnosum* complex as well as onchocerciasis have been the subject of numerous studies conducted by researchers in Nigeria (Mafuyai *et al*., 1997, Usip *et al*., 2003, Opara and Fagbemi, 2005, Ikepeama *et al*., 2006, Onyenwe *et al.*, 2007, Oluwole *et al*., 2009, Adeleke *et al.,* 2010, Okeke *et al.*, 2011, Osue *et al*., 2013, Uzoigwe *et al.*, 2013).

Although there are reported studies of onchocerciasis in Ebonyi state, these are largely epidemiological and unpublished reports (Iroha *et al.*, 2010, Nworie *et al.*, 2014, Okonkwo and Ejiofor unpublished data,). And there are no reported morphological studies of siblings of *S. damnosum* in Agba village in Ishielu L.G.A. Sequel to this, the present study was aimed at studying the morphology of adult female *S, damnosum* with the view to identifying the "types" prevalent and relating it to base line epidemiological data in the area.

MATERIALS AND METHODS

Study Area

Amagu Agba is a rural community in Ishielu local government area of Ebonyi state, Nigeria. The community lies between longitude 7^0 51' 36" E and latitude 6^0 14' 48" N (see: http://www.gps-coordinates.org/nigeria-latitude.php).

It is situated in the tropical rainforest and located at about 140km north east of Abakiliki the state capital. There is an estimated population of 32000 people in Amagu Agba according to 2006 national population census. Amagu Agba has two climate seasons, the rainy season from March to October and dry season from November to February. The annual rainfall is approximately 230 millimeters and atmospheric temperature range from (25 ± 5^0C). The people have non-functional electricity, distantly located pipe borne water and two community secondary schools. The people depend majorly on rain water, streams and rivers as alternative sources of drinking water. The river experiences lots of anthropogenic activities including sand mining at the river bank, farming and irrigation, washing and bathing respectively. The river is characterized by sand doom amid the water body; other tributaries harbour healthy vegetation with *Elaeis guinensis* dominating.

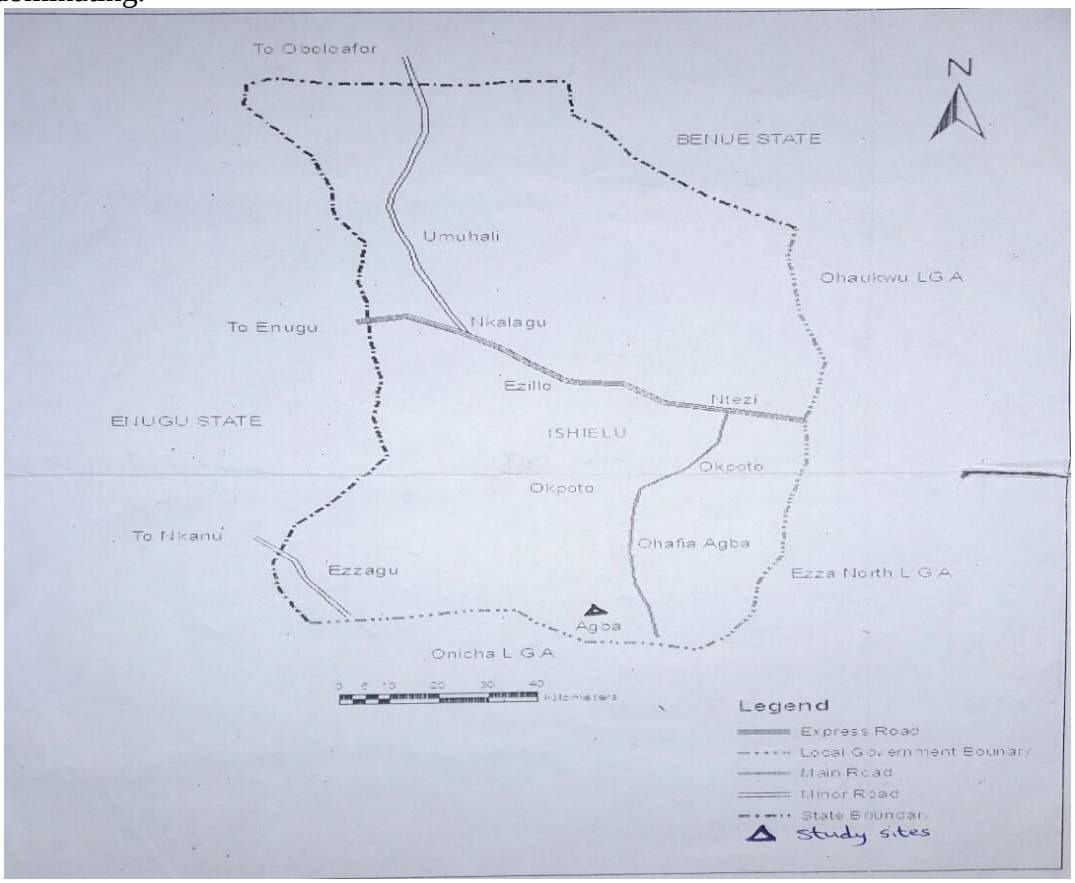

Fig 1: Map of Ishielu L.G.A showing the communities.Source :Geology and Geophysics Ebonyi state, University(2016)

2.2 Sample Collection:

Female adult flies of *S. damnosum s.l.* were collected twice monthly near breeding sites on human baits around Asu River in Agba village Ishielu L.G.A Ebonyi state, between June and July 2016. Catchers designated "fly collectors" sitting on the banks of Asu 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 carried out every fortnight for a period of 12 hours within two consecutive days. The captured flies were preserved in 70% ethanol and transported to the Zoology and Environmental Biology Laboratory of Ebonyi State University for further entomological studies.

2.3 Morphometric Studies of *Simulium damnosum s.l*:

Morphological study of ninety-one (91) members of *S. damnosum* preserved in 70% ethanol was carried out in the Applied Biology Laboratory of Ebonyi State University. The blackflies were first rinsed with distilled water and fixed on microscope slides with glycerine. The slides were then viewed under a light binocular microscope and the relevant parts were observed and measured using ocular and stage micrometer. All measurements were then transformed into millimetres as earlier described by (Okeke *et al.*, 2011), and thorax/antenna length (TL/AL) ratio obtained was used for identification and separation of *Simulium* siblings species into forest and savannah dwellers. *S. damnosum* species with thorax/antenna length ratio below 2.25mm were classified as forest dwellers while those with ratio above 2.25mm were classified as savanna-dwellers (Garms and Cheke, 1985).

RESULTS

The thorax/antenna length ratios obtained by measuring the captured flies are shown in table (1).

Table 1: Measurement of TL/AL of *Simulium* sibling species collected from the study area.

Number of flies	Thorax (mm)	Antenna(mm)	TL/AL Ratio
32	0.79	0.40	1.975
26	0.87	0.40	2.175
15	0.82	0.46	1.783
10	0.96	0.39	2.462
4	0.98	0.41	2.390
4	0.88	0.35	2.514

The TL/AL measurements range from 1.783-2.514mm. Based on this, the 91 flies measured were grouped into forest or savannah species according to the protocol of (Garms and Cheke, 1985). Thorax/Antenna ratios of 2.25 and below as mentioned earlier were grouped as forest species while, Thorax/Antenna ratios of above 2.25 were classified as savannah species. Of the 91 flies observed and measured, 73 were classified as forest species while 18 were classified as savannah species.

The mean thorax/antenna length ratios of both the forest and savannah species are shown in table (2)

Table 2: MEAN TL/AL RATIO OF FOREST AND SAVNNA FLIES

FOREST FLIES			**SAVANNA FLIES**		
NUMBER OF FLIES	TL (mm)	AL (mm)	NUMBER OF FLIES	TL (mm)	AL (mm)
32	0.79	0.40	10	0.96	0.39
26	0.87	0.40	04	0.98	0.41
15	0.82	0.46	04	0.88	0.35

Total=73			Total=18		
Mean	0.8267±0.0404	0.4200±0.0346		0.9400±0.0529	0.3833±0.0306
Mean TL/AL Ratio	1.9683			2.4524	

For the forest flies, the mean thorax length (TL) was 0.8267mm, mean antenna length (AL) was 0.4200. The savanna flies showed a mean thorax length of 0.9400 and mean antenna length of 0.3833. The mean thorax/antenna ratio for forest flies was 1.9683 while that of the savanna was 2.4524.

A total number of 91 flies were captured with the forest dwellers having the highest percentage abundance of (80.22%) and the savanna dwellers showing the least percentage abundance of (19.78%) as shown in Table (3).

Table 3: % ABUNDANCE OF FOREST AND SAVANNA FLIES

Species type	No of flies	% Abundance
Forest type	73	80.22
Savanna type	18	19.78
Total	91	100%

Discussion

According to (Post and Boakye, 1992) the main patterns of epidemiological variation are related to the taxonomy of the parasite and vector. Therefore, knowledge of the type of onchocerciasis prevalent in any climatic zone is dependent on the taxonomy of the vectors and the parasite strain they vector. This implies that not all sibling species are equally important in disease transmission. If effective vector control must be achieved there is need for identification of adult flies. Unfortunately, the propensity of members of *S.damnosum* complex to exhibit sibling speciation makes it difficult to identify the adult which actually transmit the disease. This is because sibling species re morphologically alike but reproductively isolated. Cytotaxonomic studies on larval stages have proved useful in separation of sibling species of *S.damnosum* complex and many cytotaxonomic studies have been carried out (Dunbar, 1969, Dunbar and vajime, 1972, Braide *et al.*, 1980, Mufuyai *et al.*, 1996, Onyenwe *et al.*, 2007; Post *et al.*, 2011). But cytotaxonomy relies heavily on the banding pattern found in the polytene chromosome (in the salivary gland of larval simuliids) and pay little attention to the adult which actually transmit the disease. There is therefore a huge requirement for the morphometric identification of the adult female because it is this stage that are actually involved in disease transmission. In this preliminary study, adult female *S. damnosum s.l* were classified as either forest or savannah species using the thorax/ antennae ratio. Seventy-three flies (73) were classified as forest while eighteen (18) were classified as savannah flies. Thorax/antennae ratio is generally accepted as a reliable method of separating members of *S.damnosum* complex. No other reliable method has been found to match its usefulness in separating individual female flies of S. *damnosum* complex (Wilson *et al.*, 1993). The utilization of thorax/antennae length alone in a bivariate analysis resulted in 100% separation of the savannah species and *S. sanctipauli* flies (Garms, 1978, Wilson and Baker, 1991).

The forest dwellers identified in the study area had the thorax/antennae length ratio of less than 2.25mm while the savannah dwellers had the thorax/antennae length ratio of above 2.25mm. tis

is in consonance with the work of Garms and Cheke, (1985) and Ebido *et al.*, (2011), who recorded less than 2.25mm and above 2.25mm for TL/AL of forest and savannah flies respectively. It is a well-established axiom that savannah dwelling species of *S. damnosum* complex are found mainly in the savannah zone and the forest dwelling species of *S. damnosum* restricted to the forest area (WHO, 1995). The abundant number of the forest dwelling species of *S.damnosum* complex (73) in the study area was therefore anticipated outcome because Agba village in Ishielu local government is located in the tropical rain forest of Ebonyi state. This finding is in agreement with the works of Post and Crosskey, (1985) and Adeleke *et al.*, (2010), who noted that the distribution of *S. damnosum* complex is to a larger extent related to the bioclimatic zones, of which ecology played the greatest factor.

The relatively high population of savannah dwelling species (19.78%) recorded in this study confirmed previous finding that savannah dwellers of *S. damnosum* complex migrate south-wards in search of shelter, breeding site and blood meal (Crosskey, 1990, Oluwole *et al.*, 2009). Deforestation for agricultural purposes and excessive lumbering may have also aided migration of savannah dwelling flies into the south. Onyenwe *et al.*, (2007), Oluwole *et al.*, (2009) and Post *et al.*, (2011) had reported the presence of savannah dwellers in the forest region. The invasion of savannah dwellers into the forest zones is not without the attendant public health implications. This is because savannah dwellers are known to be very efficient vectors of the severe savannah blinding strain of *Onchocerca volvolus* and inefficient vector of the less blinding forest strain also called *Onchodermatitis* (Toe *et al*., 1997).

The relevance of this study stems from the fact that it can be an efficient and rapid pointer to any trend of change in prevalence or severity of the diseases. Ibeh *et al*., (2008) noted that early warning of any such change in fly population will be very important especially in the detection of any trend of change in the prevalence or severity of Onchocerciasis. Though morphometrics alone cannot be relied on for accurate monitoring of vector dynamics, it is none the less an effective tool for planning vector control strategies. This is because it may be misleading to ascribe epidemiological importance to the different cytotaxonomic entities of the members of the *S. damnosum* complex since cytotaxonomic identifications can only be made from larvae but it is the adult females that actually transmit the parasite.

On the other hand, the extraordinary ability of members of *S. damnosum* complex to migrate from one zone to another makes it difficult to say with absolute certainty that flies caught biting alongside the river have emerged from that river. Ibeh *et al*., (2008) had observed that the admixture of a small proportion of migrant individuals of a more efficient vector may grossly distort the apparent role of a particular species in transmission.

Conclusion

Although evidence from pockets of epidemiological studies in Ebonyi state indicates that the area is endemic, the present findings not only confirm this but indicate that transmission is likely to be going on. It also indicates that the two strains of the disease causing filarial worm may co-exist in the region. The relative abundant of savannah types of flies seen in the study may be indicative of numerous ocular case documented in earlier epidemiological studies.

Recommendation

It is recommended that cytological studies be carried out in the study area to validate and confirm the present findings. While that is being done, the inhabitants are encouraged to take proper measures that will protect them from the flies. This may include staying away from the

breeding sites during the peak biting periods, and covering their body parts properly with long sleeves and head gears. They should also participate in the Community Directed Treatment with Ivermectin (CDTI) to help reduce parasite loads.

On the other hand, the Government through the Ministry of health and other agencies should educate the villagers periodically and also put logistics in place to ensure timely and adequate delivery of CDTI services.

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