STUDIES ON THE MORPHOMETRIC CHARACTERISTICS OF THE *Simulium damnosum* COMPLEX IN UZO-UWANI, ENUGU STATE, NIGERIA

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ABSTRACT

Studies on the morphometric characteristics of the Simulium vectors of onchocerciasis were carried out in Uzo-Uwani Local Government Area (LGA), Enugu State, Nigeria, between September 2008 and January 2009, with the objectives of identifying the sibling species present in the area and comparing the result obtained with cytological studies earlier done in the area. It was also meant to relate fly types with the types of onchocerciasis present in the area. The Simulium flies were collected using human baits. Seventy-five (75) flies were subjected to morphometric examinations. The thorax length, antenna length, wing length, wing width and femur length were measured and the data obtained were transformed into ratios. The morphometric studies on the Simulium damnosum populations in Nkpologu showed a higher abundance of savanna flies (54) than forest flies (21) among those sampled. The classification was done using the thorax length/antenna length (TL/AL) ratio. Discriminate function analysis revealed that 96.0% of originally grouped cases were correctly classified. The result agreed with cytotaxonomic studies on Simulium done in the area which showed predominance of savanna flies. The implications of the results are discussed.

Keywords: Morphometric, *Simulium,* Savanna flies, Forest flies, Cytotaxonomy, Onchocerciasis

INTRODUCTION

In West Africa, human onchocerciasis, which is a severely debilitating and blinding disease caused by the filarial nematode, *Onchocerca volvulus,* is transmitted exclusively by sibling species of the *Simulium damnosum* Theobald complex (Diptera: Simuliidae). Blackflies are found from arctic to tropical ecosystems, where they have significant economic impact on humans and animal production, and may reduce the fitness of wildlife (Crosskey, 1990; Adler *et al*., 2004). About 1800 species of blackflies are recognized worldwide (Crosskey, 2002) and they often reach very high abundances (Wotton, 1988) suggesting that their impact on wild animals in terrestrial landscape is significant. Blackflies are among the insects of foremost medical importance around the world, as they are vectors of filarial nematode causing onchocerciasis in inhabitants of tropical regions. They also have a veterinary importance, because they transmit protozoa and nematode to animals and are therefore a nuisance, reducing the productivity of livestock and other farm animals (NRCC, 1982).

Accurate identification of members of the Simuliidae is becoming increasingly significant as more species become the target of pest and vector control operations, or the subject of epidemiological studies on the transmission of the blackfly-borne parasites of man (*O. volvulus* and *Mansonella ozzardi*). The characterization and differentiation of species are the most important practical functions of contemporary taxonomy. At present, the overwhelming majority of blackflies are still recognized and defined on characters of their external (hard-parts) morphology, even recently described species.

Prior to 1966, *S. damnosum* was considered to be a single, but morphologically and biologically variable species. However, 25 cytotypes in this complex was discovered as a result of further chromosomal studies (Dunbar, 1969; Dunbar and Vajime, 1971; 1972; Vajime and Dunbar, 1975). These cytotypes and their distributions were summarized in tabular form in a report by WHO (1978). The West African members of this complex include *S. damnosum* s.s, *S. squamosum*, *S. sirbanum*, *S. sanctipauli*, *S. soubrense*, *S. yahense*, *S. dieguerense*, and *S. sudanense.* The latter two species are rare and of uncertain status (Vajime and Dunbar, 1975).

A detailed morphological study of the adults of the six common sibling species of the *S. damnosum* complex occurring in West Africa has been in progress since 1976. Its purpose was to try to find external characters that could be relatively easily observed and used with a high degree of reliability to separate these species (Garms, 1978).

According to current opinion, *S. squamosum*, a member of this complex described by Enderlein in 1921, is a distinct species both on the basis of chromosomal and external morphological features. Even though there is great similarity between *S. squamosum* and *S. damnosum* s.s., there are specific external characters that can be used to separate the majority of adult specimens of these two species. Presumably, it should also be possible to separate most, if not all, of the other known siblings in the complex.

The most practical procedure routinely available for distinguishing between the species of adult females belonging to the *S. damnosum* complex is the assessment of micro-morphological characters (Dang and Peterson, 1980). This method is currently in use by the World Health Organization Onchocerciasis Control Programme (WHO/OCP) involving West African countries (Wilson *et al.*, 1993; 1994). Unfortunately, the members of the *S. damnosum* complex are extremely difficult to differentiate morphologically and are usually defined by specific chromosomal characters in the larvae (Vajime and Dunbar, 1975, 1977; Crosskey, 1987; Boakye, 1993).

However, it is still important to know the morphologies of the species of the *S. damnosum* complex as this would aid in the control of the vector. The most practical procedure routinely available for distinguishing between species of adult females belonging to the *S. damnosum* complex is the assessment of micro-morphological characters (Dang and Peterson, 1980). Mafuyai *et al*. (1996) reported that for monitoring southward invasion by savanna vectors of onchocerciasis in Nigeria, it is convenient and reasonably reliable to identify adult females by means of micro-morphometric differences between sibling species of the *S. damnosum* complex. 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 (Garms, 1978). In line with the above background information the objectives of this study were to identifying the sibling species present in the area and comparing the result obtained with cytological studies earlier done in the area.

MATERIALS AND METHODS

Study Area: The study area was Nkpologu in Uzo-Uwani Local Government Area, Enugu State, Nigeria. Nkpologu lies between latitude 6^0 40′ and 6^0 50′ north and longitude 7^0 00′ and 7^0 16′ east. It belongs to the forest-savanna mosaic vegetation zone of Nigeria (Crosskey, 1981). Nkpologu is traversed by Adada River, which is a tributary of the Anambra River. Farming is the major economic activity of the area.

Fly Collection: Fly collection (adult female) took place between December 2008 and January 2009. Flies were identified (Dang and Peterson, 1980) and confirmed as member of the *S. damnosum* complex by an Entomologist in Zoology Department, University of Nigeria, where voucher specimens (SDC - 0012) were deposited in the Museum of Natural History. The flies were usually collected during the daily peak biting periods (9.00 am – 10.00 am and 5.00 pm – 6.00 pm) as reported by Ubachukwu and Anya (2001, 2005). Two main locations were used for fly collection in Nkpologu. The flies

were collected using human baits as attractant. The human bait was given 3 mg/kg mectizan as a preventive measure. The human baits sat in the fly-catching locations with their lower legs exposed. Blackflies landing on the baits for a blood meal were caught and preserved in absolute ethanol for further studies in the laboratory.

Morphometric Studies of Blackflies: Seventy-five (75) flies member of the *S. damnosum* s.l population were subjected to morphometric characterization using seven standard morphometric characters. The thorax length, antenna length, wing length, wing width and femur length were measured in millimeter. The preserved flies were first rinsed with distilled water and fixed on slides with glycerine. The slides were then viewed under binocular microscope and the different parts measured using ocular and stage micrometer. The measurements were then transformed into ratios thus: thorax length/antenna length (TL/AL), thorax length/wing length (TL/WL), antenna length/femur length (AL/FL), wing length/femur length (WL/FL), wing length/antenna length (WL/AL), thorax length/femur length (TL/FL) and thorax length/wing width (TL/WW). The 75 flies measured were further categorized as either forest or savanna species using the thorax length/antenna length ratio (Garms and Cheke, 1985). Thorax length/antenna length ratios of 2.25 and below were classified as forest species while thorax length/antenna length ratios of above 2.25 were classified as savanna species.

Statistical Analysis: The results from the morphometric studies were subjected to multivariate analysis (Discriminant Function Analysis).

RESULTS

The mean lengths of the flies measured are as follows: thorax length = 0.9821 ± 0.0904 mm, antenna length = 0.3864 ± 0.0736 mm, wing length = 1.9739 ± 0.1443 mm, wing width = 0.8357 ± 0.1800 mm and femur length = 0.5869 ± 0.0798 mm (Table 1).

Table 1: Morphometric characters of blackflies from Uzo-Uwani LGA, Enugu State, Nigeria

Morphometric Character	Mean Length
Thorax Length (TL)	0.9821 ± 0.0904
Antenna Length (AL)	0.3864 ± 0.0736
Wing Length (WL)	1.9739 ± 0.1443
Wing Width (WW)	0.8357 ± 0.1800
Femur Length (FL)	0.5869 ± 0.0798

The mean morphometric character ratios were: TL/AL = 2.6367 ± 0.5849, TL/WL = 0.4993 ± 0.0507, AL/FL = 0.6728 ± 0.1778, WL/FL = 3.4374 ± 0.6389, WL/AL = 5.2876 ± 1.0357, TL/FL = 1.7092 ± 0.3342 and TL/WW = 1.2442 ± 0.3652 (Table 2). Twenty-one (21) flies were classified as forest species while 54 were classified as savanna species. For the forest flies, the mean thorax length (TL) was 0.9162 ± 0.0889 mm, mean antenna length (AL) was 0.4524 ± 0.0602 mm, mean wing length (WL) was 1.9524 ± 0.1235 mm, mean wing width (WW) was 0.8276 ± 0.1878 mm and the mean femur length (FL) was 0.5762 ± 0.0745 mm. The savanna flies however recorded a mean thorax length of 1.0078 ± 0.0777 mm, mean antenna length of 0.3607 ± 0.0617 mm, mean wing length of 1.9822 ± 0.1519 mm, mean wing width of 0.8389 ± 0.1785 mm and mean femur length of 0.5911 ± 0.0821 mm (Table 3). The mean ratios of the different morphometric parameters are also presented (Table 4).

The stepwise discriminant function analysis revealed three major variables that contributed to the species complex differentiation. The three variables were TL/AL, TL and AL. Based on the result, TL/AL was the best single predictor of group membership and was closely followed by TL and AL respectively. These three variables were further included in a model to get the best possible prediction for group membership. At each step, the variable that minimizes the overall Wilks' lambda was entered (Table 5).

The association between the discriminant score and the groups (Eigenvalues) indicated that the function accounted for 100 % of the variance between the forest and savanna flies.

Table 2: Mean ratio of morphometric characters of blackflies from Uzo-Uwani LGA, Enugu State, Nigeria

Morphometric Character	Mean Ratio
Thorax Length/Antenna Length (TL/AL)	2.6367 ± 0.5849
Thorax Length/Wing Length (TL/WL)	0.4993 ± 0.0507
Antenna Length/Femur Length(AL/FL)	0.6728 ± 0.1778
Wing Length/Femur Length (WL/FL)	3.4374 ± 0.6389
Wing Length/Antenna Length (WL/AL)	5.2876 ± 1.0357
Thorax Length/Femur Length (TL/FL)	1.7092 ± 0.3342
Thorax Length/Wing Width (TL/WW)	1.2442 ± 0.3652

Table 3: Morphometric characters of the different groups of *Simulium damnosum* collected from Uzo-Uwani LGA, Enugu State, Nigeria

Morphometric Character	Mean Length	
	Savanna	Forest
Thorax Length (TL)	1.0078 ± 0.0777	0.9162 ± 0.0889
Antenna Length (AL)	0.3607 ± 0.0617	0.4524 ± 0.0602
Wing Length (WL)	1.9822 ± 0.1519	1.9524 ± 0.1235
Wing Width (WW)	0.8389 ± 0.1785	0.8276 ± 0.1878
Femur Length (FL)	0.5911 ± 0.0821	0.5762 ± 0.0745

Table 4: Mean ratios of the different morphometric parameters of *Simulium* collected from Uzo-Uwani LGA, Enugu State, Nigeria

Morphometric Character	Mean Ratio	
	Savanna	Forest
Thorax Length/Antenna Length (TL/AL)	2.8697 ± 0.5209	2.0375 ± 0.1531
Thorax Length/Wing Length (TL/WL)	0.5107 ± 0.0496	0.4699 ± 0.0417
Antenna Length/Femur Length(AL/FL)	0.6223 ± 0.1444	0.8027 ± 0.1924
Wing Length/Femur Length (WL/FL)	3.4296 ± 0.6533	3.4573 ± 0.6152
Wing Length/Antenna Length (WL/AL)	5.6423 ± 0.9625	4.3755 ± 0.5489
Thorax Length/Femur Length (TL/FL)	1.7447 ± 0.3446	1.6180 ± 0.2938
Thorax Length/Wing Width (TL/WW)	1.2606 ± 0.3093	1.2022 ± 0.4872

Table 5: Stepwise discriminant function analysis of the different morphometric parameters of *Simulium* collected from Uzo-Uwani LGA, Enugu State, Nigeria

		Wilks' Lambda				Wilks' Lambda Exact F			
Step	**Entered**	**Statistic**	**Df1**	**Df2**	**Df3**	**Statistic**	**Df1**	**Df2**	**Sig.**
1	**TL/AL**	0.586	1	1	73.000	51.476	1	73.000	0.000
2	**Thorax Length**	0.509	2	1	73.000	34.760	2	72.000	0.000
3	**Antenna Length**	0.334	3	1	73.000	47.235	3	71.000	0.000

Furthermore, the high value of the canonical correlation (0.816) showed that the function discriminated well (Table 6).

The classification table showed how accurately the model assigned flies to their correct groups (Table 7).

100% of the forest flies were classified accurately, while 94.4% of the savanna flies were classified accurately with only 5.6% misclassified. Generally, 96.0% of original grouped cases were correctly classified (Table 7).

Table 6: Association between the discriminant score of groups of different morphometric parameters of *Simulium* collected from Uzo-Uwani LGA, Enugu State, Nigeria

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	1.996a	100.0	100.0	0.816

Table 7: Classification table[a*] of different morphometric parameters of *Simulium* collected from Uzo-Uwani LGA, Enugu State, Nigeria

Morphometric parameters			Predicted Group Membership		
		Group	Forest	Savanna	Total
Original	Count	Forest	21.0	0.0	21.0
	(Number)	Savanna	3.0	51.0	54.0
	Percentage	Forest	100.0	0.0	100.0
	(%)	Savanna	5.6	94.4	100.0

a 96.0% of original grouped cases were correctly classified*

DISCUSSION

The major patterns of epidemiological variation in the *S. damnosum* complex are related to the taxonomy of the parasite and the vector (Post and Boakye, 1992), and so not all sibling species are equally important. Although vector taxonomy is majorly based upon the analysis of the polytene chromosomes from the larval silk glands, there is still a requirement for the identification of the adult female, because it is this stage that actually transmits the parasite.

In this study, adult female *S. damnosum* were classified as either forest or savanna species using the thorax/antenna ratio. Twenty-one flies were classified as forest while 54 were classed as savanna flies. The use of the thorax/antenna ratio in the differentiation of forest and savanna flies has proved useful. No more reliable method has been found to separate individual female flies of the *S. damnosum* complex (Wilson *et al*., 1993). The use of thorax and antenna lengths alone in a bivariate analysis resulted in 100% separation of the savanna species and *S. sanctipauli* flies (Garms, 1978; Wilson and Baker, 1991). Members of the *S. damnosum* complex are classified into forest and savanna species based on the preferred breeding habitat. The savanna-species have been known to be efficient vectors of the severely blinding savanna strain of *Onchocerca volvulus* and inefficient vectors of the less blinding forest-strain and vice versa for forest species (Duke, 1990). However, Toe *et al*. (1997) reported that this vector-parasite complex does not exist in the transition zones between the forest and the savanna. Hence the co-existence of both forest and savanna species in Nkpologu is not strange as this area belongs to the forest-savanna-mosaic vegetation belt in south-eastern Nigeria (Crosskey, 1981). Such occurrences have been reported in other parts of Africa (Opoku, 2006; Wilson *et al*., 2002; Kale, 1998).

In addition, this co-existence has been attributed to the incursion of savanna flies into massively deforested sites (Boakye, 1999; Garms *et al*., 1989; Wilson *et al*., 2002). Hence, deforestation is a very important factor in determining the kind of onchocerciasis prevalent in a particular area. Infact, available data suggest that savanna species cannot only invade deforested areas but also establish breeding populations that would persist (Post and Crosskey, 1985; Baker *et al*., 1990). Onyenwe (2007) and Onyenwe *et al*. (2007) reported the occurrence of *S. sirbanum* from cytotaxonomic examinations carried out in Ogurugu, Uzo-Uwani Local Government Area, Enugu State, Nigeria.

The importance of this study lies in the fact that it can be an efficient and fast indicator for the detection of any trend of change in prevalence or severity of the disease. Such a change might be brought about, for instance, by local deforestation and subsequent invasion by

savanna vector sibling species, which might bring the savanna strain of *O. volvulus* with them. Early warning of any such change in fly population would be very important especially in the detection of any trend of change in the prevalence or severity of onchocerciasis (Ibeh *et al*., 2008).

Furthermore, the biggest problem in ascribing epidemiological importance to the different cytotaxonomic entities of the members of the *Simulium damnosum* complex is that the cytotaxonomic identifications can only be made from larvae, but it is the adult female that actually transmits the parasite. The agility of *S. damnosum* species also makes it difficult to say with certainty that flies biting alongside a river have emerged from that river. The identification of adult flies biting man by reason of their proximity to breeding sites of known species is therefore, at best uncertain. 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 (Ibeh *et al*., 2008).

Conclusion: The morphometric studies of the *Simulium* vectors reveal a greater abundance of savanna flies as compared to forest species in Nkpologu. This suggests that there might be a greater prevalence of sight-related problems than onchocercal skin disease in the community. We recommend reduction of the rate of deforestation in this area in order to halt further incursion of savanna flies and the consequent blinding form of onchocerciasis in this area. There is also need for a more aggressive distribution of mectizan in the area to prevent irreversible blindness as a result of onchocerciasis.

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