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Full Length Research Paper

Cytotaxonomic study of the larvae of blackfly (Diptera: Simuliidae) from River-Ose, Ondo State, Nigeria

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A study of the cytotaxonomy of the larvae of blackfly was undertaken at three sites, Imeri A, Imeri B, and Ose-oba, along River Ose in Ondo State, Nigeria. Based on the morphology of the larvae and the resultant pupae, three species of blackflies were identified. These included *Simulium damnosum* complex, *Simulium alcocki*, and one yet to be identified (YI). Investigations on the polytene chromosomes from the salivary glands of each of these species revealed that the *S. damnosum* complex comprises of three cytospecies identified as *Simulium squamosum*, *Simulium damnosum* s.s, and *Simulium sirbanum*. The *Simulium alcocki* was also found to be a complex of sibling species but the YI remained as a single species. There was no significant difference between the distribution of *S. damnosum* complex and *S. alcocki.* However, these two species were different from the YI in occurrence. Results of the present study which is in conformity with earlier reports suggest that the study area (River-Ose) has been and is still a good breeding site for *S. damnosum* and *S. alcocki* species of black flies.

Key words: *Simulium damnosum*, *Simulium alcocki*, 'yet to be identified species' (YI), sibling species.

INTRODUCTION

Based on their impact on the health and economic well-being of humans, black flies are generally regarded as the second most medically important group of insects (Adler et al., 2004). They are responsible for the transmission of onchocerciasis, a debilitating disease caused by the filarial nematode *Onchocerca volvulus* Leukart (Nematoda: Filarioidea). Although, males usually feed on nectar, females obtain nourishment by feeding on the blood of humans and animals. There are several areas of the world where black flies are the most dreaded noxious arthropods due to their biting nuisance. In many rural settlements of the world they have a wide distribution particularly in Mexico, Yemen, Brazil, Venezuela, Ecuador, Colombia and Africa (Malau and James, 2009). The distribution of the flies in Africa covers the sub-tropical belt from Senegal in the West to Somalia in the East. It occurs in savanna, rainforest, plateau, and in the highland areas. The insects breed mainly in fast flowing, well-oxygenated streams and rivers. Their distribution and breeding is not even across Nigeria and their biting activities can be highly seasonal (Adeleke et al., 2011). The disease transmitted by them is prevalent in 35 countries of the world of which 28 are in Africa and Nigeria accounts for one quarter of the global infection

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rate (CDI Study Group, 2010).

Species complex occurs widely within the blackfly. A species complex (complex of sibling or cryptic species) is a group of closely related species (Crosskey and Lane, 1993). Although the species may satisfy the criterion of being reproductively isolated from each other, they are not readily or reliably distinguishable on morphological basis. This makes the use of cytological, genetic and/or ecological attributes for distinguishing between them an absolute necessity. The cytology of blackfly larvae has so far relied on studying their polytene chromosomes (Crosskey and Lane, 1993; Post et al., 2007). The micromorphology of polytene chromosomes has provided many key features for dipteran species characterization and phylogenetic investigations. The polytene chromosomes of simuliid populations have been analyzed for reasons such as the identification of species and cytotypes with vectorial capacity and the resistance or susceptibility to insecticides for the purpose of improvement of control programmes (Charalambous et al., 1995).

It has been established that not all the species of blackfly are vectors. The painful bites and disturbing effects of other species of *Simulium* in many riverine areas are intolerable nuisance. These bites could sometimes lead to blood losses and the sites could serve as portal of entry for viruses, bacteria, protozoans and nematodes which the flies may be carrying on their bodies or which are existing in the environment (Ubachukwu, 2004; Usip et al., 2006). These have consequential effects of low productivity, sickness and abandonment of infested areas.

This present study was undertaken to identify the larvae of different species of blackflies present in the study sites of River-Ose, Osun State, Nigeria, in order to reveal the different cytoforms present in the river by studying their polytene chromosomes.

METHODOLOGY

Study area

A cytotaxonomic study of the larvae of blackflies was undertaken at three breeding sites, Imeri A, Imeri B, and Ose-Oba which are located along River Ose in Ose Local Government Area of Ondo State. The segment of the river studied lie between longitude 05°66′ and 05°93′ east of the Greenwich Meridian, latitude 07°30′ and 07°31′ north of the equator. The three breeding sites (Imeri A, Imeri B, and Ose-Oba) are 224, 217 and 192 m above the sea level, respectively. The river is characterized by the presence of rocks, trees, vegetation, and sections where rocky substrata and submerged logs obstruct the flow of water, creating rapids. These rapids make the river a suitable environment for the development and survival of the aquatic larval stages of blackflies.

Sample collection

The three sites were visited biweekly for larval collection between the period of January and May 2015 during which 343 specimens of larvae were obtained. The immature stages of blackflies (larvae and pupae) were collected from submerged stones, trailing vegetations, twigs, and leaves, where the water flows rapidly, by using a pair of forceps. The larvae collected were fixed in freshly prepared cold Carnoy's fixative which contained 1:3 acetic acid and ethanol mixture, and brought to the laboratory.

Sorting of specimens

The larvae collected were sorted into different species under the objective lens of a dissecting microscope. The parameters for sorting included mainly nature of the post-genal cleft, head pigmentation patterns, pattern of gill spot, shape and length of larvae with other identification criterium such as the presence of scales on the abdominal region as described by Crosskey and Lane (1993).

Cytological studies

The larvae fixed inside Carnoy's solution were removed and blotted. The polytene chromosomes preparations were made following the method described in Sorungbe (2014) which was a modification of that of Olorode (1974). Full karyotyping and cytospecies identification were based on the criteria described by Vajime and Dunbar (1975), Boakye (1993) and Post et al. (2007).

RESULTS

Distribution of the black fly larvae

The cytotaxonomical investigation on the blackfly in the present study which was based on larvae examination revealed three species of the flies in Ose River. These included *Simulium damnosum* Theobald complex, *Simulium alcocki* group, as well as one 'yet to be identified' (YI). In all the surveyed sites of the river, members of the *S. alcocki* group were abundantly present, while *S. damnosum* were present in abundance only at Imeri sites A and B, but few at Ose-Oba site. However, a few members of the 'YI' species were present at the two sites in Imeri, but none was found at Ose-Oba site (Table 1).

Morphological identification of specimens

Matured *S. damnosum* larvae (Figure 1a) have barrel shaped structure and are usually 5 to 6.5 mm in length. The larval head capsule is strongly pigmented and spotted. The hypostomium is with 9 apical teeth. The larvae appear to be 'unshaved' as a result of the cuticular covering of small upstanding black spines and scales and they also have very prominent paired dorsal abdominal protuberances (tubercles). The setae of the larval cuticle occur even on the thoracic prolegs. The Pupae (Figure 1b) gill filaments look somewhat like hands of bananas (branching stoutly and tubular). The cocoon is shoe-shaped, closely woven, and appear blackish in colour.

The length of a matured larva of *S. alcocki* (Figure 2a)

Table 1. Overview of the abundance of various species of blackfly at the three breeding sites.

Location	No. of specimen collected	No. of male analyzed	No. of female analyzed
S. squamosum			
Imeri A	16	6	7
Imeri B	19	6	9
Ose-Oba	10	4	3
S. damnosum			
Imeri A	34	14	18
Imeri B	36	13	16
Ose-Oba	15	4	7
S. sirbanum			
Imeri A	24	10	11
Imeri B	19	9	8
Ose-Oba	12	5	5
S. alcocki			
Imeri A	50	19	25
Imeri B	53	21	23
Ose-Oba	42	18	17
YI			
Imeri A	8	3	3
Imeri B	5	2	3
Ose-Oba	-	-	-
Total	343	134	142

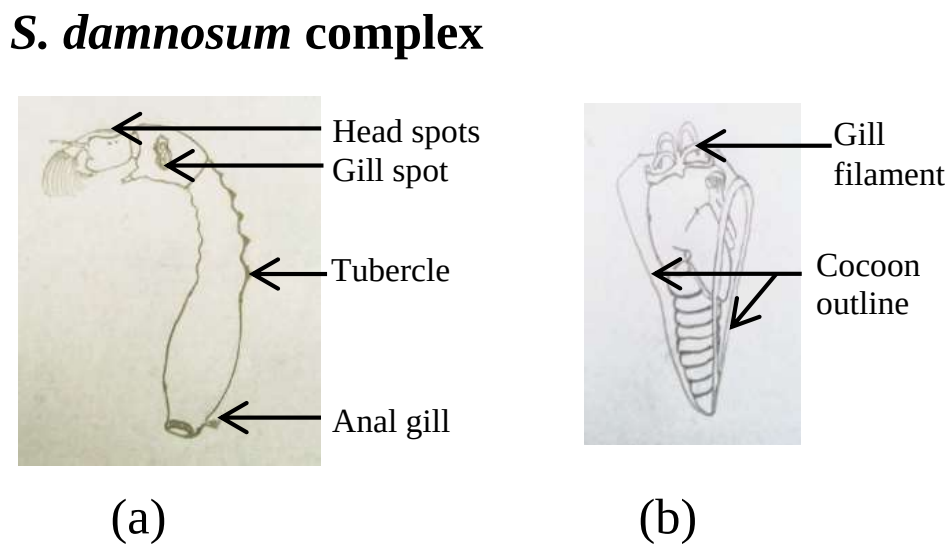

Figure 1. Lateral view of matured: (a) Larvae and (b) Pupa of *S. damnosum* (x20).

ranges between 6 and 8 mm. It has an elongated mid-section which enlarges gradually towards the posterior end. The head capsule has strong brown pigmentation. It is pale-brown but in a few of its members, the colour is

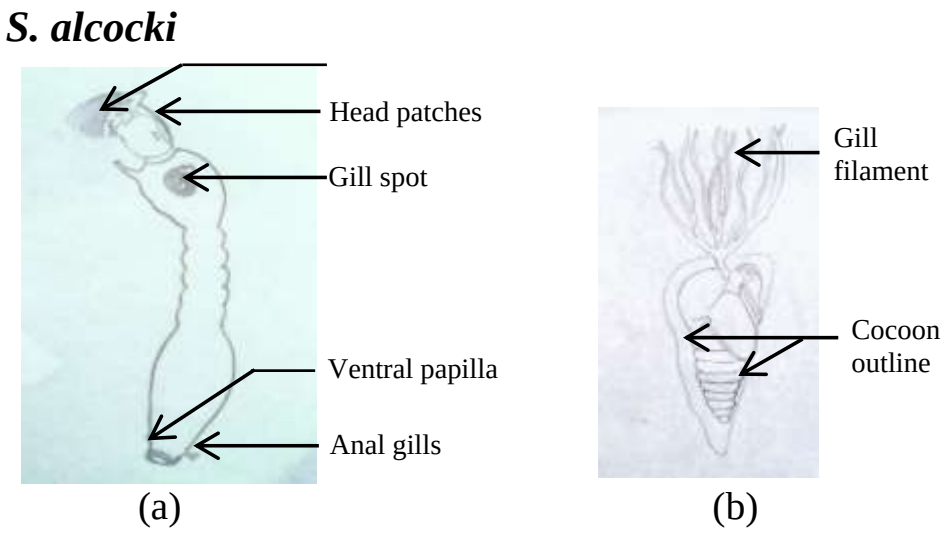

Figure 2. Lateral view of matured: (a) Larva and (b) pupa of *S. alcocki* (x20).

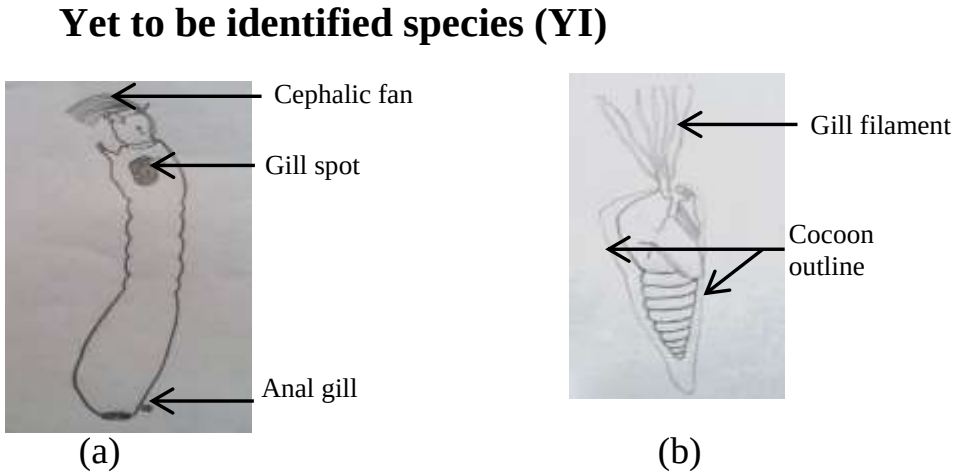

Figure 3. Lateral view of matured (a) Larva and (b) Pupa of YI species (x20).

deep-brown. The dorsal part of the abdomen is covered with setae. Each abdominal segment possesses two pairs of spots. An 'X'-like anal sclerite lies between the anus and the posterior circlet. The gill coils extensively to form a gill spot with a clear region at the center. The pupa (Figure 2b) has gills with three main branches and 9 to 12 filaments. Pupal gill arrangement is variable within samples of this species obtained in the present study. The cocoon is entirely slipper-shaped and closely woven.

Matured larvae of the yet to be identified 'YI' species (Figure 3a) were stout and with average lengths of between 4.5 and 5.5 mm. The head capsule has a clear zone with few spots but without pigmentation. Larval abdomen lacks conspicuous setae, but is covered with light brown scales. The gill is coiled extensively to form a very dark gill spot with shape somewhat similar to that of *S. alcocki* except that it has no cleared center.

The pupa (Figure 3b) of the 'YI' species has gills with 7 to 9 filaments and each of the filaments branched out right from the origin. The pupal cocoon is slipper-shaped and has darkish brown colouration.

Cytotaxonomy of the specimens

Cytological study of each of the morphologically identified species revealed three cytospecies from the *S. damnosum* complex (Figure 4). These include, *S. squamnosum* belonging to *S. squamnosum* subcomplex, *S. damnosum* s.s and *S. sirbanum* belonging to *S.*

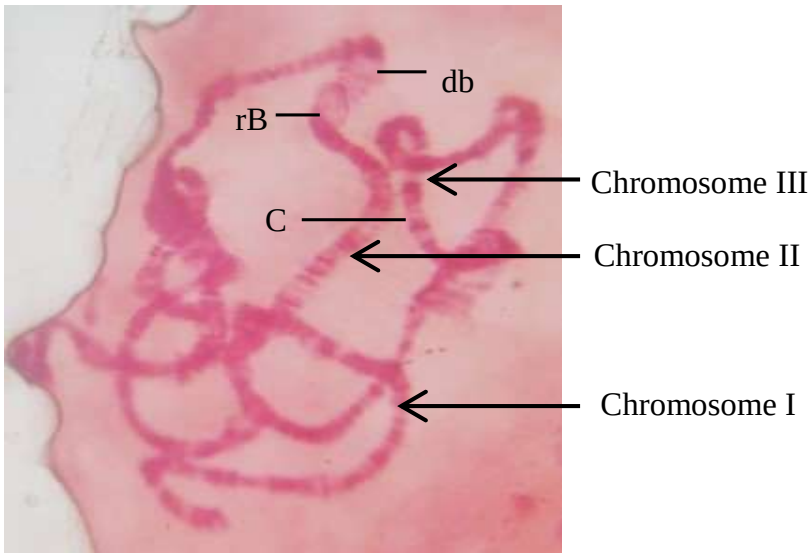

Figure 4. *S. damnosum* chromosome (x1000). db: Double bubble, rB: Ring of Balbiani, C: Centromere.

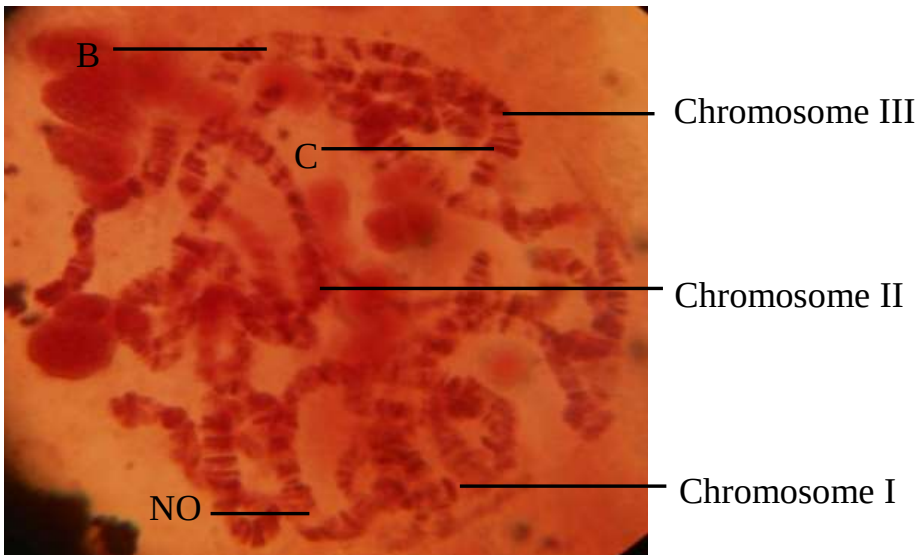

Figure 5. *S. alcocki* chromosome (x1000). B-Blister, NO-Nuclear Organizer, C- Centromere.

damnosum subcomplex. *S. squamosum* was recorded from all the breeding sites in the study area. Intraspecific chromosomal variation was observed in the *S. squamosum* encountered. Most of them showed complete synapsis in chromosome I, while some of the remaining few have short asynaptic centromeric region of chromosome I. All the specimens had homozygous inversions at both arms of chromosome I. Some specimens found at Imeri A and B showed floating inversions on IL and IIL, which was not present on any of the specimens obtained at Ose-Oba.

S. damnosum s.s were observed to be abundant in the two breading sites at Imeri (Imeri A and Imeri B). The females had homozygous inversion (C/C) on the long arm of chromosome II (IIL). Most of the specimens were homozygous, apart from some males that showed heterozygous inversion (C/C.8) on IIL (long arm of chromosome II). Amongst the *S. sirbanum* examined, inversions IL-3.1 on long arm of chromosome I was sex-linked, two inversions IS-1.3 and IIL-C.8 that were on the short arm of chromosome I and long arm of chromosome II, respectively were fixed within the specimen.

Majority of the *S. alcocki* specimens (Figure 5) had one or two inversions at either the short or long arms of

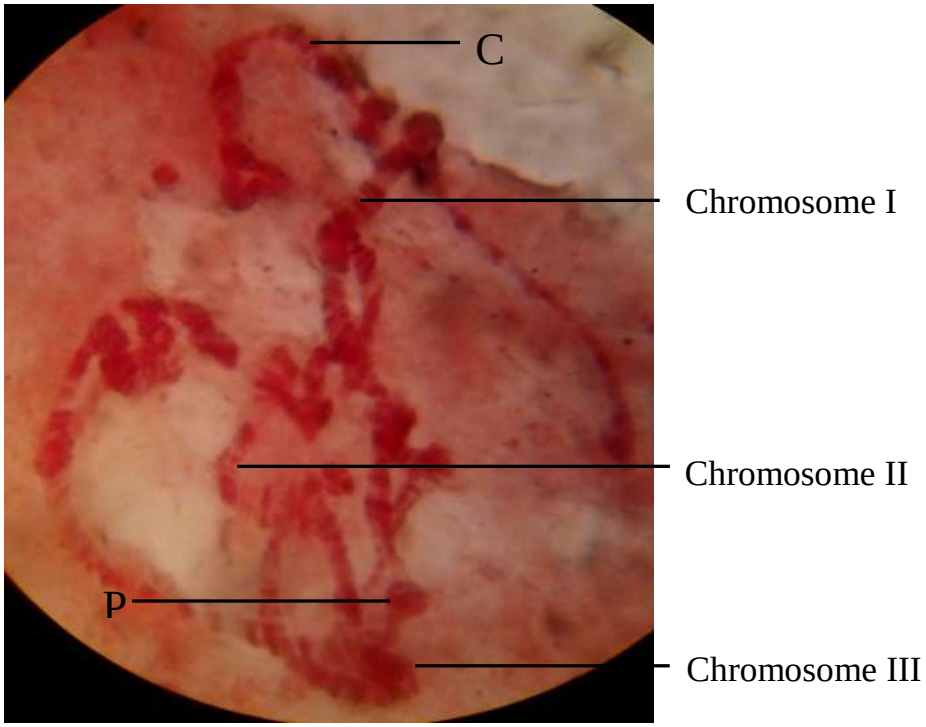

Figure 6. YI secies chromosome (x1000). C- Centromere, P-puff.

chromosome II (IIS or IIL). These inversions appeared to be fixed across specimens from each of the breeding sites, while specimens of the yet to be identified (YI) species (Figure 6) showed homogeneous and constant banding patterns. Asynaptic regions were also not frequent in this population. Arbitrary alphabets were adopted to denote inversions in *S. alcocki* and the yet to be identified species for better understanding since there was no documented standard chromosome map for comparison.

DISCUSSION

Based on morphological studies carried out on the larvae found in segments of Ose-river, findings revealed sympatric composition of three different species, although there was significant variation in the occurrence of these species along the river. This observation agrees with the earlier report of Opoku (2006) who observed that ecology plays a key role in the distribution of black flies. The most wide spread species in the three sites investigated were *S. alcocki* and *S. damnosun* which was consistent with the earlier findings of Tangjura et al. (2015) in Nasarawa State where higher occurrences of *S. damnosum* and *S. alcocki* were recorded from some rivers. The third species 'YI' was only recorded in two of the sites investigated. This species, as described in this present study, has similar morphology with the one reported earlier by Sorungbe (2014), which he denoted as unspecified species (US2) from Osun State.

The inversions on chromosomes I, II, and III of *S. damnosum* complex revealed three different cytospecies. *S. damnosum* s.s is the most wide spread member of the complex and the one having the highest occurrence in all the sites investigated. There was diversity in this sibling species across the breeding sites. Similar findings had also been reported by Post et al. (2007) and Olusi et al. (2012). This probably suggests that the flies must have invaded Ose River from different parts of the country since blackflies are reputed to undertake regular wind assisted migration covering distances of over 400 km, coupled with the fact that River OSE appears to be a good breeding site.

S. sirbanum was also found to be present in all the breeding sites and in sympatry with *S. damnosun* s.s. Their presence in this study was consistent with the work of Olusi et al. (2012); and Crosskey (1981) in a review of Nigerian cytospecies of the *S. damnosum* complex. Other workers, Wilson et al. (1994), and Onyenwe et al. (2007) also confirmed the presence of *S. sirbanum* in Nigeria. Diversity existed within the *S. sirbanum* identified in this study which was also in conformity with the findings of Vajime (1989) who synonymized *S. sirbanum* and *S. sudanense* under the name *S. sirbanum* and described them as 'sub-siblings'. However Mafuyai (1992) did not recognize the different cytotypes within *S. sirbanum*.

S. squamosum was found abundantly at Imeri A and

Imeri B sites. Its occurrence at Ose-Oba was found to be very low. Its presence was consistent with the list made available by Vajime and Gregory (1990) where eight cytospecies of *S. damnosum* complex which included *S. squamosum* were documented. Wilson et al. (1994) confirmed the presence of *S. squamosum* as reported by earlier findings.

S. alcocki were well distributed in the three different sites of the present study. However, the specimens obtained at Ose-Oba revealed more of similar inversions, and were different in parts to the inversions obtained from the other two sites of Imeri A and Imeri B. This suggests that this species comprises of more than one sibling species since there were differences in their cytology as well as breeding sites. Although the result obtained in the present study alone is not enough to justify this suggestion as there was no antecedent records to back it up, Sorungbe (2014) and Tangjura et al. (2015) had reported it's occurrence in Osun and Nassarawa States, respectively.

The specimens of 'YI' species obtained were very few. They were found mainly at imeri A and Imeri B, with no occurring in Ose-Oba. The members of this group showed little variation in distribution of inversions which suggests that they were monotypic species. This particular species was similar to the US2 species described by Sorungbe (2014) from Osun State.

Meanwhile, members of *S. damnosum* complex are solely responsible for transmission of onchocerciasis in West Africa (Boakye, 1999). Although some species like *S. alcocki* had hardly ever been reported as vectors, they can however constitute biting nuisances which can be almost unbearable. Their females, like other black flies, swarm about the head of humans in large numbers, intermittently landing and crawling on any exposed skin or darting into the eyes, ears, mouth and nostrils. All these could have implications on socio-economic well-being of the human population and consequentially culminate in overall low productivity of residents of *Simulium* infested areas. It has also been reported that the sites of bites of these flies can serve as portal of entry for bacterial infection.

The awareness of the species richness of these flies is a necessary imperative in the quest for an effective and successful control measures against them and the diseases they transmit.

Conclusion

This present study which involved identification of larvae based on morphology, as well as some pupae revealed three different species; *S. damnosum* complex, *S. alcocki,* and a yet to be identified species 'YI'. However, investigations carried out on the banding pattern of their polytene chromosomes showed that the *S. damnosum* was a complex of sibling species from which three siblings, *S. damnosum* s.s., *S. sirbanum*, and *S. squamosum* were reported, and they were widely distributed in the entire breeding sites studied along Ose-river. *S. alcocki*; as well as the yet to be identified (YI) species showed various patterns of bands with certain cytotypes having similar bands that were quite different from one another. This suggests that these species comprises of cytospecies.

Recommendation

Despite the effectiveness of Onchocerca Control Programs (OCP) and other programs directed at eliminating Onchocerciasis, blackflies still breed at such a rate that bring about great economic loss and threat to the success of any forms of control measure. Therefore, more work is indeed needed to be carried out in the aspect of the biology of the fly. Such study might probably provide better insights in carrying out more effective control measures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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