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Black fly (*Simulium* sp) composition, daytime biting activity and possible onchocerciasis infection in north-east, India

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Abstract

Pupal stages of *Simulium* were collected and identified from various breeding habitats of twelve locations in northeastern India. *Simulium* flies, while attempting to land on human were collected between 07:00- 17:00 hrs to understand the biting pattern. Seven species belonging to three sub-genera, *Eusimulium* (1), *Gomphostilbia* (1) and *Simulium* (5) belonging genus *Simulium* were encountered. Out of total seven species recorded, *S. (E) aureohirtum, S. (G) tenuistylum* and *S. (S) rufibasis* were predominant and shared 30.3%, 29.9% and 27.6% of total collection. Stream breeding habitat contributed 47.3% of total catch and was found to be preferred breeding habitat ($p<0.0001$). *S.* (*S*) *christophersi* and *S.* (*G*) *tenuistylum* were recorded for the first time from the northeastern region of India. Simuliids biting rhythm showed bimodal pattern and were more active during sunny day ($p<0.0001$). Microscopic dissection of simuliids (n=266) did not incriminate simuliids as vector of onchocerciasis.

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Introduction

Simuliids (black fly) constitute important component of stream ecosystems and breed in fast flowing streams, rivers and highly oxygenated water (Gallard and Toja, 2002). The knowledge of simuliid species composition is essential to understand its diversity and community structure in an area. Simuliids have worldwide distribution with about 1200 reported species, however in India only 52 well defined species only have been described so far (Datta, 1997). *Simulium* species are known to transmit onchocerciasis (river blindness) in many countries. Onchocerciasis has been considered of immense importance in view of associated public health and socio-economic problems (Morales and Krueger, 2009). In Africa, Mexico and many parts of America, various species of *Simulium* act as onchocerciasis vector, However in India, *Simulium* has not yet been incriminated as a vector of onchocerciasis. Simuliids are abundant in northeastern states of India and known as serious pests causing biting nuisance and local allergic reactions in human (Singh and Tripathi, 2003). In many cases, *Simulium* bite cause severe inflammation, formation of blisters and itching followed by secondary infection leaving scars on the host's body. Repeated biting to the human may cause a medically recognized syndrome called Black Fly Fever characterized by fever, headache, lymphadenitis and psychological depression (Singh and Tripathi, 2003). *Simulium* has also been found associated with output reduction and death in domestic animals (Currie and Adler, 2008). This could have implications on socio-economic well-being of the human population and consequently culminates in overall low productivity of the *Simulium* infested areas. Simuliids biting generally occur outdoor during daylight hour and prefer to bite the lower exposed body part (Opara *et al*., 2008; Hazarika *et al*., 2012). Different simuliid species may have their own preferred biting period (Opara *et al.,* 2008). The information about biting activity of *Simulium* is important in devising prevention strategy.

In India, simuliids have not yet been identified as a vector of any human disease nor has any case of onchocerciasis been reported (Singh and Tripathi, 2003). However, it is probable that current changes in settlement pattern due to migration and various development related activities may lead to the development of disease foci in this part also (WHO, 1995). The present study aimed to update the information on the diversity and distribution of black fly, *Simulium* species in northeastern parts of India.

Materials and Methods

Study site and collection of Simuliids aquatic stages : A study on distribution and biting activity of black fly, *Simulium* species were undertaken at nine places namely, Bhalukpong (stream), Bomdila (road side drain), Salari (stream and nallah), Dukumpani (stream), Rupa (stream), Tenga (stream), Tippi (stream, nallah and roadside drain), Sessa (stream and seepage water) and Seijosa (stream) in Arunachal Pradesh and three places namely, Balijan (nallah), Rajgarh (irrigation channel) and Sonapur (seepage water) in Assam during September-October 2009 and March-April 2010. Each site was visited once a year and each habitat was sampled twice during each visit. The immature stages of simuliid flies (larvae and pupae) were collected from submerged stone, trailing vegetations, twigs, leaves, with the help of forceps. Larval density was estimated using polyethylene strips (1 X 25 cm each) (Das *et al.,* 1988). The strips were placed in fast flowing streams just below the water surface by tying the strips with submerged stones at a distance of about 30 cm from each other. Observations were recorded after 24 hrs and the number of larvae attached to the strips was counted separately. Mature larvae and pupae were preserved in 70% and 90% ethanol respectively and brought to the laboratory for species identification. Species identification was done on the basis of the arrangement of gill filaments in pupae under the microscope using standard keys (Puri, 1932a, b, c and 1933a, b, c). The percent distribution of each species was determined as the number of larvae/pupae of a species collected X 100/total number collected from all sites. Species recorded were classified based on percent distribution as sparse (<5%), moderately distributed (5-20%) and widely distributed (>20%) (Shetty *et al*. 2007; Dhiman *et al*. 2009).

Monitoring of daytime biting activity : Biting activity of simuliid flies was studied near three different breeding habitats, viz; stream (Tippi, Dukumpani and Tenga), nallah (Balijan) and irrigation channel (Rajgarh) by recording human landing catches (n=4) during 07:00-17:00 hr. These sites were selected on the basis of adequate *Simulium* density and availability of the logistic support. These sites have temperature ranging from negative to 15°C in winters and 10 to 32°C in summers. Informed consents were obtained from the volunteers, who sat at least three meter apart from each other with their hands and legs exposed. The fly collectors were equipped with aspirators and timers. The simuliids perching on the exposed part for a blood meal was collected before any bite by using a small glass tube. The collected flies were stored in normal saline solution for dissection.

Detection of onchocerciasis infection : The black flies captured were immediately transferred to normal saline solution (0.65%), labeled and stored in ice till dissection. Black flies (5% from each collection site and at least 5 from such sites where 5% comes to be less than 5) were dissected in normal saline solution (0.65%) under microscope, teased with fine needle and observed for the presence of any nematode microfilaria. The remaining parts of the dissected flies were stored in phosphate buffer saline for molecular taxonomy. All dissections were carried out within 24 hrs of the simuliid flies collection.

Statistical analysis : Chi square (χ^2) test was used to compare the simuliid species collected and density in different breeding habitats. Comparison of density between the year 2009 and 2010 was made using paired student's t-test. Density among the breeding habitats was compared using two way ANOVA and Friedman test. Biting activity was compared using one way ANOVA, whereas difference in two biting peaks was compared using student's t-test.

Results and Discussion

A total 20,801, with an average of 5200.3±395.2 simuliid specimens corresponding to seven species were collected and identified from five different types of breeding habitats in the study areas during the study period. These were *Simulium* (*Eusimulium*) *aureohirtum* Brunetti, *S.* (*Gomphostilbia*) *tenuistylum* Datta, *S.* (*Simulium*) *christophersi* Puri, *S.* (*Simulium*) *himalayense* Puri, *S. (Simulium) novolineatum* Puri, *S. (Simulium) rufibasis* Brunetti and *S. (Simulium) striatum* Brunetti (Table 1). *S.* (*E*) *aureohirtum* (30.3%), *S.* (*G*) *tenuistylum* (29.9%) and *S. (S) rufibasis* (27.6%) were widely distributed and formed majority (> 85%) of the collection. *S.* (*E*) *aureohirtum* was predominant and its density was significantly higher than the other two species (χ^2=11.6; p=0.003). On the other hand, *S.* (*S*) *christophersi* Puri, *S.* (*S*) *himalayense* Puri, *S. (S) novolineatum* Puri and *S.* (*S*) *striatum* Brunetti were sparsely distributed among the study sites. *S.* (*S*) *himalayense* (1.48%) formed the lowest collection and could be recorded from Sonapur site of Assam only, whereas *S.* (*S*) *rufibasis* and *S.* (*S*) *striatum* were recorded from study sites of Arunachal Pradesh only. In the present study, stream was the preferred breeding habitat among all the breeding

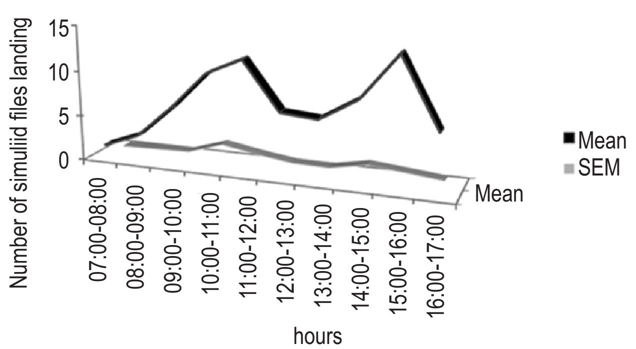

Fig. 1 : Biting activity of black fly, *Simulium* in the study sites of north east, India; SEM = Standard error mean

Table 1 : Distribution of black fly, *Simulium* species among the study sites of north east, India (2009-2010)

Collection site	***Simulium* species collected**						
	S.(E) aureohirtum	***S.(G) tenuistylum***	***S.(S) striatum***	***S.(S) christophersi***	***S.(S) novolineatum***	***S.(S) rufibasis***	***S.(S) himalayense***
Tippi	387.3±24.9	355.5±24.4	-	11.3±1.9	-	-	-
Balijan	656.5±32.2	303.0±19.8	-	25.8±2.9	138.8±11.2	-	-
Rajgarh	534.3±48.5	287.8±21.9	-	-	-	-	-
Bhalukpong	-	250.8±46.1	-	-	25.3±3.4	-	-
Salari	-	159.5±26.8	63.8±5.1	-	-	340.8±25.3	-
Seijosa	-	203.3±33.2	-	-	-	-	-
Bomdila	-	-	17.0±4.0	-	-	-	-
Dukumpani	-	-	89.3±8.8		-	532.3±40.7	-
Rupa	-	-	20.8±3.9	-	-	157.5±27.4	-
Sessa	-	-	26.8±7.0	49.3±6.8	-	-	-
Tenga	-	-	-	16.8±3.1	68.3±7.3	401.8±34.8	-
Sonapur	-	-	-	-	-	-	76.5±6.4

Values are mean of replicates ± SE

Table 2 : Breeding habitat preference of black fly, *Simulium* species in the study sites of north east, India

Simulium species	***Simulium* breeding habitat preference (%)**				
	Stream	**Nallah**	**Roadside drain**	**Irrigation channel**	**Seepage water**
S. (E) aureohirtum	12.2	49.7	4.3	33.9	0.0
S. (G) tenuistylum	43.3	19.4	18.9	18.5	0.0
S. (S) striatum	74.8	12.4	7.8	0.0	5.1
S. (S) christophersi	64.1	25.2	0.0	0.0	10.7
S. (S) novolineatum	40.1	59.9	0.0	0.0	0.0
S. (S) rufibasis	88.9	11.1	0.0	0.0	0.0
S. (S) himalayense	0.0	0.0	0.0	0.0	100.0

sites as the simuliids density was found to be highest in the stream habitat ($p=0.006$; $F=14.2$). The overall *Simulium* density recorded during the year 2009 was significantly higher than 2010 ($p=0.0004$; $t=5.0$).

The human landing rate of *Simulium* was higher during 15:00-16:00 hrs (14.2±0.8) and 11:00-12:00 hrs (12.4±1.0) (Fig. 1). The increase in human landing rate during 15:00-16:00 hrs as compared to the other landing hrs was statistically significant ($F = 22.0$; $p < 0.0001$; 95% CI = 12.5-15.9). Simuliids biting activity in the present study showed bimodal pattern and was more prominent during early evening hours. However, the activity during two biting peaks was statistically similar ($p>0.1$, $t=1.4$). No difference was observed in the daytime biting activity among the three different type of breeding habitats namely, stream, nallah and irrigation channel, where the biting activity study was carried out ($p=0.2$; $F=1.2$). The daytime biting pressure (number of flies landing per man per minute) of simuliids during the sunny day (3.5±0.4) was found to be significantly higher than shady day and rainy day ($p<0.0001$; $F=25.8$; $n=6$).

A total 266 simuliid specimens were examined at least twice by two experts for the presence of *Onchocerca* microfilaria, but could not detect any *Onchocerca* or nematode infection using microscopy.

In the presents study, *S. (S) indicum* could not be recorded from any of the study sites, unlike the earlier reports where this species was reported from many states of north east India (Lewis, 1974; Datta, 1983; Dhiman *et al.,* 2012). The *Simulium* density was significantly higher ($p < 0.0001$) in the streams (47.36%) as compared to nallah (27.7%), irrigation channels (15.8%), roadside drains (7.3%) and seepage water (1.9%) (Table 2). *S.* (*E*) *aureohirtum* and *S.* (*G*) *tenuistylum* were found to breed in fast flowing clear water in the streams, nallahs, roadside drains and irrigation channels. *S.* (*S*) *striatum* and *S.* (*S*) *christophersi* were found breeding in streams, nallahs and seepage water, while *S.* (*S*) *novolineatum* and *S.* (*S*) *rufibasis* were recorded breeding in streams and nallahs only. *S. (S) himalayense* was found breeding in slow flowing hillside seepage water. Breeding preference of simuliids is governed by many physico-chemical parameters such as turbidity, dissolved oxygen and pH of the water. In addition, water flow speed, water depth and outside environment temperature also have a critical role in the oviposition (Gallardo and Toja, 2002). Larvae of some simuliid species remain in association with those of other simuliid species, depending upon environmental conditions (Oluwole *et al.,* 2009). The stream breeding habitats carry more water discharge, have relatively low water temperature during dry season and provide long stretches for breeding, therefore considered highly supportive for *Simulium* breeding (McCall *et al.,* 1998; Gallardo and Toja, 2002). There has been no significant correlation between the altitude and diversity of *Simulium* community, whereas similarity among the simuliid communities decreases with the increasing distance between the breeding sites due to some parameters influencing the breeding and recolonisation (Gallardo and Toja, 2002). Overall *Simulium* density during 2009 was higher as compared to 2010, which may be correlated to the onset of winter during Sept-Oct 2009 and onset of summers during Mar-Apr 2010. During early winter after rainy season, increased water level and speed in the streams and other water bodies is conducive for the breeding of *Simulium* (Pachon and Walton, 2011).

The daytime biting activity was similar during the two peaks, unlike the previous studies, where this activity was found to be significantly higher in the evening as compared to the morning or vice versa (Opuku, 2006; Oluwole *et al.,* 2009). The bimodal biting behaviour observed during the present study is similar to that observed in India and some African countries but differ to that unimodal pattern observed elsewhere (Opara *et al.*, 2008; Hazarika *et al.,* 2012). Biting activity of the simuliids might be closely associated with the sunlight and outdoor activity of human (Opara *et al.,* 2008). In morning and evening hours human activity is generally increased, making them easily available for the hovering flies. Adult *Simulium* flies, for which biting activity was investigated, could not be identified due to lack of authentic keys to identify the Indian *Simulium* flies. However, it may be appropriate to assume the similar *Simulium* species composition as found by pupal identification from the five study sites, where daytime biting activity was monitored.

In India, there are only few reported case of onchocerciasis infection among human and animals (Barua *et al.,* 2011), unlike many of African and American countries, where the disease cause considerable damage to the public health (Opuku, 2006; Opara *et al.,* 2008). Several bites of infected *Simulium* are required to acquire onchocerciasis infection, for which a considerable load of microfilaria on vector (black flies) is essential. Still, the chances of *Onchocerca* infection among human and *Simulium* to act as its vector cannot be overruled in this region of India.

The present study provides new information about the black fly, simuliid species in the studied area of northeast India. *S.* (*E*) *aureohirtum* was the major species prevalent during the study. None of the dissected fly was harboring microfilaria indicating the region free from onchocerciasis.

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