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. Simulids biting rhythm showed bimodal pattern and were more active during sunny day (p<0.0001). Microscopic dissection of simulids (n=266) did not incriminate simulids as vector of onchocerciasis.

Key words
Aquatic stage, Black fly, Breeding habitat, Microfilaria, Vector

Introduction
Simulids (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 simulid species composition is essential to understand its diversity and community structure in an area. Simulids 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. Simulids 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. Simulids 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 simulid 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, simulids 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).
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 Simuliiids 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), Raigarh (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 simuliiids 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 simuliiids flies was studied near three different breeding habitats, viz; stream (Tippi, Dukumpani and Tenga), nallah (Balijan) and irrigation channel (Raigarh) 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 simuliiids 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 simuliiids flies collection.

Statistical analysis : Chi square (χ²) test was used to compare the simuliiids 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 simuliiids 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 (χ²=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 areas during the study period. These were 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 (χ²=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
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

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

<table>
<thead>
<tr>
<th>Collection site</th>
<th>S.(E) aureohirtum</th>
<th>S.(G) tenuistylum</th>
<th>S.(S) striatum</th>
<th>S.(S) christophersi</th>
<th>S.(S) novolineatum</th>
<th>S.(S) rufibasis</th>
<th>S.(S) himalayense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tippi</td>
<td>387.3±24.9</td>
<td>355.5±24.4</td>
<td></td>
<td>11.3±1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balijan</td>
<td>658.5±32.2</td>
<td>303.0±19.8</td>
<td></td>
<td>25.8±2.9</td>
<td>138.8±11.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rāggar</td>
<td>534.3±48.5</td>
<td>287.8±21.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bhālukpong</td>
<td>-</td>
<td>250.8±46.1</td>
<td></td>
<td>25.3±2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salari</td>
<td>-</td>
<td>159.5±26.8</td>
<td></td>
<td></td>
<td></td>
<td>340.8±25.3</td>
<td></td>
</tr>
<tr>
<td>Seijosa</td>
<td>-</td>
<td>203.3±33.2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bomdila</td>
<td>-</td>
<td>17.0±4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dukumpani</td>
<td>-</td>
<td>89.3±6.8</td>
<td></td>
<td></td>
<td></td>
<td>532.3±40.7</td>
<td></td>
</tr>
<tr>
<td>Rupa</td>
<td>-</td>
<td>20.8±3.9</td>
<td></td>
<td></td>
<td></td>
<td>157.5±27.4</td>
<td></td>
</tr>
<tr>
<td>Sessa</td>
<td>-</td>
<td>26.8±7.0</td>
<td></td>
<td>49.3±6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenga</td>
<td>-</td>
<td>16.8±3.1</td>
<td></td>
<td>68.3±7.3</td>
<td>401.8±34.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sonapur</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>76.5±6.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean of replicates ± SE

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

<table>
<thead>
<tr>
<th>Simulium species</th>
<th>Stream</th>
<th>Nallah</th>
<th>Roadside drain</th>
<th>Irrigation channel</th>
<th>Seepage water</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. (E) aureohirtum</td>
<td>12.2</td>
<td>49.7</td>
<td>4.3</td>
<td>33.9</td>
<td>0.0</td>
</tr>
<tr>
<td>S. (G) tenuistylum</td>
<td>43.3</td>
<td>19.4</td>
<td>18.9</td>
<td>18.5</td>
<td>0.0</td>
</tr>
<tr>
<td>S. (S) striatum</td>
<td>74.8</td>
<td>12.4</td>
<td>7.8</td>
<td>0.0</td>
<td>5.1</td>
</tr>
<tr>
<td>S. (S) christophersi</td>
<td>64.1</td>
<td>25.2</td>
<td>0.0</td>
<td>0.0</td>
<td>10.7</td>
</tr>
<tr>
<td>S. (S) novolineatum</td>
<td>40.1</td>
<td>59.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>S. (S) rufibasis</td>
<td>88.9</td>
<td>11.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>S. (S) himalayense</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>
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 simulid species remain in association with those of other simulid 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 simulid 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 (Opoku, 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 simulids 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 (Opoku, 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, simulid 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.

Acknowledgments

The authors are thankful to all the human volunteers and Gamburas (village headman) of villages where study was carried out. The logistic help rendered by concerned state government is also acknowledged.

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