

## Research Note

# Distribution and abundance of black flies (Diptera: Simuliidae) in recreation parks in Selangor State, Peninsular Malaysia

Chen, C.D.\*, Takaoka, H., Tan, P.R., Lau, K.W., Low, V.L., Leong, C.S., Karen-Chia, H.M. and Sofian-Azirun, M.

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

\*Corresponding author e-mail: chen\_ctbr@um.edu.my

Received 21 September 2015, received in revised form 13 December 2015; accepted 15 December 2015

**Abstract.** A preliminary survey of larvae and pupae of black flies (Diptera: Simuliidae) was conducted in three recreation parks [Templer Recreation Park (TRP), Congkak River Forest Reserve (CRFR) and Ampang Forest Reserve (AFR)] located in Selangor State, which is located 18 to 35 km from Kuala Lumpur city center, Malaysia. This study was initiated to determine the distribution and abundance of filarial vectors, *Simulium* spp. A total of 12 species of black flies belonging to three subgenera (*Gomphostilbia*, *Simulium* and *Nevermannia*) were collected. *Simulium* (*Simulium*) *nobile* was incriminated as the most dominant species in all recreation parks. This study is a first report on the distribution and abundance of black flies obtained from recreation parks in Malaysia.

The biting Diptera is two-winged flying insects that suck blood from human and animals. Blackflies (Diptera: Simuliidae) are one of the most important groups of biting Diptera. The female black flies are also well-known to cause medical and veterinary problems, including transmissions of filarial diseases of humans and animals, such as human and zoonotic onchocerciasis (Adler *et al.*, 2004; Takaoka *et al.*, 2012a). In addition, black flies are a nuisance in many parts of the world because of their bites and sometimes enormous numbers involved in the attacks (Rozendaal, 1997). They attack livestock causing economic recession result of reduction in milk and beef production (Montagna *et al.*, 2012).

Blackflies have been reported to occur all around the world and there are 2,151 nominal species (Adler & Crosskey, 2014). A total of 59 species have been reported in Peninsular Malaysia (Adler & Crosskey, 2014;

Takaoka *et al.*, 2014a,b,c; Ya'cob *et al.*, 2014). During immature stage, black flies inhabit in fast-flowing streams, which have high oxygen supply (Chalifour *et al.*, 1990) and become part of the fish and other aquatic insect's diet (Bertaza & Figueiró, 2012). Black flies also attract environmental biologists and ecologists because their immature stages breed only in clean running fresh waters, and larvae are one of the principal processors of plant debris in streams.

Black flies are widely distributed, from the tropics to the Arctic Circle (Araújo-Coutinho *et al.*, 2005). Nevertheless, to our knowledge, research regarding the distribution and abundance of black flies has never been reported in Malaysia. Most of the blackfly research in Malaysia were centered to the exploitation of the fauna describing new species found in the country (Takaoka *et al.*, 2014; Takaoka *et al.*, 2013; Takaoka *et al.*, 2012b,c).

A distributional pattern of an organism is a fundamental task of community ecology (Corkum & Currie, 1987). According to Hamada & McCreadie (1999), distribution pattern of blackflies is greatly associated with environmental factors. Determination of the factors which influence the abundance and distribution of species will be a first step towards documenting the various types of *Simulium* spp. Thus, further prediction of blackflies distribution can be carried out based on similar environmental components.

This study reports the distribution and abundance of black flies, *Simulium* spp. in three recreation parks located in Selangor State, namely Templer Recreation Park (TRP), Congkak River Forest Reserve (CRFR) and Ampang Forest Reserve (AFR) (Figure 1). All study sites are located approximately 18 to 35 km from Kuala Lumpur city center, Malaysia, and with elevation ranging from 100 to 200 meters above sea level. The geographical information and ecological description of each study site are presented in Table 1.



Figure 1. Location of study sites for black fly sampling.

Table 1. Geographical and ecological description of study sites

District	Study Sites	Geographical Information	Ecological Descriptions
Gombak	Templer Recreation Park (TRP)	<ul style="list-style-type: none"> <li>• N3°17'11.2", E101°38'59.3"</li> <li>• Elevation: 130-170m a.s.l.</li> <li>• Approximately 25km from Kuala Lumpur city center</li> </ul>	<ul style="list-style-type: none"> <li>• All study sites are located in the secondary forest, consist of streams and trails.</li> <li>• All sampling sites were partially shaded by trees and scrubs.</li> </ul>
Hulu Langat	Congkak River Forest Reserve (CRFR)	<ul style="list-style-type: none"> <li>• N3°12'32.5", E101°50'36.6"</li> <li>• Elevation: 170-200m a.s.l.</li> <li>• Approximately 35km from Kuala Lumpur city center</li> </ul>	<ul style="list-style-type: none"> <li>• Water temperature for the streams were between 20-22°C.</li> <li>• No human dwelling was found within the study sites.</li> </ul>
Ampang	Ampang Forest Reserve (AFR)	<ul style="list-style-type: none"> <li>• N3°9'27.5", E101°47'56.2"</li> <li>• Elevation: 100-130m a.s.l.</li> <li>• Approximately 18km from Kuala Lumpur city center</li> </ul>	<ul style="list-style-type: none"> <li>• High human activities due to all study sites are tourism important.</li> <li>• Several amenities are available in the recreation parks, such as picnic grounds, camping ground, car parks and public toilets.</li> </ul>

Five collectors were involved in the sampling. All collectors surveyed larvae and pupae of black flies in the same collection point (50 meters in diameter) at the streams for 30 minutes simultaneously. A total of 5 collection points (each collection point was approximately 500 to 1000 meters apart from each other) were surveyed in each study site. Larvae and pupae of black flies were collected according to the methods described by Takaoka (2003). The larvae were manually collected from available substrates such as trailing grasses, stems, twigs, stones and rocks. All collected larvae were preserved in 80% ethanol or Carnoy's solution (ethanol: acetic acid, 3:1). Pupae that attached on substrates were individually isolated and kept alive in vials until adults emerged. The vials with the pupae were kept in a cooler box with ice. Emerged adults and their pupal exuviae and cocoons were preserved in 80% ethanol for further examination. All specimens of adults, pupal exuviae, cocoons and both mature and immature larvae were brought to the laboratory for further examination. All samples were examined in a Petri dish under a microscope for identification at subgenus, species-group and species levels according to Takaoka and Davies (1995).

A total of 12 species of black flies belonging to three subgenera (*Gomphostilbia*, *Simulium* and *Nevermannia*) were collected as presented in Table 2. Seven

species were collected from Templer Recreation Park (TRP), which are *S. (S.) nobile* (81.61%), followed by *S. (N.) aureohirtum* (12.79%), *S. (G.)* sp. (nr. *sheilae*) (1.87%), *S. (G.) parahiyagum* (1.68%), *S. (S.) tani* (1.21%), *S. (G.) angulistylum* (0.75%) and *S. (G.) tahanense* (0.09%). On the other hand, seven species of black flies collected from Congkak River Forest Reserve (CRFR) are *S. (S.) nobile* (58.61%), *S. (G.) angulistylum* (28.93%), *S. (S.) tani* (7.94%), *S. (S.) hirtinervis* (2.65%), *S. (G.)* sp. (nr. *sheilae*) (1.13%), *S. (G.) parahiyagum* (0.67%) and *S. (G.) tahanense* (0.07%). A total of 9 species of immature black flies were collected from Ampang Forest Reserve (AFR) with the highest number of *S. (S.) nobile* (88.69%), followed by *S. (G.)* sp. (nr. *sheilae*) (3.62%), *S. (G.) angulistylum* (2.26%), *S. (G.) roslhashimi* (1.36%), *S. (G.) sheilae* (1.36%), *S. (S.) tani* (1.36%), *S. (G.) cheongi* (0.45%), *S. (G.) decuplum* (0.45%) and *S. (N.) aureohirtum* (0.45%). Two and 103 immature larvae obtained from TRP and AFR were unidentified, respectively.

Four species of black flies were found to occur in all study sites, namely *S. (G.) angulistylum*, *S. (G.)* sp. (nr. *sheilae*), *S. (S.) nobile* and *S. (S.) tani*. Four species of black flies were solely found in AFR, i.e. *S. (G.) cheongi*, *S. (G.) decuplum*, *S. (G.) roslhashimi* and *S. (G.) sheilae*; and one species was solely recorded from CRFR, i.e. *S. (S.) hirtinervis*. The occurrence of certain

Table 2. Species composition and relative abundance of black flies obtained from three recreation parks in Selangor State, Peninsular Malaysia

Species	Study Sites					
	Templer Recreation Park (TRP) 130-170m a.s.l.		Congkak River Forest Reserve (CRFR) 170-200m a.s.l.		Ampang Forest Reserve (AFR) 100-130m a.s.l.	
	No. specimen	Percentage (%)	No. specimen	Percentage (%)	No. specimen	Percentage (%)
<b>Subgenus <i>Gomphostilbia</i></b>						
<i>S. (G.) angulistylum</i>	8	0.75	820	28.93	5	2.26
<i>S. (G.) cheongi</i>					1	0.45
<i>S. (G.) decuplum</i>					1	0.45
<i>S. (G.) parahiyangum</i>	18	1.68	19	0.67		
<i>S. (G.) roslihashimi</i>					3	1.36
<i>S. (G.) sheilae</i>					3	1.36
<i>S. (G.) tahanense</i>	1	0.09	2	0.07		
<i>S. (G.) sp. (nr. sheilae)</i>	20	1.87	32	1.13	8	3.62
<b>Subgenus <i>Simulium</i></b>						
<i>S. (S.) hirtinervis</i>			75	2.65		
<i>S. (S.) nobile</i>	874	81.61	1,661	58.61	196	88.69
<i>S. (S.) tani</i>	13	1.21	225	7.94	3	1.36
<b>Subgenus <i>Nevermannia</i></b>						
<i>S. (N.) aureohirtum</i>	137	12.79			1	0.45
Total number of specimens / percentage (%)	1,071	100	2,834	100	221	100

Note: No of unidentified immature larvae obtained from TRP and AFR were 2 and 103, respectively.

Table 3. Comparison of Shannon-Weiner Index ( $H'$ ) and Evenness Index ( $J'$ ) of black fly fauna among recreation parks in Selangor State, Peninsular Malaysia

	Templer Recreation Park (TRP)	Congkak River Forest Reserve (CRFR)	Ampang Forest Reserve (AFR)
Shannon-Weiner Index ( $H'$ )	0.6686	1.0585	0.5607
Evenness Index ( $J'$ )	0.2607	0.4127	0.2186
Number of species obtained	7	7	9
Dominant Species*	<i>S. (S.) nobile</i>	<i>S. (S.) nobile</i>	<i>S. (S.) nobile</i>

\* According to the percentage of specimens obtained from the respective recreation parks.

species of black flies in specific geographical or ecological habitats may be affected by environmental factors such as temperature, substrate, food for the larvae, water velocity, depth, width, discharge and pH level, as reported by Pramual & Wongpakam (2010), Hamada *et al.* (2002), Couceiro *et al.* (2014) and Carlsson *et al.* (1997).

The comparison of Shannon-Weiner Index ( $H'$ ) and Evenness Index ( $J'$ ) of black fly fauna among recreation parks in Selangor State, Peninsular Malaysia is presented in Table 3. Shannon-Weiner Index ( $H'$ ) and Evenness Index ( $J'$ ) indicated the occurrence of dominant species in the populations and the diversity of black flies which were not

distributed evenly in all study sites. *Simulium* (*S.*) *nobile* was the most dominant species recorded in all study sites.

According to Takaoka (2004), the subgenus *Simulium* can be found all over the world with an exception in Africa and Australia. The immature stages of *Simulium* are generally distributed in various altitudes with clean running water ranging from trickles to large rivers. Substrates such as dead leaves, trailing grasses, stones or rocks in the water were the places they would likely to attach. However, the detail factors that cause the occurrence of high density of *S.* (*S.*) *nobile* in the streams remain unknown.

*Gomphostilbia* (8 species out of 12 species of black flies) was the most abundant subgenus found in this study, as expected by the fact that *Gomphostilbia* is a dominant subgenus of black flies in Malaysia, represented by 34 of 59 species of black flies recorded in Peninsular Malaysia. The breeding site of this subgenus is usually associated with streams with moderate current velocity in various altitudes from sea level to high mountains over 2000 meters, but still can be occasionally found in large lotic river (Takaoka, 2004).

Subgenus *Nevermannia* is a small taxon, with only five species recorded in Peninsular Malaysia. Only one species of the subgenus *Nevermannia* was recorded in this survey, namely *S.* (*N.*) *aureohirtum*. The immature stages of this species are often found in very slow-flowing water breeding sites and withstand high water temperature and polluted waters (Takaoka, 2004).

This study provides a snapshot of the patterns, distribution and abundance of black flies obtained. There is a knowledge gap on this research topic and it is necessary to conduct more comprehensive studies on the ecological habitats of blackflies. In addition, the medically and veterinary important black fly species in our study site and Malaysia have not yet been identified. Information on blackflies involved in filariasis transmission in Malaysia is absent, and the female host preferences still remain unknown.

Tropical regions are always found to be higher in species richness than in temperate regions (Hamada *et al.*, 2002). However,

studies on the distribution and abundance of blackflies in Malaysia have just begun. Our study documented the distribution and abundance of blackflies in recreation parks for the first time in Malaysia. The current study not only provides fundamental information but also is a preliminary step towards understanding the abundance and diversity of black fly in tropical streams. A much higher diverse nature of black flies in Peninsular Malaysia will be clarified by further extensive surveys.

*Acknowledgements.* The authors would like to thank University of Malaya for funding on this study (Project No. RP021A/16SUS).

## REFERENCES

- Adler, P.H. & Crosskey, R.W. (2014). World blackflies (Diptera: Simuliidae): a comprehensive revision of the taxonomic and geographical inventory. <http://www.clemson.edu/cafls/biomia/pdfs/blackflyinventory.pdf>.
- Adler, P.H., Currie, D.C. & Wood, D.M. (2004). *The black flies (Simuliidae) of North America*. Cornell University Press, Ithaca, New York.
- Araújo-Coutinho, C.J.P.C., Figueiró, R., Viciani, A.P., Nascimento, É.S. & Cavados, C.F.G. (2005). A bioassay method for black flies (Diptera: Simuliidae) using larvicides. *Neotropical Entomology* **34**(3): 511-513.
- Bertazo, K. & Figueiró, R. (2012). Spatial distribution of black fly (Diptera: Simuliidae) immature in a water current velocity gradient in Aracruz/ES, Brazil. *Revista de Ciências da Vida, RJ, EDUR* **32**(2): 91-101.
- Carlsson, M., Nilsson, L.M., Svensson, B.J., Ulfstrand, S. & Wotton, R.S. (1977). Lacustrine seston and other factors influencing the blackflies (Diptera: Simuliidae) inhabiting lake outlets in Swedish Lapland. *Oikos* **29**: 229-238.
- Chalifour, A., Boisvert, J. & Back, C. (1990). Optimization of insecticide treatments in rivers: an application of graph theory for planning a black fly larvae control



- program. *Canadian Journal of Fisheries and Aquatic Sciences* **47**(10): 2049-2056.
- Corkum, L.D. & Currie, D.C. (1987). Distributional patterns of immature Simuliidae (Diptera) in north western North America. *Freshwater Biology* **17**: 201-221.
- Hamada, N. & McCreadie, J.W. (1999). Environmental factors associated with the distribution of *Simulium perflavum* (Diptera: Simuliidae) among streams in Brazil Amazonia. *Hydrobiologia* **397**: 71-78.
- Hamada, N., McCreadie, J.W. & Adler, P. (2002). Species richness and spatial distribution of blackflies (Diptera: Simuliidae) in streams of Central Amazonia, Brazil. *Freshwater Biology* **47**(1): 31-40.
- Montagna, C.M., Gauna, L.E., D'Angelo, A.P.D. & Anguiano, O.L. (2012). Evolution of insecticide resistance in non-target black flies (Diptera: Simuliidae) from Argentina. *Memorias do Instituto Oswaldo Cruz* **107**(4): 458-465.
- Pramual, P. & Wongpakam, K. (2010). Seasonal variation of black fly (Diptera: Simuliidae) species diversity and community structure in tropical streams of Thailand. *Entomological Science* **13**: 17-28.
- Rozendaal, J.A. (1997). *Vector control: Methods for use by individuals and communities*. World Health Organization, Geneva.
- Takaoka, H. (2003). *The black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya*. Kyushu University Press, Fukuoka, Japan.
- Takaoka, H. (2004). Insecta: Diptera, Simuliidae. In Yule, C.M. & Yong, H.S. (Eds.), *Freshwater Invertebrates of Malaysia Region*. pp. 673-682. Academy of Sciences Malaysia, Kuala Lumpur.
- Takaoka, H. & Davies, D.M. (1995). *The black flies (Diptera: Simuliidae) of West Malaysia*. Kyushu University Press, Fukuoka, Japan.
- Takaoka, H., Fukuda, M., Otsuka, Y., Aoki, C., Uni, S. & Bain, O. (2012a). Blackfly vectors of zoonotic onchocerciasis in Japan. *Medical and Veterinary Entomology* **26**: 372-378.
- Takaoka, H., Sofian-Azirun, M., Hashim, R., Ya'cob, Z. & Chen, C.D. (2012b). Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Peninsular Malaysia. *Journal of Medical Entomology* **49**(4): 803-812.
- Takaoka, H., Sofian-Azirun, M. & Ya'cob, Z. (2012c). A new species of *Simulium* (*Simulium*) (Diptera: Simuliidae) from Langkawi Island, Peninsular Malaysia. *Journal of Medical Entomology* **49**(6): 1198-1205.
- Takaoka, H., Sofian-Azirun, M., Ya'cob, Z. & Hashim, R. (2014). Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Cameron's Highlands, Peninsular Malaysia, with keys to 21 species of the *Simulium asakoe* species-group. *Zootaxa* **3765**(1): 054-068.
- Takaoka, H., Sofian-Azirun, M. & Ya'cob, Z. (2013). A new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Langkawi Island, Malaysia. *Journal of Medical Entomology* **50**(4): 701-708.
- Ya'cob, Z., Takaoka, H. & Sofian-Azirun, M. (2014). *Simulium ledangense*, a new species of the *Simulium feuerborni* species-group of the subgenus *Nevermannia* (Diptera: Simuliidae) from Mount Ledang, Peninsular Malaysia. *Zootaxa* **3881**(3): 228-236.