

Short Communication

Review of Malaysian black flies (Diptera: Simuliidae): note on new record on *Simulium kalimantanense* from Sabah

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Abstract

Black flies are the two-winged, small-bodied (3mm to 6mm) and blood-sucking insects of medical and veterinary importance. Female of certain species play a role as a vector of several disease agents including, *Onchocerca volvulus*, causative agent of human onchocerciasis. Despite their medical significance, the biodiversity of black flies in the Borneo part of Malaysia as well as other Southeast Asian countries (except Thailand, Vietnam and Indonesia) are unknown. Our recent visit to the Kangkawat Reserve within the Imbak Canyon Conservation Area (ICCA) has successfully discovered one new record from Malaysia, *Simulium kalimantanense* of the *S. banauense* species-group and one from Sabah, *Simulium sarawakense* of the *S. epistum* species-group. Based on these current findings, the total number of species and species-group of black flies inhabiting Malaysia has increased to 96 and 22 respectively.

Keywords: black fly, Simuliidae, Malaysia, Sabah, biodiversity, vector, Onchocerciasis

Introduction

To date, there are 2,335 black fly species of five genera discovered worldwide (Adler & Crosskey, 2018). The genus *Simulium* is the most diverse group containing the largest species number (1,919) and occurs in almost all places, however, it represents the only genus in the Oriental region. Black flies are the two-winged, small-bodied (3mm to 6mm) and blood-sucking insects of medical dan veterinary importance.

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Female of certain species in the genus *Simulium* play a role as a vector of filaria parasites including *Onchocerca volvulus*, an agent of human Onchocerciasis in Sub-Saharan Africa, Latin America and Yemen (Ishii et al, 2008; Adler et al. 2010).

Black flies in the order Diptera and family Simuliidae is widely distributed across all zoogeographical regions and the preimaginal black flies generally inhabit unpolluted running water (Takaoka, 1995; Currie & Adler, 2008). In fact, preimaginal black flies are important components of the stream ecosystem (Hamada et al., 2002; Currie & Adler, 2008; Pramual & Kuvangkadilok, 2009). They act as the keystone species in the ecology of running water because they are usually present as a major component of stream macroinvertebrates (Malmqvist et al., 2004) and have the ability to filter dissolved organic matter and make it available in the food chain (Currie & Adler, 2008). Black flies are also important in the monitoring of freshwater contamination because larvae and pupae are susceptible to both organic and inorganic pollutions (i.e. insecticides and fertilizers) (Currie & Adler, 2008).

Despite their medical and ecological significance, the biodiversity of black flies in most parts of the Southeast Asian region, except Thailand, Vietnam and Indonesia are unknown. In 2014, led by our team, University of Malaya Simuliidae Research Project (UMSRP), a comprehensive survey on the biodiversity of black flies was conducted across the west (Peninsular Malaysia) and partly in the east (Sabah) Malaysia. As an outcome, 30 new species have been discovered (23 and seven in the west and east Malaysia respectively), bringing the total number of Malaysian species to 95 (see Table 1).

Biodiversity of black flies in the Borneo part of Malaysia (Sabah and Sarawak) is understudied. Currently, there are only 28% of the total Malaysian black fly species from 11 species-groups recorded in Sabah (see Table 1 and Table 2). Owing to various bio-geographical landscapes in the Borneo part of Malaysia and Indonesia, we hypothesized that more significant species are yet to be explored which may consequently, contribute to increased biodiversity of black flies and identification of vector of human and animal onchocerciasis. Therefore, in this scientific expedition, we aim to explore the biodiversity of black flies residing in a remote area in Kangkawat Reserve within the Imbak Canyon Conservation Area (ICCA) in the state of Sabah. The outcome of this entomological survey would provide updated biodiversity data for further scientific research in related disciplines (i.e. breeding habitat preference, vector-borne diseases and epidemiology).

Table 1. The updated list of black fly species from Malaysia

Classification	Total	West Malaysia		East Malaysia	
		Peninsular Malaysia	Sabah	Sarawak	
Genus					
<i>Simulium</i> Latreille	94	63	27	25	
Subgenus					
<i>Daviesellum</i> Takaoka & Adler	1	1	0	0	
<i>Gomphostilbia</i> Enderlein	47	35	9	15	
<i>Nevermannia</i> Enderlein	7	5	3	1	
<i>Simulium</i> Latreille	39	22	15	9	

Table 2. Diversity of black fly species in Malaysia based on species-group level

Genus	Subgenus	Species-group	Total	West Malaysia		East Malaysia	
				Peninsular Malaysia	Sabah	Sarawak	
	<i>Daviesellum</i>	-	1	1	0	0	
		<i>Asakoe</i>	8	8	1	1	
		<i>Batoense</i>	14	12	2	4	
		<i>Ceylonicum</i>	5	4	2	2	
	<i>Gomphostilbia</i>	<i>Darjeelingense</i>	2	0	1	1	
		<i>Epistum</i>	11	4	2	6	
		<i>Gombakense</i>	3	3	0	0	
		<i>Varicorne</i>	4	4	0	1	
		<i>Banauense</i>	1	0	1	0	
		<i>Feuerborni</i>	4	2	2	0	
	<i>Nevermannia</i>	<i>Ruficorne</i>	1	1	1	1	
		<i>Vernum</i>	2	2	0	0	
<i>Simulium</i>		<i>Argentipes</i>	2	1	1	1	
		<i>Christophersi</i>	3	3	0	0	
		<i>Crocinum</i>	2	2	0	0	
		<i>Griseifrons</i>	1	1	0	0	
		<i>Grossifilum</i>	1	1	0	0	
	<i>Simulium</i> S. str.	<i>Melanopus</i>	10	1	8	4	
		<i>Multistriatum</i>	3	3	0	0	
		<i>Nobile</i>	2	1	1	0	
		<i>Striatum</i>	3	3	0	0	
		<i>Tuberosum</i>	11	5	5	4	
		<i>Variegatum</i>	1	1	0	0	

Methodology

Sample collection

Streams were chosen based on the presence of flow and accessibility. The stream was sampled from downstream to upstream (20 m). Larvae and pupae attached on aquatic substrates such as grasses, leaves and stems, twigs, plant

roots and rocks were collected by hand using fine forceps. These sampling protocols could represent the species occurrence in a locality (McCreadie et al. 2005; McCreadie & Colbo, 1991). Pupae attached on similar substrates were individually kept alive in vials until emergence. The adults, together with their pupal exuviae and cocoons were preserved in 80% ethanol for identification at the subgenus, species-group or species level. The methods of collection and identification followed those of Takaoka (2003) and Adler et al. (2010).

Stream measurement

For ecological data collection, the following stream physicochemical parameters were measured at the time of each collection: depth (m), width (m), velocity (m/s) (one to three measurements along the collection path), water temperature (°C), acidity (pH), conductivity (mS/cm) and dissolved oxygen (mg/l). The values of pH, temperature, conductivity and dissolved oxygen were taken using a portable multi-probe parameter (Hanna HI 9828). Meter tape and steel ruler were used to measure stream width and depth respectively, while cork and a timer watch were used to measure stream velocity; the time is taken for a cork to move one meter in distance. Velocity, depth and width measurements were used to estimate discharge (McCreadie et al. 2006). The ecological and physicochemical measurement protocols including those for major streambed particles, riparian vegetation, and canopy cover followed those of McCreadie et al. (McCreadie et al. 2016). For each fixed-stream site, the latitude and longitudinal coordinates were taken once and recorded using a hand held global positioning system (GPS) instrument (Garmin International Inc., Olathe, KS).

Table 3. Details of sampling sites from the Kangkawat Reserve within the Imbak Canyon Conservation Area (ICCA), Sabah, Malaysia.

Trail (stream code)	Latitude Longitude	Riparian vegetation	Streambed particle	Canopy cover	Specimen collected
Nepenthes (St-1)	05° 04.928'N 117° 03.099'E	Forest	Boulder	Partial	Larvae
Nepenthes (St-2)	05° 05.005'N 117° 03.046'E	Forest	Pebble	Complete	Larvae and Pupae
South Rim (St-3)	05° 04.056'N 117° 03.049'E	Forest	Pebble	Complete	Larvae and Pupae
South Rim (St-3/2)	05° 04.056'N 117° 03.049'E	Forest	Pebble	Partial	Larvae
Pelajau (St-4)	05° 05.007'N 117° 03.545'E	Forest	Pebble	Complete	Larvae and Pupae
Kangkawat (St-5)	05.07704° 117.05758°	Forest	Sand/mud	Complete	Larvae

Table 4. Physicochemical characteristics of surveyed streams

Stream Code	Width (m)	Depth (m)	Flow (velocity)	pH	Temperature (°C)	Conductivity (µS/cm)
St-1	4.00 - 5.00	0.50	Moderate	6.37	24.4	41.3
St-2	5.00	0.30	Moderate	5.86	24.3	25.2
St-3	4.50	0.50	Moderate	6.53	24.0	54.1
St-3/2	2.00	0.07	Slow	6.54	23.9	50.8
St-4	2.00	0.05	Moderate	5.58	23.9	10.7
St-5	1.50	0.20	Slow	6.69	25.5	67.4

Results and Discussion

A total of 81 black fly specimens consisting of two stages, larvae and pupae of three species (Table 4) were successfully collected from streams inside the Kangkawat Forest Reserve within the Imbak Canyon Conservation Area (ICCA). *Simulium kalimantanense* was the most abundantly collected species (69.1%) followed by *S. sarawakense* (28.3%) and *S. tahanense* (2.5%).

Table 5. Total species and the specimen collected from Kangkawat Forest Reserve, ICCA.

Species	Larvae	Pupa	Total specimen
<i>Simulium (Gomphostilbia) sarawakense</i>	5	18	23
<i>Simulium (Gomphostilbia) kalimantanense</i>	50	6	56
<i>Simulium (Gomphostilbia) tahanense</i>	2	-	2
Total	57	24	81

Table 6. The diversity and abundance of three black fly species collected from Kangkawat, Imbak Canyon Conservation Area (ICCA), in the state of Sabah, Malaysia

Stream	Species					
	<i>Simulium kalimantanense</i>		<i>Simulium sarawakense</i>		<i>Simulium tahanense</i>	
	Larva	Pupa	Larva	Pupa	Larva	Pupa
St-1	2	-	-	-	-	-
St-2	10	2M	-	-	-	-
St-3	17	3M, 1F	2	1M, 1PS	2	-
St-3/2	8	-	-	-	-	-
St-4	10	-	3	8M, 3F, 5F	-	-
St-5	3	-	-	-	-	-

Notes on newly recorded species

***Simulium (Gomphostilbia) kalimantanense* Takoaka & Sofian-azirun, 2016: 798-806 (female, male, pupa and larva)"**

Simulium kalimantanense was originally described on the basis of females, males, pupae, and mature larvae from East Kalimantan, Indonesia. This species is assigned in the *Simulium banauense* species group and is the first member reported from Malaysia. There are only 15 members of the *S. banauense* species-group recorded worldwide. Of these, 13 were reported in the Philippines, and one species each from Sulawesi and Kalimantan, Indonesia (Adler & Crosskey 2018).

Simulium kalimantanense is distinct with all existing species in Malaysia by the pupal gills composed of only four long filaments on each side. Both adults of this species are characterized by their lack of hairs on the pleural membrane or its vicinity and medium-sized female claw tooth. Pupal of this species have conical terminal hooks while larva with a medium-sized postgenal cleft (Takaoka et al. 2016).

***Simulium (Gomphostilbia) sarawakense* Takaoka, 2001: 247-250 (female and pupa).**

This study also discovered a new locality record from Sabah, *Simulium sarawakense* of the *S. epistum* species-group. It was originally described from Mount Pueh, Sarawak. Current data has provided new distribution information of this species which extends to the heart of Sabah region. *Simulium sarawakense* can be distinguished with other existing species through the pupa gill with one long and seven short filaments which are about half the length and thickness of the longer filament (Takaoka 2001). This species is closely similar to *Simulium pegalanense* however, differ by pupal terminal hooks not serrated (Takaoka, 2001).

Distributions of preimaginal black flies (i.e. larva and pupa) are highly influenced by their associated stream habitats (Ya'cob et al. 2016a, b). Therefore, an abnormal stream discharge due to heavy rainfall might wash all stream inhabitants including pupae and larvae of black flies. Due to this limitation, the aim of the current study was not fully achieved in terms of recording the actual biodiversity of black flies inside the Kangkawat reserve forest. Further entomological observations need to be carried out outside the

monsoon period in order to reveal its actual biodiversity from various landscapes and habitat types.

Conclusion

In conclusion, this one-time black fly survey has provided new insight into the black fly species composition in Malaysia. *Simulium kalimantanense* of the *S. banauense* species-group is officially added to the list of the fauna of black flies in Malaysia which brings the total number of species and species-group to 96 and 22 respectively. Basic information obtained in this study will be useful in predicting more species, including those new to science, and yet to be discovered in Malaysia. Additionally, this first survey will be the stepping stone to promote more black fly studies including identification of vector species and its associated pathogens from Sabah as well as in Borneo Island as a whole.

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