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Breeding habitat preference of preimaginal black flies (Diptera: Simuliidae) in Peninsular Malaysia

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ABSTRACT

To investigate the breeding habitat preference of black flies, a comprehensive black fly survey was conducted for the first time in Peninsular Malaysia. Preimaginal black flies (pupae and larvae) were collected manually from 180 stream points encompassing northern, southern, central and east coast of the Peninsular Malaysia. A total of 47 black fly species were recorded in this study. The predominant species were *Simulium trangense* (36.7%) and *Simulium angulistylum* (33.3%). Relatively common species were *Simulium cheongi* (29.4%), *Simulium tani* (25.6%), *Simulium nobile* (16.2%), *Simulium sheilae* (14.5%) and *Simulium bishopi* (10.6%). Principal Component Analysis (PCA) of all stream variables revealed four PCs that accounted for 69.3% of the total intersite variance. Regression analysis revealed that high species richness is associated with larger, deeper, faster and higher discharge streams with larger streambed particles, more riparian vegetation and low pH ($F = 22.7$, d.f. = 1, 173; $P < 0.001$). Relationship between species occurrence of seven common species (present in >10% of the sampling sites) was assessed. Forward logistic regression analysis indicated that four species were significantly related to the stream variables. *S. nobile* and *S. tani* prefer large, fast flowing streams with higher pH, large streambed particles and riparian trees. *S. bishopi* was commonly found at high elevation with cooler stream, low conductivity, higher conductivity and more riparian trees. In contrast, *S. sheilae* was negatively correlated with PC-2, thus, this species commonly found at low elevation, warmer stream with low conductivity and less riparian trees. The results of this study are consistent with previous studies from other geographic regions, which indicated that both physical and chemical stream conditions are the key factors for black fly ecology.

1. Introduction

Adult black flies (Diptera: Simuliidae) are one of the most important groups of blood-sucking Diptera (Takaoka, 1983). The females of certain species, when they bite and draw blood, not only cause severe skin diseases to humans and animals, but also serve as a vector of filarial parasites (Ishii et al., 2008). The Simuliidae is widely distributed across all zoogeographical regions and the preimaginal black flies generally inhabit unpolluted running water (Takaoka, 1995; Currie and Adler, 2008). In fact, preimaginal black flies are important components of the stream ecosystem (Hamada et al., 2002; Currie and Adler, 2008; Pramual and 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 an ability to filter dissolved organic matter and make it available in the food chain (Currie and Adler, 2008). Black flies are also important in the monitoring of freshwater contamination because larvae and pupae are susceptible to both organic and inorganic pollution (i.e., insecticides and fertilizers) (Currie and Adler, 2008).

Although black flies are well known for their medical and ecological importance, the significance of Malaysian black flies remains unexplored. By contrast, the spatial distribution of preimaginal black flies has been well-studied in many parts of the world, for example in the temperate region (McCreadie and Adler, 1998, 2006; McCreadie et al., 2005), the tropical region of South America (Grillet and Barrera, 1997; Hamada and McCreadie, 1999; Hamada et al., 2002; McCreadie et al., 2004) and the Oriental region, notably Thailand (Pramual and Kuvangkadilok, 2009; Pramual and Wongpakam, 2010). These previous studies reported that the

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species richness and distribution of preimaginal black flies have been primarily associated with stream physicochemical conditions.

Ecological studies of black flies in the Oriental region, particularly South East Asia are still scarce except in Thailand, where the advances in simuliid morphotaxonomy (Takaoka and Choochote 2004), cytotaxonomy (Phasuk et al., 2005; Kuvangkadilok et al., 2008; Tangkawanit et al., 2009; Pramual et al., 2012; Pramual and Adler, 2014) and phylogenetics (Pramual et al., 2011; Pramual and Adler, 2014) have allowed researchers to gain a better understanding of their ecology (Pramual and Kuvangkadilok, 2009; Pramual and Wongpakam, 2010). Many countries in Southeast Asia are experiencing extremely slow growth in the taxonomic knowledge of black flies, as a consequence, nothing is known about their ecology, biology and other related information. In Malaysia, morphotaxonomic studies of black flies have been progressing well in recent years, (Takaoka and Davies, 1995; Takaoka, 2012) thus, allowing us to make our first attempt to obtain insight into the black fly ecology in association with their habitat characteristics. Exploring the fauna of black flies is a prerequisite for larval habitat control programs. Hence, the current study aims to investigate the breeding habitat preference of preimaginal black flies for the first time, across four geographical regions in Peninsular Malaysia.

2. Material and method

2.1. Study sites

This study was carried out at 180 stream points across Peninsular Malaysia, encompassing four geographical regions and 10 states namely East coast (Kelantan, Terengganu and Pahang), Northern (Perlis, Kedah, Penang and Perak), Central (Selangor) and Southern (Negeri Sembilan and Johore) (Fig. 1). The sampling sites included forests, recreational areas, agricultural sites (oil palm and rubber plantations), and residential areas. Details on sampling sites and collections are presented in Table 1.

2.2. Preimaginal sampling and identification

Samplings were conducted within March 2013 until February 2015 (Table 1). There is no distinct wet or dry season throughout the year and rain is experienced every single month in Malaysia with an average 2000 to 3500 mm per year. Seasonal rainfall variation occurred in every state of Malaysia during the northeast and northwest monsoon seasons (Low et al., 2012). We ensured that our sampling periods were free from its influence. All potential breeding sites for black flies were chosen based on accessibility and the presence of flow (i.e., streams, waterfalls and ditches). Each stream was sampled once from downstream to upstream (30 m), for approximately 1 h, by two people. 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 and Colbo, 1991; McCreadie et al., 2005). 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. (2004).

2.3. Physicochemical measurement

The following stream physicochemical parameters were measured at the time of each collection: width (m), depth (m), velocity (m/s) (one to three measurements along the collection path), 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 a cork and a timer watch were used to measure stream velocity; the time 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 physicochemical measurements protocols including those for major streambed particles, riparian vegetation, and canopy cover followed those of McCreadie et al. (2006). For each study site, the latitude and longitudinal co-ordinates were taken and recorded using a hand held Global positioning system (GPS) instrument (Garmin International Inc., Olathe, KS).

2.4. Data analysis

The frequency of species occurrence was designated in percentages. The presence or absence of a species was expressed on a binary scale (0 = absent, 1 = present), as in previous studies (Hamada and McCreadie, 1999; McCreadie et al., 2004 and Pramual and Kuvangkadilok, 2009). Because stream variables are inter-correlated, Principal Components Analysis (PCA) was used to reduce the number of variables into groups of independent components. Principal Components (PCs) with eigenvalues greater than 1.0 were retained as variables. To interpret the PCs, Spearman's rank correlations were used to detect the relationship between principal components and stream variables using a significance level of $P < 0.001$. Forward logistic regression analysis was used to examine the relationships between spatial distribution and the PCs. Only species that occurred at more than 10% of the sites were considered in regression analyses (Hamada et al., 2002) because those present at a lower frequency have resulted in the lack of statistical power (large number of zero values were observed) (McCreadie et al., 2005). Linear regression was used to test the relationship between species richness (i.e., number of species in each sampling site) and the stream variables of the sampling sites (i.e., PC scores). All collections (180 stream points) were subjected to PCA, and the PC scores were used for regression analysis.

3. Result

Forty-seven species were found from 180 sampled streams throughout Peninsular Malaysia (Table 2). At the subgeneric level, *Gomphostilbia* (31 species) was the largest subgenus in our collection, followed by *Simulium* s. str. (12 species) and *Nevermannia* (4 species). The current study also successfully revealed 16 out of the 18 species-groups recorded in Malaysia. The *Simulium batoense* species-group (9 species) was the most abundant, followed by the *S. asakoae* species-group (7 species), the *S. ceylonicum* species-group and the *S. epistum* species-group (4 species each). Other species-groups were represented by one to three species.

The predominant species were *S. trangense* (36.7%) and *S. angulistylum* (33.3%). Relatively common species were *S. cheongi* (29.4%), *S. tani* (25.6%), *S. nobile* (16.2%), *S. sheilae* (14.5%) and *S. bishopi* (10.6%). Other species were collected at frequencies lower than 10% (Table 2). Based on the current results, 85.1% (40 species) of total collected species had frequency of occurrence less than 10% and were considered as rare (Table 2). Of these, 12 species were collected only once (0.6%) (Table 2). The maximum number of black fly species collected in a single stream for all samples (180 sampling points) was 10 and minimum was one with the mean number per stream for the total sample sites was 3.0 ± 0.1 (SE).

The PCA of all collections (180 sampling points) revealed four PCs which had eigenvalues >1.0, accounted for 69.3% of the total intersite variance of the stream condition variables (Table 3). Spear-