RESEARCH

Biodiversity, seasonal abundance, and distribution of blackflies (Diptera: Simuliidae) in six different regions of Thailand

Wichai Srisuka1, Hiroyuki Takaoka2, Yasushi Otsuka3, Masako Fukuda4, Sorawat Thongsahuan5, Kritsana Taal6 and Atiporn Saeung7*

Abstract

**Background:** Blackflies are an important medical and veterinary group of small blood-sucking insects. Ninety-three blackfly species have been reported in Thailand. However, information on their biodiversity and population dynamics in each region is lacking. The main aim of this study was to assess the regional biodiversity, seasonal abundance and distribution of blackflies in six eco-geographically different regions in the country.

**Methods:** Blackfly larvae and pupae were sampled monthly from 58 sites between May 2011 and April 2013. Diversity parameters, seasonal abundance, regional distribution and frequency of species occurrence in stream sites were analyzed.

**Results:** A total of 19,456 mature larvae representing 57 species, and belonging to six subgenera in the genus *Simulium* Latreille (s.l.), were found. The five predominant taxa were *S. fenestratum* (8.6%), the *S. asakoae* complex (8.3%), *S. nakhonense* (7.5%), the *S. siamense* complex (7.4%) and the *S. doipuiense* complex (6.7%). The most frequent taxa at all sites were the *S. asakoae* complex (84.5%), followed by *S. fenestratum* (82.8%), the *S. siamense* complex (75.9%), *S. decuplum* (60.3%), *S. nakhonense* (58.6%) and the *S. tani* complex (48.3%). The richness of regional species was highest (40 species) in the north and predominated in the cold season. However, blackflies in the south predominated during the hot season. The highest numbers of blackflies collected from central, northeastern, eastern and western regions of the country were observed in the rainy season. Overall, the mean number of blackflies collected across the six regions during the rainy and cold season had no statistically significant difference, but it differed significantly in the hot season.

**Conclusions:** Blackflies in Thailand were surveyed in all three seasons across six geographical regions. These findings demonstrated that blackfly communities at each stream site varied with seasonality, and the regional relative abundance of blackflies differed markedly in the hot season. It was also found that the occurrence and distribution of blackflies in each region were associated strongly with elevation.

**Keywords:** Blackfly, *Simulium*, Biodiversity, Shannon diversity index, Regional distribution, Thailand

---

* Correspondence: atisaeung.no@gmail.com
1Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
Full list of author information is available at the end of the article

© The Author(s). 2017 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background

Blackflies (Diptera: Simuliidae) are distributed widely in all zoogeographical regions and found almost everywhere with running water that is suitable as a habitat for their aquatic stages [1]. Larvae and pupae are aquatic, and attach themselves to various submerged objects in many types of lotic environments, ranging from large rivers to tiny spring-fed trickles, and from swift currents to water that barely moves [2]. The choice of habitat usually varies between species. Due to their blood-sucking habits, adult females of certain blackfly species are of a medical and veterinary importance. Blackflies have been considered as vectors of many pathogens, such as filarioid nematodes of the genus *Onchocerca* in humans, cattle and deer, the genus *Dirofilaria* in bears, the genus *Splendidofilaria* in ducks; blood protozoans of the genera *Leucocytozoon* and *Trypanosoma* in birds; and viruses (rift valley fever, vesicular stomatitis) in horses and cattle; as well as chlamydial bacteria that cause blindness in sheep and abortion in cattle [1–3]. Furthermore, blackfly bites can cause other severe problems in humans, since they frequently inflicts pain, localized swelling, chronic dermatitis and inflammation accompanied by intense irritation that lasts for several days or even weeks [2].

In Thailand, a total of 93 blackfly species belonging to six subgenera, including *Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Montisimulium*, *Nevermannia* and *Simulium*, have been reported ([4], W. Srisuka, unpublished observations), with most new blackfly species being discovered in the northern part of the country. Remarkably, although the above information reflects rich species diversity, there are only a few reports of simulids from other regions in Thailand, for instance, *S. otsukai*, *S. thongsahuai*, *S. dafaense* and *S. trangense*, in the south [5–7]; *S. vanellum* from the west [8], *S. atipornae* and *S. lomkaoense* from central Thailand [9, 10]; and *S. kuvangkadilokae* from the northeast [11, 12]. Notably, there were no reports on regional biodiversity, seasonal abundance or distribution of blackflies in macro-scale areas of Thailand, apart from only the hotspot area in the tropical rainforest at Doi Pha Hom Pok National Park, in the northern region [13]. Additionally, human-biting blackfly species are found in large numbers, and cause irritation in domestic environments and to indigenous people and tourists [14], thus, most previous studies focused on the annual biting activity of adult females at Doi Inthanon and Doi Suthep-Pui National Park as well as in the village of Ban Pang Faen, Chiang Mai Province, northern Thailand [14–17].

Hence, the main aim of this study was to determine the seasonal abundance and dynamics of blackflies in six geographically and ecologically different regions of Thailand.

Methods

Study areas and sampling

This study was carried out at 58 fixed-stream sites in 41 provinces in six regions across Thailand, including 15, 10, 10, 7, 8 and 8 sites in the north (9 provinces), central (7 provinces), northeast (7 provinces), east (5 provinces), west (5 provinces) and south (8 provinces), respectively (Fig. 1, Additional file 1: Table S1). A total of 696 collections were made in this study (12 at each 58 fixed-stream sites at monthly intervals) from May 2011 to April 2013 which covered all seasons for each region. Larvae and pupae were hand sampled using fine forceps from available substrates in streams, such as fallen leaves, mud or rock surfaces, and trailing grasses. Forty-five minutes exactly were spent for the collection of larvae and pupae by the same person (one person) at each stream site. Larvae were preserved in 80% ethanol. The substrates were cut into pieces so that each part harboured a single pupa. Matured pupae were maintained individually in a plastic tube (10 cm long and 1.7 cm in diameter) with very little water at the bottom until adults emerged. After emergence, adult flies were kept alive in the same tube for at least 24 h, to secure hardening and colouring of their body and legs. Adult flies, associated with their pupal exuviae, were used to confirm the species identification of the larvae.

Meteorology and regions

The classification of the season in each region follows the Thai Meteorological Department, which bases its records on rainfall and air temperature data. Thus, each year is divided climatically into three seasons in the following regions: North region, with hilly and mountainous areas ranging from 392 to 2210 m in height, total rainfall of approximate 1287 mm and an average air temperature of 25 °C; West region, with mostly mountainous areas in the range of 111–560 m in height, similar to the north, and total rainfall and average air temperature of 1243 mm and 27.13 °C, respectively; Central region, with largely low-level plains and a few mountains ranging from 167 to 1550 m high in the northern and western part of the region, with a total rainfall and average air temperature of 1377 mm and 26.1 °C, respectively; Northeast region, which is a naturally high-level plain called the northeast plateau; Northwest-southeast region, which has Phu Phan Ridge oriented in the northeastern portion that separates this area into two basins, the first one is a large high-level plain in the west and the other smaller and sloped towards the east with an elevation ranging from 110 to 1337 m; this region has a total rainfall and average air temperature of 1589 mm and 25.1 °C, respectively; East region, which is mountainous in the northeast, and its eastern area is close to the Gulf of Thailand with an...
elevation ranging from 76 to 409 m, and total rainfall and an average air temperature of 2903 mm and 26.7 °C, respectively; South region, which has a peninsula mountain spine that is very steep. Its eastern area is close to the Gulf of Thailand and its western region near the Andaman Sea. It has an elevation ranging from 111 to 560 m, and total rainfall and an average air temperature of 2601 mm and 28.9 °C, respectively.

Species identification
Species identification was based on morphological characteristics of last-instar larvae (matured), pupae and reared adults by using the standard keys of Takaoka & Choochote [18], which covered 45 blackfly species, and additional keys that dealt with blackflies in Thailand [5–12, 19–26]. When formally named species were known to consist of cryptic species, they were referred to as species complex [4]. All specimens of blackflies from this study were deposited at the Entomology Section, Queen Sirikit Botanic Garden (QSBGE), Chiang Mai Province, Thailand.

Data analysis
Species and relative abundance of mature larvae at each site were recorded. The frequency of blackfly species was calculated by the total number of species occurrence
Results

Species composition of blackflies

A total of 19,456 mature larvae, representing 57 blackfly species of six subgenera, were collected from 58 stream sites across six regions in Thailand (Table 1, Additional file 1: Table S1). At the subgenus level, Simulium was the most diverse (28 species), followed by Gomphostilbia (16 species), Nevermannia (7 species), Asiosimulium (3 species), Montisimulium (2 species) and Daviesellum (1 species). Simulium fenestratum, the S. asakoa complex, S. nakhonense, the S. siamense complex, and the S. doipuiense complex were the five predominant taxa, each representing 8.6% (n = 1681), 8.3% (n = 1608), 7.5% (n = 1451), 7.4% (n = 1441) and 6.7% (n = 1298) of those collected. The most frequent taxa at all sites were the S. asakoa complex (84.5%, 49/58 sites), followed by S. fenestratum (82.8%, 48/58 sites), the S. siamense complex, (75.9%, 44/58 sites), S. decuplum (60.3%, 35/58 sites), S. nakhonense (58.6%, 34/58 sites) and the S. tani complex, (48.3%, 28/58 sites).

Species diversity, richness and distribution pattern

Species diversity and richness of blackflies in each region are shown in Fig. 2, with the highest in the northern region (H = 3.1, J' = 0.8) and lowest in the southern (H = 2.1, J' = 0.5). Of 58 stream sites, the Shannon diversity index (H) was highest at Rom Klaok (Phitsanulok Province), followed by Doi Phu Kha (Nan Province), Phu Rua (Loei Province) and Mae Wong (Kamphaeng Phet Province), which represented 2.4, 2.4, 2.3 and 2.3, respectively (Additional file 2: Table S2).

The species accumulation (rarefaction) curves (Fig. 3) showed the expected and observed richness of species occurring in all sites, with a total from all collections being 71 and 57 species, respectively. The expected (± SE) and observed species richness were 52 ± 4.5 and 40 spp. in northern, 45 ± 4.1 and 34 spp. in the central, 38 ± 2.9 and 28 spp. in western, 31 ± 2.8 and 22 spp. in northeastern, 25 ± 4.3 and 17 spp. in southern and 24 ± 1.8 and 15 spp. in eastern regions, respectively (Fig. 4).

The detrended correspondence analysis (DCA) for the distribution of blackfly species associated with sampling sites (axis 1: eigenvalue 0.8; axis 2: eigenvalue 0.5) is presented in Fig. 5. Overall, the S. asakoa complex and S. fenestratum were common species found in all sites. The distribution of the S. doipuiense complex, S. intanonoense, S. fruticosum, S. chiangdaensoe, S. maeaiense, S. (Montisimulium) sp., S. vessabutrae, S. atipornae and S. lonskaoense were associated strongly with high elevation (site nos. 8, 13, 14, 17, 21 and 22) in the northern and central region, while S. quinquestratrum, S. siamense complex, S. decuplum and S. dentistyllum were associated with low elevation (site nos. 20, 23, 24, 25, 29, 30, 31, 35, 38, 40, 41 and 42) in the central and northeastern regions. However, S. lampangense, S. weji, S. prayongi and S. takense were associated with calcareous waterfalls (site nos. 5, 12 and 44).

Seasonal dynamics

Overall, S. fenestratum was the dominant species during all three seasons (Additional file 3: Table S3). Almost all of the species in the north were dominant in the cold season, and S. chumpornense and S. phayaoense were recorded only during that time, while S. pahangense was collected only during the rainy season (Fig. 6, Additional file 3: Table S3). Most species in the central region were discovered in the rainy season, and S. oblongum, S. aureohirtum, S. bullatum, S. phukaense and the S. tani complex were found only during that time, whereas S. yongi was only recorded in the cold season (Fig. 6, Additional file 3: Table S3). A greater number of blackflies were collected during the rainy season in the northeastern, eastern and western regions (Figs. 7, 8, Additional file 3: Table S3). Simulium oblongum, S. aureohirtum and S. yuphae were found only during the rainy season in the eastern region,
similar to species found in the central region. Remarkably, blackflies in the southern region were more dominant during the hot season (Fig. 8, Additional file 3: Table S3). The mean number of blackflies collected across the six regions during the rainy (Kruskal-Wallis test, $H = 6.242, df = 5, P = 0.283$) and cold season (Kruskal-Wallis test, $H = 8.650, df = 4, P = 0.070$) had no statistically significant difference, but it differed significantly in the hot season (Kruskal-Wallis test, $H = 26.589, df = 5, P < 0.0001$).

### Regional relative abundance

#### Northern region

The most frequent taxa at all sites were *S. asakoae* complex (80%) and *S. fenestratum* (80%), followed by *S. yuphæae* (73.3%) and the *S. siamense* complex (66.7%). In addition, the *S. doipuiense* complex, *S. inthanonense*, the *S. asakoae* complex, *S. decuplum/S. fenestratum* and *S. chiangdaensoe* were the five predominant taxa, representing 15.4% ($n = 860$), 11.6% ($n = 647$), 7.0% ($n = 389$), 6.4% ($n = 358$) and 5.3% ($n = 298$), respectively (Additional file 4: Table S4). The hot season had significantly lower mean numbers when compared to the rainy (Kruskal-Wallis

### Table 1

| Species | Total collected | %flies | %SO |
|---------|----------------|--------|-----|
| *Simulium* (asi*ossimulium*) furvum | 34 | 0.2 | 1.7 |
| *Simulium* (asi*ossimulium*) oblongum | 487 | 2.5 | 17.2 |
| *Simulium* (asi*ossimulium*) wanchaii | 34 | 0.2 | 3.5 |
| *Simulium* (Daviesellum) pahangense | 9 | 0.1 | 5.2 |
| *Simulium* (Gomphostilbia) angulistyllum complex | 515 | 2.7 | 22.4 |
| *Simulium* (Gomphostilbia) asakoae complex | 1608 | 8.3 | 84.5 |
| *Simulium* (Gomphostilbia) burtoni | 523 | 2.7 | 24.1 |
| *Simulium* (Gomphostilbia) chiangdaensoe | 515 | 2.7 | 10.3 |
| *Simulium* (Gomphostilbia) chumporomense | 182 | 0.9 | 27.6 |
| *Simulium* (Gomphostilbia) curtatum | 235 | 1.2 | 12.1 |
| *Simulium* (Gomphostilbia) decuplum | 1175 | 6.0 | 60.3 |
| *Simulium* (Gomphostilbia) dentistyllum | 367 | 1.9 | 37.9 |
| *Simulium* (Gomphostilbia) duolongum | 450 | 2.3 | 22.4 |
| *Simulium* (Gomphostilbia) gambakense | 90 | 0.5 | 13.8 |
| *Simulium* (Gomphostilbia) inthanonense | 753 | 3.9 | 19.0 |
| *Simulium* (Gomphostilbia) piroaene | 52 | 0.3 | 1.7 |
| *Simulium* (Gomphostilbia) kuvangkadilakae | 98 | 0.5 | 3.5 |
| *Simulium* (Gomphostilbia) parahyangum | 3 | 0.1 | 1.7 |
| *Simulium* (Gomphostilbia) shelae | 556 | 2.9 | 44.8 |
| *Simulium* (Gomphostilbia) siamense complex | 1441 | 7.4 | 75.9 |
| *Simulium* (Montisimulium) nanense | 51 | 0.3 | 1.7 |
| *Simulium* (Montisimulium) sp. | 57 | 0.3 | 1.7 |
| *Simulium* (Nevermannia) aureohirtum | 408 | 2.1 | 22.4 |
| *Simulium* (Nevermannia) tangense | 22 | 0.1 | 1.7 |
| *Simulium* (Nevermannia) feuerborni complex | 142 | 0.7 | 8.6 |
| *Simulium* (Nevermannia) fruticosum | 329 | 1.7 | 17.2 |
| *Simulium* (Nevermannia) khunklangense | 73 | 0.4 | 1.7 |
| *Simulium* (Nevermannia) maeaiense | 241 | 1.2 | 8.6 |
| *Simulium* (Nevermannia) vessabutrae | 7 | 0.1 | 1.7 |
| *Simulium* (Simulium) atipornae | 98 | 0.5 | 3.5 |
| *Simulium* (Simulium) baimaii | 69 | 0.4 | 1.7 |
| *Simulium* (Simulium) brevipar | 16 | 0.1 | 1.7 |
| *Simulium* (Simulium) bullaturn | 61 | 0.3 | 10.3 |
| *Simulium* (Simulium) chamlongi | 217 | 1.1 | 20.7 |
| *Simulium* (Simulium) chiangmaiense | 79 | 0.4 | 3.5 |
| *Simulium* (Simulium) doipuiense complex | 1298 | 6.7 | 25.9 |
| *Simulium* (Simulium) fenestratum complex | 1681 | 8.6 | 82.8 |
| *Simulium* (Simulium) gigas | 15 | 0.1 | 1.7 |
| *Simulium* (Simulium) lampangense | 139 | 0.7 | 5.2 |
| *Simulium* (Simulium) longkaoense | 98 | 0.5 | 3.5 |
| *Simulium* (Simulium) malayense | 21 | 0.1 | 1.7 |
| *Simulium* (Simulium) manooni | 196 | 1.0 | 8.6 |
| *Simulium* (Simulium) niihoreense | 1451 | 7.5 | 58.6 |
| *Simulium* (Simulium) nigrogilum | 17 | 0.1 | 5.2 |
| *Simulium* (Simulium) nobile | 843 | 4.3 | 17.2 |
| *Simulium* (Simulium) robusum | 301 | 1.5 | 15.5 |
| *Simulium* (Simulium) phayaense | 54 | 0.3 | 6.9 |
| *Simulium* (Simulium) prasyongi | 35 | 0.2 | 1.7 |
| *Simulium* (Simulium) phuakense | 37 | 0.2 | 6.9 |
| *Simulium* (Simulium) quinquestriatum | 614 | 3.2 | 34.1 |
| *Simulium* (Simulium) siripoonense | 19 | 0.1 | 1.7 |
| *Simulium* (Simulium) takense | 69 | 0.4 | 1.7 |
| *Simulium* (Simulium) tani complex | 385 | 2.1 | 48.9 |
| *Simulium* (Simulium) thailandicum | 228 | 1.2 | 12.1 |
| *Simulium* (Simulium) weji | 609 | 3.1 | 6.9 |
| *Simulium* (Simulium) yongi | 38 | 0.2 | 3.5 |
| *Simulium* (Simulium) yuphæae | 311 | 1.6 | 36.2 |

Total 19,456 100

*The most frequent taxa at all sites

bThe most predominant taxa
test, $H = 21.195$, $df = 2$, $P = 0.021$) and cold seasons (Kruskal-Wallis test, $H = 34.122$, $df = 2$, $P < 0.0001$).

**Central region**

The most frequent taxa at all sites were *S. asakoae* complex (90%) and the *S. siamense* complex (90%), followed by *S. decuplum* and *S. fenestratum* (80%). Additionally, the *S. asakoae* complex, the *S. doipuiense* complex, the *S. siamense* complex, *S. nakhonense* and *S. ducuplum*, were the five predominant taxa, representing 10.3% ($n = 353$), 10% ($n = 345$), 7.4% ($n = 254$), 7.3% ($n = 251$) and 6.3% ($n = 218$), respectively (Additional file 5: Table S5).

![Fig. 2 Diversity parameters, Shannon-Wiener index (H), evenness (Pielou J') and expected H value (Exp H) for blackflies in the six regions of Thailand](image1)

![Fig. 3 Species accumulation (rarefaction) curves for blackflies from 696 collections overall at 58 sites in the six regions of Thailand](image2)
Fig. 4 Species accumulation and species richness curves representing the observed (obs) and estimated (est) number of blackflies collected from 58 sites across the six regions of Thailand (northern: 180 collections; central: 120 collections; northeastern: 120 collections; eastern: 84 collections; western: 96 collections; southern: 96 collections)

Fig. 5 Ordination diagrams extracted by detrended correspondence analysis (DCA) of blackflies distributed in 58 sites
There were significant differences in the mean number of blackflies captured in the central region during the hot season, when compared to the rainy (Kruskal-Wallis test, $H = 30.265, df = 2, P < 0.0001$) and cold seasons (Kruskal-Wallis test, $H = 19.456, df = 2, P = 0.020$).

**Northeastern region**

The most frequent taxa at all sites were $S$. asakoae complex and $S$. siamense complex (100%), followed by $S$. decuplum, $S$. fenestratum and $S$. quinquestriatum (90%). In addition, the $S$. asakoae complex, $S$. oblongum, $S$. fenestratum, the $S$. siamense complex and $S$. quinquestriatum were the five predominant taxa, representing 15.7% ($n = 415$), 13.4% ($n = 353$), 12.8% ($n = 338$), 11% ($n = 290$) and 8.3% ($n = 219$), respectively (Additional file 6: Table S6). There was a significant difference in the mean number of blackflies collected among the three seasons (Kruskal-Wallis test, $H = 28.687, df = 2, P < 0.0001$).

**Eastern region**

The most frequent taxa at all sites were $S$. decuplum, $S$. dentistylum, the $S$. siamense complex, $S$. fenestratum and the $S$. tani complex (100%), followed by the $S$.
asakoae complex and S. nakhonense (85.7%). Additionally, S. fenestratum, the S. siamense complex, S. decuplum, S. dentistylum and S. nakhonense were the five predominant taxa, representing 24% ($n = 479$), 19.6% ($n = 391$), 18.5% ($n = 369$), 8.1% ($n = 163$) and 7% ($n = 140$), respectively (Additional file 7: Table S7). The mean number of blackflies captured in the hot season was lower than that in the rainy (Kruskal-Wallis test, $H = 21.195$, $df = 2$, $P = 0.021$) and cold season (Kruskal-Wallis test, $H = 34.122$, $df = 2$, $P < 0.0001$). There was a significant difference in the mean number of the blackflies collected in this region between the hot and rainy seasons (Kruskal-Wallis test, $H = 15.033$, $df = 2$, $P < 0.005$).

Western region
The most frequent taxa at all sites were S. nakhonense and S. tani complex (75%), followed by the S. angulistylum complex, the S. asakoae complex, S. sheilae, the S. siamense complex and S. fenestratum (62.5%). In addition, S. nakhonense, S. weji, the S. angulistylum complex, S. duolongum and the S. siamense complex were the five predominant taxa, representing 16.1% ($n = 548$), 12% ($n = 406$), 9.3% ($n = 317$), 7.5% ($n = 255$) and 6.5% ($n = 221$), respectively (Additional file 8: Table S8). The mean number of blackflies captured was lower during the hot season than that during the rainy (Kruskal-Wallis test, $H = 27.071$, $df = 2$, $P < 0.0001$) and cold season (Kruskal-Wallis test, $H = 22.589$, $df = 2$, $P = 0.002$).

Southern region
The most frequent taxa at all sites were S. burtoni, S. sheilae, S. nakhonense and S. nobile (100%), followed by the S. asakoae complex and S. fenestratum (87.5%). Additionally, S. nobile, S. burtoni, S. nakhonense, S. sheilae and the S. asakoae complex were the five predominant taxa, representing 31.1% ($n = 746$), 19.8% ($n = 474$), 12.4% ($n = 297$), 12.1% ($n = 289$) and 5.2% ($n = 124$), respectively (Additional file 9: Table S9). There were no significant differences in the mean number of blackflies captured between the hot and rainy seasons (Mann-Whitney U-test, $U = 128.000$, $P = 0.570$).

Discussion
Species composition, species richness, seasonal abundance and diversity
The number of blackflies reached its highest during the cold season, according to a previous report by Srisuka et al. [13], who studied the seasonal biodiversity of blackflies at Doi Pha Hom Pok, northern Thailand. The greatest number of blackflies in the southern region was in the hot season. This study found that seven of seventeen species identified from this region increased their populations approximately two to three times during this season. This observation agrees with the study of blackflies in northern Sweden, where they were higher in the summer than other seasons [29]. The highest number of blackflies collected from central, northeastern, eastern and western regions of Thailand peaked in the rainy season. The findings in this study were consistent with those in a previous report by Pramual & Wongpakam [30], who studied the seasonal variation of blackflies at Phu Phan mountain range in northeastern Thailand. They demonstrated that the species abundance was higher in rainy seasons than in others and blackfly communities at each stream site varied with seasonality, i.e. S. nakhonense, the S. angulistylum complex and S.
kuvangkadilokae were more dominant in the rainy season, whereas the S. asakoe complex, S. aureohirtum and S. trangense were dominant in the hot and cold season [30]. Likewise, blackflies were caught in higher numbers during the rainy season in Nigeria, Africa [31]. Takoaka [32] showed that seasonal abundance patterns of adult populations of S. ochraceum, the vector of onchocerciasis in Guatemala, Central America, differ by localities depending on the availability of permanent and temporary streams suitable for its immature stages.

In addition to seasons and geographical locations, elevation also can influence blackfly populations. The results of this study showed that the Shannon diversity index was highest in areas with high elevations, i.e. Rom Klao (1047 m), Doi Phu Kha (1629 m), Phu Rhea (1337 m) and Mae Wong (1274 m). It was found that 36 species manifested in optimal or unique environments that had suitable factors for their breeding habitats. For example, S. baimaii breeds at Phu Kradueng, Loei Province in only high mountains, with slow-flowing streams exposed to sunlight. Likewise, all species members in the subgenus Montisimulium are restricted to high elevations at Doi Inthanon National Park, Chiang Mai Province. In contrast, S. gombakense has a wide vertical distribution range from a height of 500 m in small streams in the foothills to 2100 m near the summit of Doi Pha Hom Pok National Park [13], and it is also found at an elevation of 412 m at Mae Klang Waterfall, Doi Inthanon National Park [33]. In addition, the S. asakoe complex, S. fenestratum, the S. siamense complex, S. decuplum, S. nakhonense, and the S. tani complex were the most common taxa found in this study, which is similar to previous reports by Pramual & Kuvangkadi-kok [34], and Pramual & Wongpakam [30].

Relationship of subgenera to elevation
The subgenus Asiosimulium is a small and endemic subgenus in the Oriental region. It is represented by four species, of which three, S. oblongum, S. wanchaii and S. furvum, have been reported in Thailand [35–37], and the remaining one, S. suchitrae, in Nepal [38]. The first three species were found in lowland streams, flowing slowly over rock surfaces exposed to the sun during the rainy season, while S. suchitrae was found at high elevation (1826 m) in a small stream flowing slowly over rocks [38]. Both S. furvum and S. wanchaii were restricted to their sites, but S. oblongum was distributed widely in and near the northeastern, central and eastern regions. The subgenus Daviesellum is represented by two species, S. pahangense and S. courtneyei, in Thailand [39]. Only S. pahangense was distributed at high elevation from northern to central regions along the boundary with Myanmar, and also in lowland streams in the southern region. Most species of Gomphostilbia, such as those of the S. batoense, S. ceylonicum, S. epistum, S. gombakense and S. varicone species-groups, are the second largest subgenus in Thailand and distributed at low elevations. This study found S. sheilae, the S. siamense complex and S. chumponense in all six regions of Thailand, with their breeding habitats mostly in lowland streams as previously reported [7, 30]. In contrast, species of the S. asakoe and S. darjeelingense species-groups were found in highland streams, except for the S. asakoe complex, which was distributed widely from low elevations to 2500 m at the summit of Doi Inthanon National Park, Chiang Mai Province and in other Asian countries, such as Malaysia, China (Hong Kong) and Vietnam [7, 13, 21, 25, 40–42].

The subgenus Montisimulium is represented by six species in Thailand. Two of them, i.e. S. nanense and S. (Montisimulium) sp., were discovered in this study at high elevations, as reported by Takoaka & Somboon [43] and Takoaka et al. [44], who collected three species of this subgenus at high elevations ranging from 2229 to 3720 m in Bhutan and 1750 m in Vietnam, respectively. The remaining species have been found only on Doi Inthanon and Doi Pha Hom Pok in Chiang Mai Province [45, 46]. Of ten species of the subgenus, Nevermannia reported in Thailand, seven were found in this study. Most species were collected at high elevations, for example, the high mountains of Chiang Mai Province, northern Thailand [13, 26, 47, 48]. Other reports from several other Asian countries, such as Malaysia, Myanmar, Vietnam, Indonesia and Bhutan, indicated that members of this subgenus were associated with high elevations ranging from 1000 to 2532 m [43, 49–53]. The subgenus Simulium is the largest subgenus in Thailand, including 45 described species, of which 28 (62% of total species) were collected. Most of the common taxa, such as S. fenestratum, S. nakhonense, S. quinquestriatum, and the S. tani complex, breed in lowland streams. The findings of this study were in accordance with those reported by Takoaka et al. [44], Srisuka et al. [13] and Pramual & Wongpakam [30]. Species of the S. Christophersi, S. malyschevi and S. variegatum species-groups were distributed in middle to high elevations (1200–2200 m), as previously studied in Vietnam and Thailand [44, 54]. In contrast, most of the species within the S. multistriatum, S. nobile and S. striatum species-groups occurred in lowland streams, as reported by Srisuka et al. [13] and Ishii et al. [17]. Members of the S. griseifrons species-group colonized streams at low to high elevations (200–2210 m).

Conclusion
The findings of this study demonstrated that the richness and relative abundance of blackflies were different between regions, and blackfly communities at each
stream site varied with seasonality. Also, the elevation of sampling sites, which ranged from high mountainous to lowland streams as well as covering all mainland steams, influenced the distribution of blackflies in the country. Concurrent species, population dynamics and seasonal abundance in each area are important as useful information for pest species management and control programs, and especially for ecotourism in forests, by waterfalls and in high mountainous areas, where the number of tourists increases yearly.

Additional files

Additional file 1: Table S1. Names of sampling sites, geographical coordinates, altitudes, and environmental variables for blackfly collections at 58 sampling sites in six regions of Thailand. (DOCX 38 kb)

Additional file 2: Table S2. Diversity parameters for blackflies at 58 sampling sites in the six regions of Thailand. (DOCX 19 kb)

Additional file 3: Table S3. Seasonal abundance and species richness of blackfly species at 58 sampling sites representing six regions in Thailand. (DOCX 44 kb)

Additional file 4: Table S4. Regional distribution and relative abundance of blackflies at 15 sampling sites in northern Thailand. (DOCX 30 kb)

Additional file 5: Table S5. Regional distribution and relative abundance of blackflies at 10 sampling sites in central Thailand. (DOCX 24 kb)

Additional file 6: Table S6. Regional distribution and relative abundance of blackflies at 10 sampling sites in northeastern Thailand. (DOCX 20 kb)

Additional file 7: Table S7. Regional distribution and relative abundance of blackflies at 7 sampling sites in eastern Thailand. (DOCX 18 kb)

Additional file 8: Table S8. Regional distribution and relative abundance of blackflies at 8 sampling sites in western Thailand. (DOCX 21 kb)

Additional file 9: Table S9. Regional distribution and relative abundance of blackflies at 8 sampling sites in southern Thailand. (DOCX 19 kb)

Abbreviations

DCA: Detrended correspondence analysis; Exp: H: Expected value of H; Pi: Lou J: evenness; H: Shannon-Wiener index

Acknowledgements

We are grateful to Dr Suyannee Vessabutr, Director of Queen Sirikit Botanic Garden, Chiang Mai, Thailand, for her interest and support in this study. Thanks go to Mr. Sumrith Suriya, Mr. Raewat Saksod, Ms. Sunantha Pilakantha and Ms. Chayanit Surin for their kind help in the field surveys and preparation of specimens in the laboratory. This article is dedicated to the late Professor Wej Choochote who kindly provided invaluable suggestions and supported this study.

Funding

This study was financially supported by the Thailand Research Fund (TRF Senior Research Scholar: grant number RTA5480006) to WC, and the Faculty of Medicine Research Fund, Chiang Mai University (CMU) to AS. This study was in part supported by the research grant from the Thailand Research Fund (TRF) and the Office of the Higher Education Commission (OHEC) through the Research Grant for New Scholar (grant number MRG5880101) to A5, and the University of Malaya (R0021/16SUS) to HT.

Availability of data and materials

The data sets supporting the conclusions of this article are included within the article and its additional files.

Authors’ contributions

WS and AS conceived and designed the study. WS and AS performed field and laboratory experiments, analyzed the data, interpreted the findings and wrote the manuscript. HT participated in species identification, data analysis and revised the manuscript. YO and MF helped to revise the manuscript. ST and KT participated in field experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Entomology Section, Queen Sirikit Botanic Garden, P.O. Box 7, Chiang Mai 50180, Thailand. 2Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia. 3Research Center for the Pacific Islands, Kagoshima University, Kagoshima 890-8580, Japan. 4Division of Life Science Research, Research Promotion Institute, Oita University, Hasama, Oita 879-5593, Japan. 5Faculty of Veterinary Science, Prince of Songkla University, Songkhla 90110, Thailand. 6Faculty of Veterinary Medicine, Western University, Kanchanaburi 71170, Thailand. Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

Received: 9 March 2017 Accepted: 23 October 2017

Published online: 21 November 2017

References

1. Takaoka H. Insecta: Diptera, Simuliidae. In: Yule CM, Yong HS, editors. Freshwater invertebrates of the Malaysian region. Kuala Lumpur, Malaysia: The Academy of Sciences Malaysia; 2004. p. 673–82.

2. Srisuka W. Species diversity of black flies in Thailand, and the evaluation of ecological factors influencing black-fly species diversity in Doi Phahompok National Park. PhD Thesis, Chiang Mai University; 2015.

3. Murdock CC, Adler PH, Frank J, Perkins SL. Molecular analyses on host-seeking blackflies (Diptera: Simuliidae) reveal a diverse assemblage of Leucocytozoon (Apicomplexa: Haemospororida) parasites in an alpine ecosystem. Parasit Vectors. 2015;8:343.

4. Adler PH, Crosseby RW. World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory. Available at: http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf. Accessed 20 Feb 2017.

5. Takaoka H, Otsuka Y, Choochote W, Thongsahuan S. Two new and one newly recorded species of Simulium (Gomphostilbia) (Diptera: Simuliidae) from southern Thailand. Med Entomol Zool. 2009;60:259–68.

6. Takaoka H, Otsuka Y, Choochote W, Thongsahuan S. A new species of Simulium (Simulium) (Diptera: Simuliidae) from southern Thailand. Med Entomol Zool. 2010;61:17–25.

7. Jitklang S, Kuvangkadilok C, Baimai V, Takaoka H, Adler PH. Cytogenetics and morphoanatomy of the Simulium (Gomphostilbia) ceylonicum species group (Diptera: Simuliidae) in Thailand. Zootaxa. 2008;1971:71–28.

8. Huang YT, Phasuk J, Chanpaisaeng J, Adler PH. A new species of black fly in the subgenus Simulium (Diptera: Simuliidae) from Thailand. Med Entomol Zool. 2010;61:49–58.

9. Takaoka H, Srisuka W, Otsuka Y, Choochote W. A new species and species-group of Simulium (Simulium) (Diptera: Simuliidae) from Thailand. J Med Entomol. 2014;51:275–32.

10. Takaoka H, Srisuka W, Saeng U, Otsuka Y, Choochote W. Simulium (Simulium) lomkaoense, a new species of black fly (Diptera: Simuliidae) from Thailand. J Med Entomol. 2014;51:1109–15.

11. Pramual P, Tangkawanit U. A new species of Simulium (Gomphostilbia) (Diptera: Simuliidae) from northeastern Thailand. Med Entomol Zool. 2008;59:297–303.

12. Takaoka H, Srisuka W. Description of the female of Simulium (Gomphostilbia) kuvangkadilokiae (Diptera: Simuliidae) from Thailand. Med Entomol Zool. 2010;61:39–47.
