

# Trait-based and phylogenetic community ecology of black flies (Diptera: Simuliidae) in tropical streams of Thailand

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**Abstract** A primary aim of community ecology is to determine mechanisms that shape community structure. In this study, we used trait-based and phylogenetic community ecology approaches to determine the factors underlying the species assemblage of black fly larvae in tropical streams of Thailand. Ecological data were obtained from 150 collections. Morphological traits were measured from 35 species. The phylogenetic relationship of these species was inferred from cytochrome *c* oxidase I, II, and 18S rRNA/ITS1 sequences. Phylogeny-based community analyses indicated that species in the community did not skew toward phylogenetic clustering or overdispersion. However, the trait-based community structure analysis

based on labral fan morphology revealed that species in most (>78%) communities were more similar morphologically than expected by chance. As labral fan morphology is strongly related to habitat exploitation, it is considered that habitat filtering is a major factor shaping community structure of black fly larvae in tropical streams of Thailand.

**Keywords** Aquatic insects · Black fly · Community structure · Simuliidae · *Simulium*

## Introduction

Understanding factors underlying biological community structure is one of the primary goals of community and evolutionary ecology. Two principal mechanisms have been proposed as factors shaping community structure—habitat filtering (e.g., Weiher & Keddy, 1995; Chesson, 2000) and competitive exclusion (MacArthur & Levins, 1967). Habitat filtering and competitive exclusion predict different outcomes of morphological trait similarity and phylogenetic relatedness of the coexisting species. Phylogenetic community ecology, which integrates phylogenetic and morphological traits as well as species assemblage data, can be used to examine the relative importance of these mechanisms (Webb et al., 2002; Emerson & Gillespie, 2008; Cavender-Bares et al., 2009; Vamosi et al., 2009). If the coexisting species possess similar traits and are more phylogenetically related than a

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random community, the community structure can be interpreted as a result of habitat filtering. On the other hand, if the co-occurring species in the community are different in morphological traits and are phylogenetically distantly related, competitive exclusion is suggested as the principal factor determining community (Cavender-Bares et al., 2004; Vamosi et al., 2009). Several studies have now used phylogenetic community ecology to test hypotheses about the factors underlying biological community structure (e.g., Kozak et al., 2005; Vamosi & Vamosi, 2007; Willis et al., 2010).

Black flies are insects belonging to the family Simuliidae, in the order Diptera. A total of 2177 species have been reported worldwide and 92 species have been recorded in Thailand (Adler & Crosskey, 2015). Adult female black flies feed on vertebrate blood, and some species are important pests of both humans and livestock (Crosskey, 1990; Adler et al., 2004). However, the immature stages are aquatic and are significant components of stream ecosystems (Cummins, 1987). Ecological studies revealed that the distribution of black fly species is determined mainly by environmental factors, such as stream size, flow velocity, pH, water conductivity, temperature, altitude, riparian forest cover, and presence of impoundments (Hamada & McCreadie, 1999; Hamada et al., 2002; Scheder & Waringer, 2002; McCreadie et al., 2004; Illéšová et al., 2008; Pramual & Kuvangkadilok, 2009). Among these factors, stream size, velocity, and presence of water impoundments play particularly important roles. In addition to individual species' distributions, species richness and species assemblage are also affected by the physical and chemical factors of the stream (McCreadie & Adler, 2006, 2012; Pramual & Kuvangkadilok, 2009; Pramual & Wongpakam, 2010).

Although the patterns of the species distribution and species assemblages of larval black flies are well documented in terms of their relationship to stream variables, the pattern reflecting phylogenetic relatedness, morphological similarity, or only environmental conditions has rarely been examined. As knowledge of the taxonomy and ecology of black flies in Thailand is well developed, they provide a good model for phylogenetic community ecology. In this study, we investigated the relationship between species assemblage, morphology, and phylogenetic relationships among black fly species in tropical streams of Thailand. The

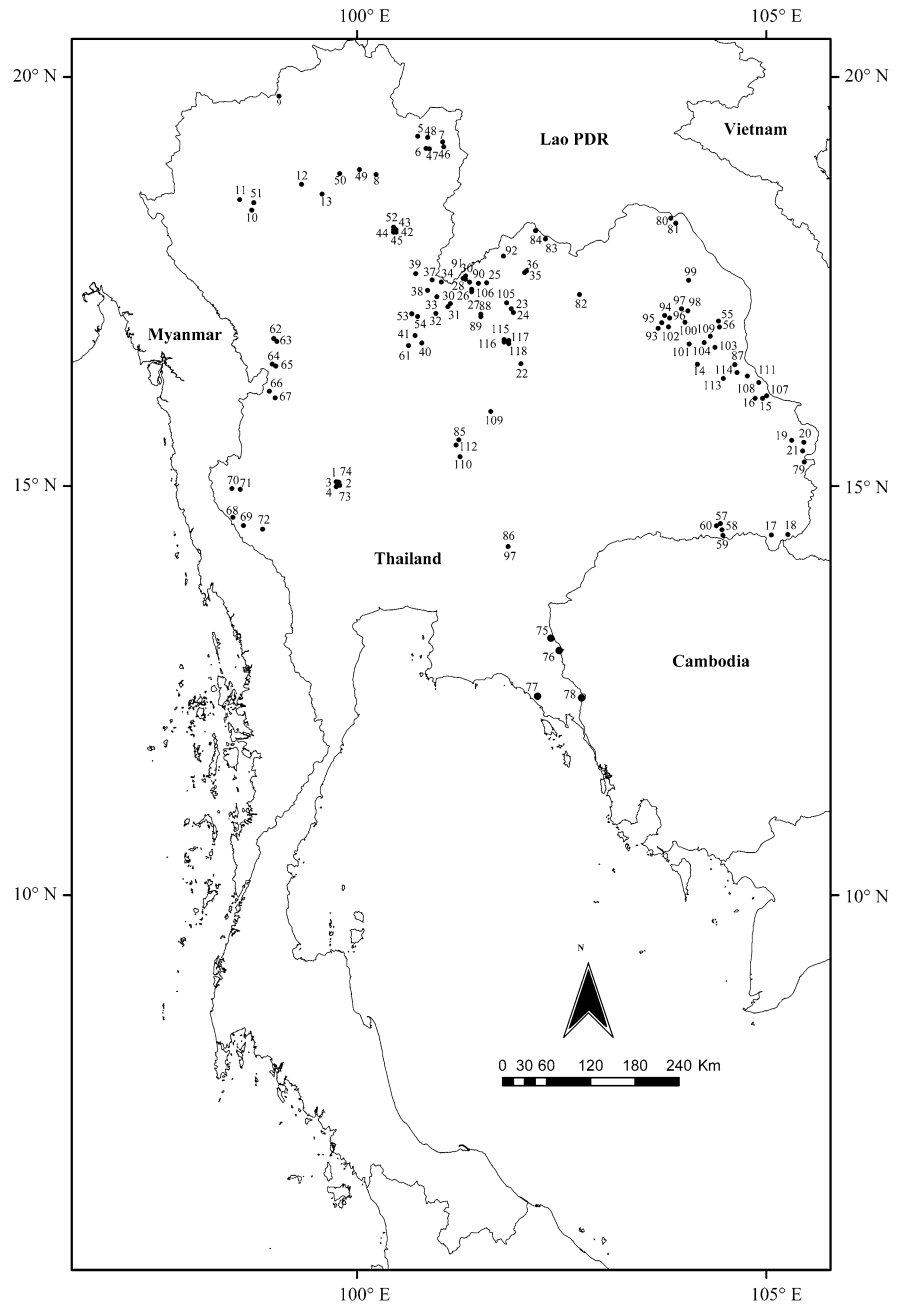
labral fan was selected as the morphological character for our study as it has been shown in previous studies that this character was strongly related to habitat exploitation (Zhang & Malmqvist, 1996; Palmer & Craig, 2000). The labral fan is a food-filtering organ of the larva which comprises numerous rays with the size and number of the rays being related to the current velocity of the stream. Species that occupy slow flowing water usually have larger fan rays than species in fast flowing streams (Zhang & Malmqvist, 1996; Palmer & Craig, 2000). Our purpose is to examine the following questions: (i) are black fly species that share a habitat more similar in labral fan morphology or phylogeny? and (ii) does the labral fan morphology relate to species phylogeny?

## Materials and methods

### Sample collection and identification

A total of 150 black fly collections [87 collections from present study and 63 collections from previous publications (Pramual & Kuvangkadilok, 2009; Pramual & Wongpakam, 2010)] were made from 118 sites in Thailand (Fig. 1; Table S1). Larvae and pupae were collected by hand from the substrates (such as submerged leaves or trailing grass), using fine forceps. Previous studies have demonstrated that samples collected by this procedure are representative of species occurring in a locality (McCreadie & Colbo, 1991; McCreadie et al., 2004). Larvae and pupae were preserved in modified Carnoy's solution (3:1, 95% ethanol/acetic acid). The fixative was changed twice within 1 h and again after 24 h. Species were identified morphologically using the keys and descriptions of black fly species in Thailand and Southeast Asia (e.g., Takaoka & Suzuki, 1984; Takaoka & Davies, 1995; Takaoka & Choochote, 2004). We measured the physical parameters of the larval habitats that previous studies found were associated with black fly species distribution (elevation, stream width, depth, discharge, velocity, water conductivity, dominant streambed particle size, canopy cover, and riparian vegetation) (McCreadie & Colbo, 1991; McCreadie & Adler, 1998; Hamada & McCreadie, 1999; McCreadie et al., 2004; Pramual & Kuvangkadilok, 2009). Types of streambed particles were

**Fig. 1** Map showing approximate locations of 118 collection sites of black flies in Thailand. Details of sampling sites are given in Table S1



classified into six categories based on the size of the dominant particle as described in McCreadie et al. (2006). Riparian vegetation was classified into three types (open, brush, and forest) according to the vegetation forms (McCreadie et al., 2006). Canopy cover over the stream was visually estimated using three criteria (complete, partial, and open canopy) following the description of McCreadie et al. (2006).

#### Black fly trait data

We selected the primary labral fan rays as the morphological trait for trait-based phylogenetic community structure analysis. Previous studies have demonstrated that the labral fan morphology was related to stream variables (Zhang & Malmqvist, 1996; Palmer & Craig, 2000). Therefore, this larval character

is the principal morphological trait that determines species distribution (Palmer & Craig, 2000). Last instar larvae were used for morphological trait analysis. Labral fan morphology was assessed by measuring the fan ray width and length and number of rays of the completely opened primary fan, under a compound microscope using a microscopic micrometer. As intraspecific variation of the labral fan morphology has been reported (Lucas & Hunter, 1999; Adler et al., 2004), we selected larvae from several sampling sites for morphological measurement. For each species, at least 30 larvae were measured. The only exceptions were larvae of *Simulium siripoomense* Takaoka & Saito and *S. malayense* Takaoka & Davies where only five and ten larvae were available respectively. To test whether the sampling size was enough to capture intraspecific morphological variation, we compared the means of morphological characteristics between groups with different sampling sizes (i.e., 30 and larger) in eight geographically widespread species. All comparisons revealed no significant differences in the mean morphological characteristics, which indicates that an increase in sampling sizes is unlikely to alter the results and that our sampling size is suitable to capture intraspecific morphological variation.

#### DNA extraction, polymerase chain reaction, and sequencing

Most (32 species) of the DNA samples were obtained from previous studies (Pramual et al., 2012; Pramual & Adler, 2014; Sriphirom et al., 2014). Larval specimens of three species (*Simulium baimaii* Kuvangkadilok & Takaoka, *S. Chiangmaiense* Takaoka & Suzuki and *S. siripoomense*) were obtained in the present study. For molecular work, we selected one representative specimen of each species from one sampling site that was included in morphological trait analyses. Previous phylogenetic studies (Pramual et al., 2012; Pramual & Adler, 2014; Sriphirom et al., 2014) revealed that the specimens included in our analyses were from monophyletic species. In addition, we also found no significant morphological differentiation within species across different sampling sites (data not shown). Thus, we assumed that the specimens are representative of the species. Genomic DNA was extracted from the larvae following the method of Collins et al. (1987). Polymerase chain reaction (PCR) methods were conducted for

cytochrome *c* oxidase subunit I (COI) using primers LCO1490 (5'-GGTCAACAAATCATA AAGATATT GG-3') and HCO2198 (5'-TAAACTTCAGGGTGAC CAAAAATCA-3') (Folmer et al., 1994); cytochrome *c* oxidase subunit II (COII), using primers TL2-J-3034 (5'-ATTATGGCAGATTAGTGCA-3') and TK-N-3785 (5'-GTTTAAGAGACCAGTACTT G-3'); and 18S/ITS1, using primers 18 s/sd5' (5'-TG GTGCATGGCCGTTCTTAG-3') and 5.8 s/sd3' (5'-GTCGATGTTTCATGTGTCCTGC-3') (Simon et al., 1994). PCR conditions were as described by Conflitti et al. (2010). PCR products were checked with 1% agarose gel electrophoresis and cleaned using the HiYield Gel/PCR DNA Extraction Kit (RBC BIOSIENCE, Taiwan). Sequencing was performed at Macrogen (Seoul, Korea) using the same primers as in the PCR.

#### Phylogenetic analysis

DNA sequences for COI, COII, and 18S/ITS genes of 35 species were included in the phylogenetic analysis (Table S2). The combined dataset was 1875 bp (586 bp for COI, 697 bp for COII and 592 bp for 18 S/ITS1). Most (138) sequences were available from previous publications (Pramual et al., 2012; Pramual & Adler, 2014; Sriphirom et al., 2014). The remaining 34 sequences (Table S2) were obtained in this study. Phylogenetic relationships were analyzed by Bayesian methods using MRBAYES 3.04b (Huelsenbeck & Ronquist, 2001). The best-fit model for Bayesian analysis was selected by hierarchical likelihood ratio tests implemented in MrModeltest (Nylander, 2004). The general time-reversible (GTR) model was selected as the best fitting model. Bayesian analysis was run for 20,000,000 generations, with a sampling frequency of 100 generations. *Parasimulium crosskeyi* Peterson was used as an outgroup because the morphological evidence indicates that it is a member of the most primitive lineage of black flies (Adler et al., 2004).

#### Analytical framework of phylogenetic community structure

The phylogenetic community structure of black flies in Thailand was examined following the analytical framework of Cavender-Bares et al. (2004). Three sets of analyses are required to infer the mechanisms underlying the community structure. Firstly, the

hypothesis that co-occurring black fly species are more closely related than expected was tested. Secondly, labral fan morphology was used to test the hypothesis that co-occurring black fly species are more morphologically similar than expected (i.e., they are morphologically clustered). Then the phylogenetic signal of the labral fan morphology was examined. A combination of the results of these analyses was used to infer the mechanism underlying the community structure [see Fig. 2 of Cavender-Bares et al. (2004)] of black flies in Thailand. If the phylogenetic signal analysis indicates that the labral fan morphology is conserved (i.e., closely related species possess similar fan morphology or phylogenetic conservatism) and the co-occurring species are morphologically clustered, then habitat filtering is a primary factor determining community structure. However, if the co-occurring species are morphologically overdispersed, then the community structure will be dominated by competitive exclusion. If the phylogenetic signal analysis indicates convergence of the labral fan morphology and the co-occurring species are morphologically divergent, then the random process is the primary factor that governs community structure. Otherwise, if the co-occurring species are morphologically clustered, then habitat filtering is the primary mechanism determining the community structure (Webb et al., 2002; Cavender-Bares et al., 2004).

#### Phylogenetic community structure

The computer package Phylocom version 4.2 (Webb et al., 2008) was used to calculate mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD). MPD measures the mean pairwise phylogenetic distance between each taxon within the community. MNTD measures the average distance to the closest relative for each taxon. These two measures were standardized into a net relatedness index (NRI) and nearest related taxon index (NTI), respectively (Webb et al., 2002). The NRI and NTI describe the differences between phylogenetic distance in the observed and null communities. Positive values of both indexes indicate that species in the community were more phylogenetically related than by chance. Alternatively, negative values reflect that species are less phylogenetically related than by chance (Webb et al., 2002). High level of trait conservatism at a deep phylogenetic level will result

in high NRI and NTI. Conservatism at more terminal levels significantly increases NTI relative to NRI (Webb et al., 2002). The independent swap method was used to generate null communities by considering all species represented in the communities as the potential source pool. *T* tests were used to determine if the means of the NRI and NTI differed from zero (Ingram & Shurin, 2009; Razafindratsima et al., 2013), which demonstrates that the community is either clustering (positive) or overdispersed (negative).

#### Trait-based community structure

Phylocom was used to measure morphological trait dispersion within samples across the range of communities and species pool. The matrix from the mean pairwise trait distance among taxa (MPD) and mean distances to nearest-neighbor trait distance (MNTD) were used to measure trait dispersion of the community. Dispersion values in observed and null distributions were used with standardized effect size (SES) of the trait dispersion metrics relative to the null distribution communities (Weiher & Keddy, 1995; Webb et al., 2008). These values provided a signature of the trait clustering or evenness. Negative values for SES indicate trait clustering. Positive values of SES indicate overdispersion of the trait. We used *t* tests to determine whether that mean SES values across the community were significantly different from zero (Ingram & Shurin, 2009; Razafindratsima et al., 2013).

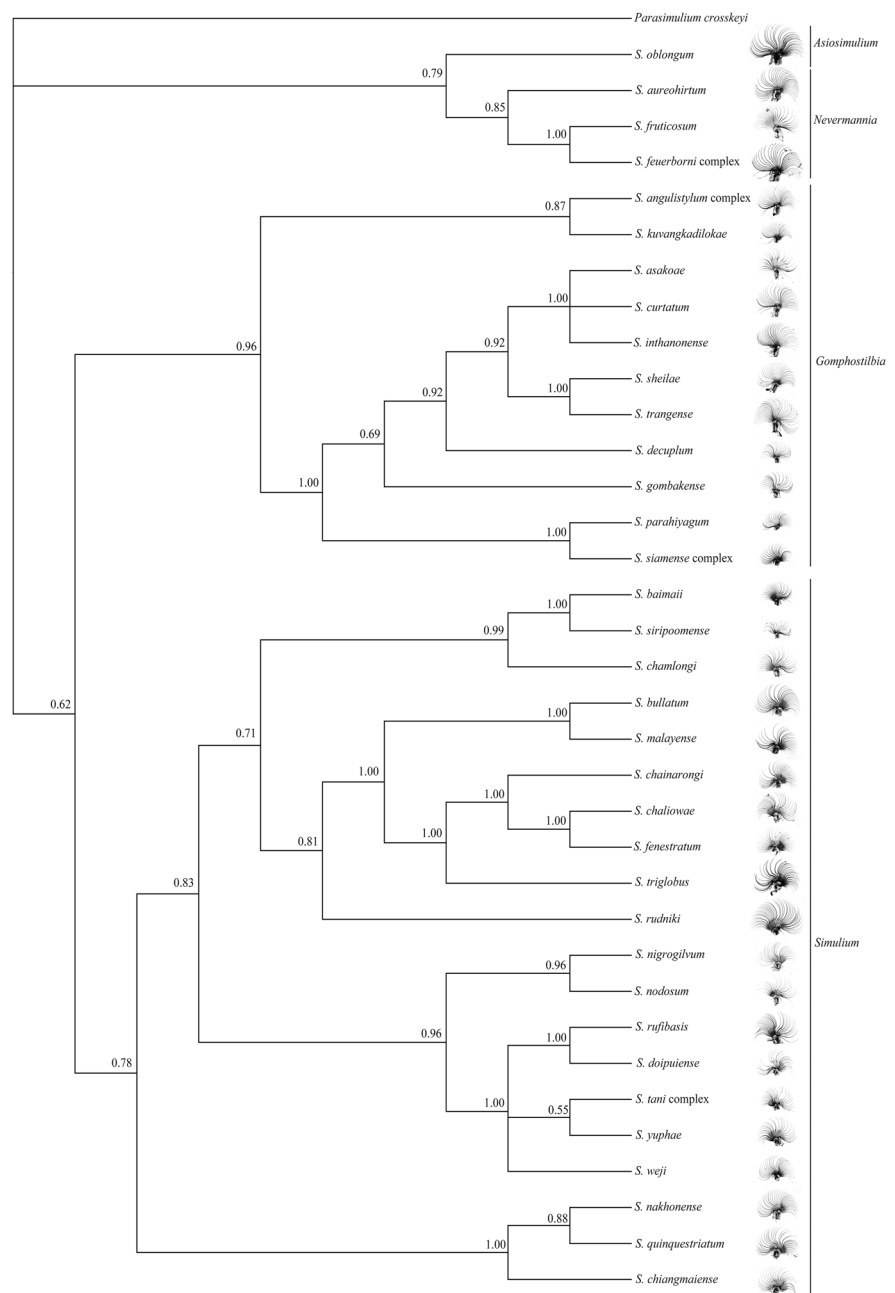
We also tested the phylogenetic signal of the labral fan morphology. The analysis of trait (aot) module in Phylocom was used to test the tendency for closely related species to share similar morphology (i.e., phylogenetic signal). The phylogenetic signal measured the magnitude of the trait variation of species relative to the phylogenetic position. If closely related species are more similar to each other, there will be small variance when compared to the expectation under the null model of randomly swapping trait values across the tree (Webb et al., 2008).

## Results

#### Phylogenetic relationships

The Bayesian tree of 35 black fly species included in the analysis revealed two main clades (Fig. 2). A

**Fig. 2** Bayesian tree for 35 species of black flies in Thailand based on 1875 bp sequences combined dataset of COI, COII, and 18S/ITS1 genes. Posterior probabilities for Bayesian analysis are shown above or near branches. Labral fan of each species was placed near tip of tree. Scale bar represent 200  $\mu\text{m}$



single species of the subgenus *Asiosimulium* (*Simulium oblongum* Takaoka & Choochote) formed a distinct clade with the members of subgenus *Nevermannia*. Subgenera *Gomphostilbia* and *Simulium* formed a second large clade that further divided into two subclades. The 11 species of the subgenus *Gomphostilbia* included in this study formed a monophyletic clade with strong (0.96) support. Members of

the subgenus *Simulium* (20 species) formed a monophyletic clade although with moderate (0.78) support.

#### Phylogenetic community structure

Black fly assemblages from a total of 150 sites were included in this study. There were 35 species recorded (Table S2). The number of species in each assemblage



**Table 2** Mean of morphological characteristics of primary labral fan for 35 species of black flies in Thailand

Species ( <i>n</i> ) <sup>a</sup>	Fan ray width (±SE) (μm)	Fan ray length (±SE) (μm)	Number of fan rays (±SE)
Subgenus <i>Asiosimulium</i>			
<i>S. oblongum</i> Takaoka & Choochote (62)	1516.94 (14.21)	762.90 (7.66)	42.63 (0.51)
Subgenus <i>Gomphostilbia</i>			
<i>S. angulistylum</i> Takaoka & Davies complex (42)	1032.14 (17.27)	539.29 (9.79)	33.50 (0.69)
<i>S. asakoae</i> Takaoka & Davies (66)	1202.27 (20.86)	603.41 (6.67)	37.80 (0.71)
<i>S. curtatum</i> Jitklang, Kuvangkadilok, Baimai, Takaoka & Adler (30)	1310.83 (17.23)	673.33 (9.66)	38.57 (0.36)
<i>S. decuplum</i> Takaoka & Davies (30)	924.14 (13.16)	488.79 (5.05)	29.86 (0.34)
<i>S. doipuiense</i> Takaoka & Choochote (30)	1116.67 (16.23)	583.33 (9.84)	36.97 (0.66)
<i>S. gombakense</i> Takaoka & Davies (30)	1037.93 (12.07)	532.76 (5.56)	40.97 (0.59)
<i>S. inthanonense</i> Takaoka & Suzuki (30)	1235.00 (21.73)	624.17 (10.75)	31.30 (0.56)
<i>S. kuvangkadilokae</i> Pramual & Tangkawanit (30)	820.00 (18.39)	421.67 (6.86)	33.30 (0.57)
<i>S. parahiyangum</i> Takaoka & Sigit (30)	880.00 (6.93)	441.67 (7.80)	26.73 (0.43)
<i>S. sheilae</i> Takaoka & Davies (30)	1146.55 (15.83)	547.41 (9.15)	40.48 (0.83)
<i>S. siamense</i> Takaoka & Suzuki complex (51)	1019.12 (15.04)	510.29 (7.71)	37.88 (0.83)
<i>S. trangense</i> Jitklang, Kuvangkadilok, Baimai, Takaoka & Adler (30)	1059.38 (11.14)	582.29 (11.70)	39.63 (0.53)
Subgenus <i>Nevermannia</i>			
<i>S. aureohirtum</i> Brunetti (55)	1262.27 (23.99)	619.55 (11.19)	37.22 (0.95)
<i>S. feuerborni</i> Edwards complex (30)	1298.33 (37.56)	701.67 (16.03)	35.70 (0.42)
<i>S. fruticosum</i> Takaoka & Choochote (30)	1410.00 (12.43)	739.17 (5.32)	26.40 (0.32)
Subgenus <i>Simulium</i>			
<i>S. baimaii</i> Kuvangkadilok & Takaoka (30)	1351.67 (18.76)	700.83 (7.34)	55.40 (0.48)
<i>S. bullatum</i> Takaoka & Choochote (30)	1387.50 (27.51)	706.67 (13.34)	46.13 (0.58)
<i>S. chainarongi</i> Kuvangkadilok & Takaoka (38)	1172.37 (12.85)	590.13 (6.83)	43.61 (0.59)
<i>S. chaliowae</i> Takaoka & Bookemtong (30)	1115.83 (10.75)	555.83 (7.55)	40.00 (0.22)
<i>S. chamlongi</i> Takaoka & Suzuki (30)	631.67 (11.37)	565.83 (26.76)	41.10 (0.48)
<i>S. Chiangmaiense</i> Takaoka & Suzuki (30)	1015.00 (26.39)	570.83 (11.50)	39.23 (0.41)
<i>S. fenestratum</i> Edwards (70)	1258.93 (17.45)	596.79 (9.58)	43.03 (0.71)
<i>S. malayense</i> Takaoka & Davies (10)	1081.82 (26.56)	545.45 (16.10)	43.91 (1.54)
<i>S. nakhonense</i> Takaoka & Suzuki (61)	1052.46 (12.20)	532.17 (5.67)	40.67 (0.85)
<i>S. nigrogilvum</i> Summers (30)	1187.50 (20.08)	592.50 (9.90)	39.20 (0.88)
<i>S. nodosum</i> Puri (30)	1133.33 (7.98)	573.33 (4.31)	37.00 (0.21)
<i>S. quinquestriatus</i> Shiraki (30)	1221.67 (25.90)	613.33 (16.11)	47.43 (1.16)
<i>S. rudnicki</i> Takaoka & Davies (30)	1310.00 (34.19)	695.83 (17.21)	46.40 (1.17)
<i>S. rufibasis</i> Brunetti (30)	1335.83 (9.79)	629.17 (7.00)	43.57 (0.54)
<i>S. siripoomense</i> Takaoka & Saito (5)	418.75 (11.97)	875.00 (0.00)	37.50 (0.29)
<i>S. tani</i> Takaoka & Davies complex (30)	1024.17 (20.04)	531.67 (10.38)	32.37 (0.98)
<i>S. triglobus</i> Takaoka & Kuvangkadilok (30)	1355.83 (21.40)	620.00 (12.48)	57.00 (0.37)
<i>S. weji</i> Takaoka (30)	1021.67 (16.86)	541.67 (14.35)	36.00 (0.58)
<i>S. yuphae</i> Takaoka & Choochote (30)	1066.67 (27.63)	555.83 (13.54)	39.87 (0.74)

<sup>a</sup> Number of larvae used for morphological measurement



**Table 3** Pearson correlation coefficients between labral fan morphological traits of 35 black fly species in Thailand

	Fan ray width	Fan ray length	Number of fan ray
Fan ray width	–		
Fan ray length	0.632*	–	
Number of fan ray	0.431*	0.317*	–

\*  $P < 0.01$ 

However, the fan ray length had significant conservatism across the phylogeny ( $P = 0.03$ ).

## Discussion

Our results indicate that the community structure of black flies in tropical streams of Thailand was not skewed toward phylogenetic clustering or overdispersion. Phylogenetic community structure analysis revealed similar proportions in the communities that show signals of phylogenetic clustering and overdispersion. In contrast, the trait-based community structure analysis found that black fly species assemblage was largely determined by habitat filtering mechanisms. Members of the black fly species in most (>78%) communities are more similar in labral fan morphology than would be expected from the null community. The results are counter intuitive as it has been suggested that closely related species usually possess similar morphological characteristics (Webb et al., 2002; Emerson & Gillespie, 2008). Therefore, phylogenetic clustering was expected with the habitat filtering. However, habitat filtering does not always produce phylogenetic clustering if the trait under study is governed by convergent evolution (Losos et al.,

2003; Cavender-Bares et al., 2004; Kraft et al., 2007). Previous studies found that black fly labral fan morphology was not related to the species phylogenetic relationships (Zhang & Malmqvist, 1996; Palmer & Craig, 2000; Joy & Conn, 2001). The results of our phylogenetic conservatism analysis for fan ray width and number of fan ray support the previous findings and indicate that convergent evolution could be a factor responsible for the species' differences in these labral fan traits.

Non-phylogenetic conservatism of the labral fan morphology implies that closely related species could have differences in this trait. Differences in labral fan morphology allow closely related species to exploit different habitats, thus it could be a factor promoting species divergence (Zhang & Malmqvist, 1996; Joy & Conn, 2001). For example, two closely related species of the subgenus *Gomphostilbia* in Thailand, *Simulium inthanonense* Takaoka & Suzuki and *S. curtatum* Jitklang, Kuvangkadilok, Baimai, Takaoka & Adler, occur in high elevation habitats (>1000 m above sea level), but the two species utilized streams with different velocities (0.49 m/s for *S. curtatum* and 0.23 m/s for *S. inthanonense*) (Pramual et al., 2012). Comparisons of the labral fan morphology indicated significant differences between the two species (fan ray length,  $t = 2.734$ ,  $P = 0.008$ ; fan ray width,  $t = 3.401$ ,  $P = 0.001$ ; number of fan rays,  $t = 10.914$ ,  $P < 0.001$ ). The smaller labral fan in *S. curtatum* is consistent with the prediction that this species occupies faster streams. As these species are closely related according to molecular evidence (Pramual et al., 2012), the differences in larval habitats due to morphological divergence (or vice versa) could possibly be a driving force of speciation in black flies (Joy & Conn, 2001; Pramual et al., 2012).

**Table 4** Pearson correlation coefficients between morphological traits of 35 black fly species and stream variables from 150 sampling sites in Thailand

Morphological trait	Stream variable								
	Width	Depth	Velocity	Discharge	Elevation	Conductivity	Streambed particle	Canopy cover	Riparian vegetation
Fan ray width	−0.131*	−0.139*	−0.091*	−0.161*	0.305*	0.020	0.025 <sup>a</sup>	−0.006 <sup>a</sup>	0.126 <sup>*,a</sup>
Fan ray length	−0.147*	−0.064	−0.095*	−0.133*	0.236*	0.019	−0.021 <sup>a</sup>	−0.010 <sup>a</sup>	0.090 <sup>*,a</sup>
Number of fan ray	0.032	−0.161*	0.162*	−0.149*	0.201*	0.118*	0.189 <sup>*,a</sup>	0.062 <sup>a</sup>	0.206 <sup>*,a</sup>

\*  $P < 0.01$ <sup>a</sup> Spearman's rank correlation

Previous studies as well as our data have shown that labral fan morphology is correlated with stream velocity (Zhang & Malmqvist, 1996; Malmqvist et al., 1999; Palmer & Craig, 2000), and it has been suggested that stream velocity is one of the most important factors determining black fly species distribution (Palmer & Craig, 2000). Shared morphological characteristics related to habitat usage indicate that environmental conditions are the major factors that select species into a community (i.e., habitat filtering). Thus, stream velocity could be primarily an environmental factor filtering black fly species in the community. Our finding that habitat filtering is a primary mechanism determining black fly species assemblage was consistent with several previous studies. Pramual & Wongpakam (2010) found that species that require similar ecological niches co-exist in several communities in tropical streams of northeastern Thailand. Environmental factors associated with black fly assemblage have also been reported in other geographic areas such as Europe and North America. Malmqvist et al. (1999) found that species assemblages of black fly larvae in 56 Swedish streams were significantly associated with stream variables such as stream width, depth, velocity, and streambed particle size. McCreadie & Adler (2006) found that species assemblages of North American black flies were determined primarily by ecological factors such as pH, stream velocity, and streambed particle size.

In conclusion, the results suggest that environmental conditions in the habitat play an important role in black fly community assemblages. Thus, habitat filtering is likely to be the most important mechanism determining black fly community structure in Thailand. Nonetheless, our sampling regimes restricted us to analyzing data that could only be used to examine the spatial aspect of the community. However, the temporal dynamics of black fly species assemblages has also been reported (Pramual & Wongpakam, 2010; McCreadie & Adler, 2012) and further studies should consider including temporal factors. Our results also indicate that morphological divergence among the closely related species was related to habitat exploitation and it could be the factor promoting speciation. Therefore, integrating phylogenetic and morphological characters of the closely related species could be used to test this hypothesis in a future study.

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