

# **Species Diversity of Black Fly in Southeast Asia: Phylogenetic Analysis of Three Subgenera, *Asiosimulium*, *Daviesellum*, and *Wallacellum*, of the Genus *Simulium* s. l.**

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## **Abstract**

Black flies (Diptera: Simuliidae) are distributed worldwide. The biting habits of black flies are known to cause medical and veterinary problems including transmissions of filarial diseases of humans and animals. In Southeast Asia (Thailand, Malaysia, Philippines, and Indonesia), eight subgenera (*Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Montisimulium*, *Morops*, *Nevermannia*, *Simulium* s. str., and *Wallacellum*) of genus *Simulium* s. l. are known. Of these subgenera, three subgenera (*Asiosimulium*, *Daviesellum*, and *Wallacellum*) are small, endemic only in the Oriental region (except one species of *Wallacellum*), and recently established. To confirm the subgeneric status, phylogenetic analysis was conducted using mitochondrial 16S rRNA sequences. As results, the three subgenera were clearly separated from other subgenera. Their subgeneric status was confirmed both by phylogenetic analysis and morphological characters.

**Keywords:** *Asiosimulium*, black fly, *Daviesellum*, phylogeny, *Simulium*, *Wallacellum*

## **Introduction**

In a revision of supraspecific taxa of the genus *Simulium* Latreille s. l. which includes about 10 subgenera in the Oriental region, two subgenera: *Nevermannia* Enderlein and *Himalayum* Lewis have already been evaluated by phylogenetic analysis of mitochondrial 16S ribosomal RNA (rRNA) gene sequences (OTSUKA *et al.* 2001, 2003). Genus *Simulium* s. l. is the largest genus in the family Simuliidae, having 1,745 species in the 37 subgenera (ADLER and CROSSKEY 2014). Among these, ten subgenera are reported from the Oriental region. Moreover, in Southeast Asia (Thailand, Malaysia, Philippines, and Indonesia), eight subgenera (*Asiosimulium* Takaoka and Choochote, *Daviesellum* Takaoka and Adler, *Gomphostilbia* Enderlein, *Montisimulium* Rubtsov, *Morops* Enderlein, *Nevermannia*,

*Simulium* Latreille s. str., and *Wallacellum* Takaoka) are known (Table 1). Of these subgenera, three subgenera (*Asiosimulium*, *Daviesellum*, and *Wallacellum*) are small, endemic only in the Oriental region (except one species of *Wallacellum*), and recently established (Fig. 1). To confirm the subgeneric status, phylogenetic analysis was conducted using mitochondrial 16S rRNA sequences.

Table 1. Number of black flies in countries of Southeast Asia.

Genus	Subgenus	Thailand	Malaysia	Philippines	Indonesia
<i>Simulium</i>	<i>Asiosimulium</i>	3	0	0	0
	<i>Daviesellum</i>	2	1	0	0
	<i>Gomphostilbia</i>	23	26	33	44
	<i>Montisimulium</i>	6	0	0	0
	<i>Morops</i>	0	0	0	26
	<i>Nevermannia</i>	2	7	4	8
	<i>Simulium</i>	43	31	34	40
	<i>Wallacellum</i>	0	0	14	2

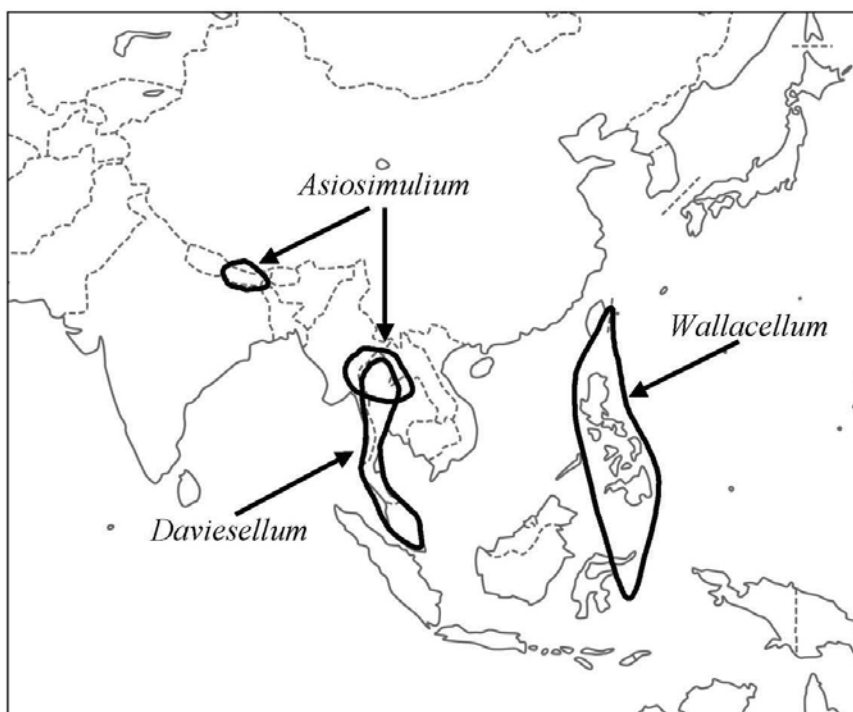


Fig. 1. Distribution of three subgenera, *Asiosimulium*, *Daviesellum*, and *Wallacellum*.

## Materials and Methods

Phylogenetic analysis was conducted using the 16S mitochondrial sequences of 28 species (OTSUKA *et al.* 2001, 2003, 2007) (Table 2). The sequences were aligned by the program CLUSTAL W, and the alignment was then adjusted manually. Sites containing alignment gaps were excluded in the following analysis. The number of nucleotide substitution per site was estimated between each pair of the sequences, using Jukes-Cantor methods. Construction and bootstrap probability estimation of the neighbor-joining tree were performed by MEGA6 (TAMURA *et al.* 2013).

Table 2. Black fly species used for the phylogenetic analysis.

Species	Locality	Accession number
<i>Simulium (Asiosimulium) oblongum</i>	Muk Da Han, Thailand	AB334089
<i>S. (Daviesellum) courtneyi</i>	Doi Phu Kha, Thailand	AB334090
<i>S. (D.) pahangense</i>	Peninsular Malaysia	AB334091
<i>S. (Gomphostilbia) palauense</i>	Palau	AB056742
<i>S. (G.) whartoni</i>	Peninsular Malaysia	AB056743
<i>S. (Hellichella) chiharuuae</i>	Kyoto, Japan	AB334092
<i>S. (Montisimulium) kobayashii</i>	Mikurajima, Japan	AB334093
<i>S. (Mon.) merga</i>	Doi Inthanon, Thailand	AB334094
<i>S. (Morops) farciminis</i>	Irian Jaya, Indonesia	AB056744
<i>S. (Nevermannia) aureohirtum</i>	Peninsular Malaysia	AB056736
<i>S. (N.) feuerborni</i>	Peninsular Malaysia	AB056729
<i>S. (N.) ornatipes</i>	Irian Jaya, Indonesia	AB056737
<i>S. (N.) uchidai</i>	Oita, Japan	AB056740
<i>S. (Simulium) eximium</i>	Flores, Indonesia	AB093100
<i>S. (S.) malayense</i>	Peninsular Malaysia	AB093112
<i>S. (S.) nobile</i>	Peninsular Malaysia	AB093115
<i>S. (S.) tani</i>	Peninsular Malaysia	AB093123
<i>S. (Wallacellum) cabrerai</i>	Luzon, Philippines	AB093128
<i>S. (W.) carinatum</i>	Luzon, Philippines	AB093129
<i>S. (W.) celebesense</i>	Sulawesi, Indonesia	AB334095
<i>S. (W.) claveriaense</i>	Luzon, Philippines	AB334096
<i>S. (W.) recurvum</i>	Luzon, Philippines	AB334097
<i>S. (W.) spinosibranchium</i>	Luzon, Philippines	AB334098
<i>S. (W.) suyoense</i>	Luzon, Philippines	AB334099
<i>S. (W.) tuyense</i>	Luzon, Philippines	AB334100
<i>S. (W.) yonakuniense</i>	Yonakuni, Japan	AB334101
<i>Austrosimulium bancrofti</i>	Australia	AB093130
<i>Prosimulium kiotoense</i>	Oita, Japan	AB056747

The sequences were determined in OTSUKA *et al.* (2001, 2003, 2007).

## Results and Discussions

Sequences of the mitochondrial 16S rRNA gene region of the 26 species of genus *Simulium* s. l. were aligned with the two species from other genera as outgroup (Table 2). To reveal the evolutionary relationship among the species of the genus *Simulium* s. l., a neighbor-joining tree was made (Fig. 2). The tree shows that all subgenera, except for subgenus *Nevermannia*, occur in a clade, and supports the independence of the subgenera *Asiosimulium*, *Daviesellum*, and *Wallacellum*, which have been recently established. OTSUKA *et al.* (2001) reported that *ruficorne* species-group of *Nevermannia*, which includes *S. aureohirtum* Brunetti and *S. ornatipes* Skuse, was genetically and morphologically different from the other species-groups, and suggested that revision of the definition of *Nevermannia* was needed.

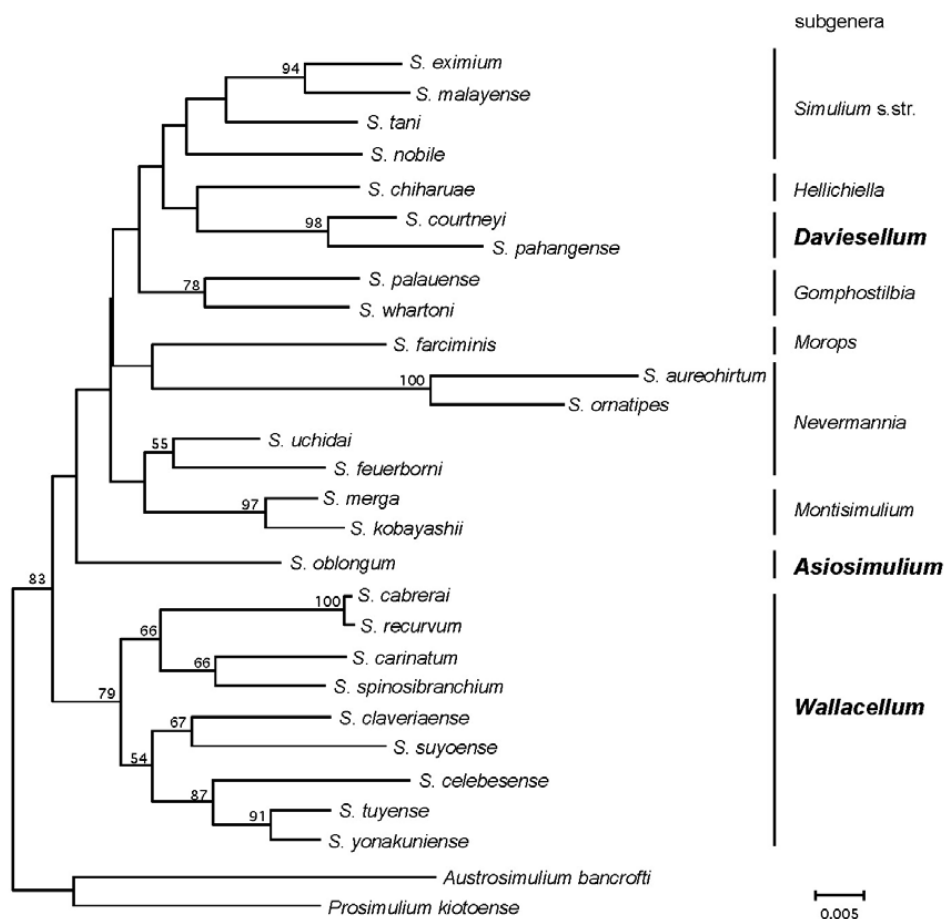


Fig. 2. Phylogenetic tree obtained by neighbor-joining method for a partial mitochondrial 16S rRNA sequence of 28 black fly species. The values at branch points indicate the percentage support for a particular node after 1,000 bootstrap replicates were performed. Values less than 50% are not shown.

*Asiosimulium* was recently established based on a unique species, *S. (A.) oblongum* Takaoka and Choochote, found in Thailand, and was reported to have more similarities morphologically to three subgenera, namely: *Boreosimulium* Rubtsov and Yankovsky in the Holarctic Region, *Inseliellum* Rubtsov in Micronesia and Polynesia, and *Nevermannia*, a cosmopolitan heterogeneous taxon (TAKAOKA and CHOOCHOTE 2005). *Daviesellum* is also a small subgenus, represented by only two species, *S. (D.) pahangense* Takaoka and Davies, and *S. (D.) courtneyi* Takaoka and Adler, collected from Peninsular Malaysia and Thailand, and has distinctive genitalia in both sexes, suggesting no close relationship to other subgenera of the genus *Simulium* s. l. (TAKAOKA and ADLER 1997). On the other hand, *Wallacellum*, represented by 11 species mostly from the Philippines, seems to have a close relationship to an Australasian subgenus *Morops* by having hairs on both katepisternum and pleural membrane (TAKAOKA 2003). However, the phylogenetic tree in this study did not show a close relationship between *Wallacellum* and *Morops*. Phylogenetic analysis of black flies using the mitochondrial 16S rRNA region can be a tool in determining the identity of the subgenus of *Simulium* s. l., but does not seem to resolve relationships among the subgenera due to low bootstrap values. More information on other genes as well as cytogenetic study is needed to clarify the relationships among subgenera of *Simulium* s. l.

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