Phylogenetic analysis of three subgenera: *Asiosimulium*, *Daviesellum* and *Wallacellum*, of the genus *Simulium* s. l. endemic in the Oriental Region

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Abstract: The subgenera *Asiosimulium*, *Daviesellum*, and *Wallacellum* of the genus *Simulium* s. l. are endemic to the Oriental Region. Their subgeneric status was confirmed by phylogenetic analysis using the mitochondrial 16S ribosomal RNA gene sequences.

Key words: *Simulium*, black fly, *Asiosimulium*, *Daviesellum*, *Wallacellum*, phylogeny

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 and 2003). Using the same method, we phylogenetically studied three relatively small subgenera of *Simulium* s. l., i.e., *Asiosimulium* Takaoka and Choochote, *Daviesellum* Takaoka and Adler, and *Wallacellum* Takaoka, all known to be endemic to the Oriental Region (except for one species of *Wallacellum* found in the Australasian Region) (Takaoka, 2003; Takaoka and Choochote, 2005).

The results of our phylogenetic analysis of these three subgenera confirm their subgeneric status and suggest the possible relationships among these three and other subgenera in the Oriental Region.

MATERIALS AND METHODS

Black fly species used in this study were listed in Table 1. DNA extraction, polymerase chain reaction, sequencing and phylogenetic analysis were done as described previously by Otsuka et al. (2003). Primer A (5'-CGCCTGTTTATC-AAAAACAT-3') and primer B (5'-CTCCG-GTTTGAAGTCAGATC-3') were used to amplify the partial mitochondrial 16S rRNA gene region (Xiong and Kocher, 1991). Newly determined sequences were deposited into GenBank/EMBL/DDBJ under accession number AB334089-AB334101.

RESULTS AND DISCUSSION

Sequences of the mitochondrial 16S rRNA gene region of 13 species of genus *Simulium* s. l. were determined and aligned with sequences already reported for 15 other species of black flies (Table 1, Fig. 1). To reveal the evolutionary relation-

Table 1. Black fly species used in this study.

Species	Locality	Accession number
<i>Simulium</i> (<i>Asiosimulium</i>) <i>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. (Hellichiella) chiharuae</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.) suyoenae</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

* Newly determined sequences.

ship 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* and *S. ornatipes*, was genetically and morphologically different from the other species-groups, and suggested that revision of the definition of *Nevermannia* was needed.

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 sub-

genera, namely: *Boreosimulium* Rubtsov and Yankovsky in the Holarctic Region, *Inseliellum* Rubtsov in Micronesia and Polynesia, and *Nevermannia* Enderlein, 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* Enderlein by having hairs on both katapisternum and pleural membrane

[illegible]

Fig. 1. Variable sites detected in the mitochondrial 16S rRNA region among the 28 black fly species. The nucleotides identical to the sequence of *S. oblongum* are represented by dots. Dashes mean gap sites.

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.

(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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REFERENCES

- Otsuka, Y., Aoki, C., Suzuki, H., Saito, K., Hadi, U. K. and Takaoka, H. 2001. Phylogenetic analyses of a blackfly subgenus *Simulium* (*Nevermannia*) based on mitochondrial 16S ribosomal RNA gene sequences. *Jpn. J. Trop. Med. Hyg.*, 29: 261–266.
- Otsuka, Y., Takaoka, H., Aoki, C. and Choochote, W. 2003. Phylogenetic analysis of the subgenus *Himalayum* within the genus *Simulium* s. l.

- (Diptera: Simuliidae) using mitochondrial 16S rRNA gene sequences. *Med. Entomol. Zool.*, 54: 113–120.
- Takaoka, H. 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. xxii+581 pp., Kyushu University Press, Fukuoka.
- Takaoka, H. and Adler, P. H. 1997. A new subgenus, *Simulium* (*Daviesellum*), and a new species, *S. (D.) courtneyi* (Diptera: Simuliidae) from Thailand and Peninsular Malaysia. *Jpn. J. Trop. Med. Hyg.*, 25: 17–27.
- Takaoka, H. and Choochote, W. 2005. A new subgenus and a new species of *Simulium* s. l. (Diptera: Simuliidae) from Thailand. *Med. Entomol. Zool.*, 56: 33–41.
- Xiong, B. and Kocher, T. D. 1991. Comparison of mitochondrial DNA sequences of seven morpho-species of black flies (Diptera: Simuliidae). *Genome*, 34: 306–311.