# CHAPTER 1

# Introduction

## 1.1 Statement and Significance of the Problem

Black flies (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. This family is one of the most important medical and economic groups among blood-sucking insects. Some species can transmit pathogens to humans, livestock and poultry, and bites from adult females cause a wide range of problems for humans and animals. To date, the study of black files has advanced markedly, especially in the systematics of Simuliidae, including cytotaxonomy, molecular taxonomy and phylogeny. In addition, several studies have been performed on the salivary glands (SGs) of black flies. SGs are of interest because they produce various secreted components in saliva, which help black flies in blood-feeding and transmitting pathogens during blood meals. Also, their immunogens may cause allergic reactions to humans and animals. However, only a few ecological studies have been carried out on this family.

Between 1984 and 2012, at least 88 species of black-fly fauna, comprising 6 subgenera in the genus *Simulium* Latreille s.l. (*Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Montisimulium*, *Nevermannia* and *Simulium* s. str.) were reported so far from 34 provinces across 6 regions (northern, southern, central, northeastern, eastern and western) of Thailand. Among these, 12 species (*Simulium asakoae*, *Simulium barnesi*, *Simulium chamlongi*, *Simulium doipuiense*, *Simulium fenestratum*, *Simulium maenoi*, *Simulium manooni*, *Simulium mediocoloratum*, *Simulium nakhonense*, *Simulium nigrogilvum*, *Simulium nodosum* and *Simulium rufibasis*) have been incriminated as man-biting, of which only 4 were ranked as main human-biters that seemingly exist in species-specific localities. Based on the recovery of infective stages ($L_3$ larvae) of filarial worm from wild-caught females, 2 species, i.e., *S. nigrogilvum* and

*S. nodosum*, were incriminated recently as natural vectors of *Onchocerca* spp., which can probably cause zoonotic filarial infection in the Thai population, and this was reported for the first time in Thailand and/or the Southeast Asian region. Remarkably, there is no report on the seasonal biodiversity of black flies or the evaluation of ecological factors influencing their distribution in mountainous regions of northern Thailand, despite these areas being very significant. Since most new Thai black-fly species (approximately 80 species) have been discovered in these areas, it is presumed that these diverse microhabitats are very important in supporting black-fly diversity. In addition, anthropophilic black-fly species are found to occur in large numbers, causing irritation to indigenous people, tourists and domestic environments.

Although the above information reflected very rich species diversity, pattern of distribution, and medical and/or veterinary significance of Thai black flies from 34 provinces across 6 regions of Thailand; much still needs to be studied in the remaining provinces, particularly concerning the following 2 aspects. (1) Diversity and distribution characteristics of seasonal black flies should be researched intensively and systematically in relation to various environmental factors regarding each representative province, since no such data have been available, except for two national parks (Doi Inthanon and Doi Suthep-Pui) in northern Thailand. (2) For a reliable representative model, detailed ecological factors that influence black-fly species diversity should be studied in a specific location, where richness of biodiversity is still conserved, such as Thailand's second highest mountain in Doi Phahompok National Park, Chiang Mai, northern Thailand.



Doi Phahompok National Park contains the second highest mountain in Thailand (altitude 2,285 m) and covers 524 km² of mountainous terrain in the Daen Lao Range on the Thai-Myanmar border. It has a wide range of mountainous peaks with heights of 500 m to 2,285 m from the foot to the summit. It also is characterized by a diverse plant community and various microhabitats, especially in both seasonal and permanent running streams, which are presumed to support species diversity and abundance of black flies. However, little is known about the environmental factors influencing the diversity and distribution pattern of black flies in this National Park.

The reasons for selecting Doi Phahompok National Park as a reliable representation depend upon preliminary surveys at 6 altitudes in this location (i.e., 2,100 m: Doi Phahompok; 2,000 m: Kiewlom; 1,500 m: Ban pu-meun; 1,400 m: Banlek; 700 m: Huai bon; and 500 m: Huai ma-yom), which clearly show richer species diversity than the other 2 areas (Doi Inthanon and Doi Suthep-Pui) in terms of both numbers of species composition and diversity of microhabitats. These, in particular, are unique microhabitats that favor conditions for the existence of rare species [e.g., 1,900-2,500 m: *Simulium merga*, *Simulium surachaii*, *Simulium phahompokense*, *Simulium angkaense*, *Simulium suchariti*; 1,400-1,800 m: *Simulium laoleense*, *S. manooni*, *Simulium fangense*, *Simulium maeaiense*; 500-1,300 m: *Simulium inthanonense, Simulium courtneyi, Simulium chomthongense*; <500 m *Simulium chiangdaoense*] and/or endemic species [e.g., 500-1,300 m: *Simulium wanchaii* and *Simulium furvum*]. The richness in species diversity at 6 different altitudes from high to low at this location was used to compare with complementary altitudes of other locations across 6 regions.

It is expected that much data obtained from this study achieved a better understanding of species diversity of black-fly fauna in Thailand, when relating to changes of certain natural environmental factors. Also, the data explained fulfillment of the fauna itself and patterns of species distribution for further insight into the mechanism that functions for species assemblages. All this information is necessary for future studies of population-genetic structures, roles of black flies as disease vectors and/or vicious biters to humans and animals, and the formation of reliably effective control measurements at specific locations and/or across a comparatively wide-range geography.



## 1.2 Literature Review

### 1.2.1 Classification and distribution

Black flies (Diptera: Simuliidae), also called buffalo gnats, coffee flies, Turkey gnats, jejen, and Kriebelmücken, are small vicious blood-sucking insects. They are classified into the Kingdom Animalia, Phylum Arthropoda, Class Insecta, Order Diptera, Suborder Nematocera (or Eudiptera) and Family Simuliidae. The family Simuliidae is classified into two subfamilies: the Parasimuliinae, comprising five evolutionarily basal, non blood-sucking black flies endemic to the forest of North America's Pacific Northwest, and the Simuliinae, accounting for the vast remainder of the world's species. There are 2 tribes: the Prosimuliini and Simuliini, within the Simuliinae. The largest and most widely distributed genus is *Simulium* with nearly 1,800 species arranged in 40 subgenera (Adler and Crosskey 2014). Throughout the world, at least 2,163 formally named species (2,151 living and 12 fossil species) comprising 26 genera of black flies have been discovered, and only 4 genera (*Austrosimulium*, *Cnephia*, *Prosimulium* and *Simulium*) were reported to be man-biting and economically important (Service 1980; Adler and Crosskey 2014). The number of black-fly species is greater in temperate areas than in tropical ones (Adler 2005). The Simuliidae are rich in species complexes and most of them are recognized by using the polytene chromosome from their larval salivary glands. For example, the African black fly, *Simulium damnosum* complex comprises about 40 sibling species (Adler 2005). Regarding the Oriental region, at least 410 black-fly species of the genus *Simulium* Latreille s.l., which is the largest group of the world's fauna in this family, have been discovered. Among these, at least 18 species have been incriminated as anthropophilic black flies, such as, *Simulium indicum*, which is distributed widely at the southern foot of the Himalayas, from Pakistan in the west, through India and Nepal, to Myanmar and southern China in the east (Lewis 1974; Datta 1992); *Simulium asishi*, *Simulium himalayense*, *S. nodosum* and *Simulium tenuistylum* in India (Datta 1992); and *Simulium japonicum* in the Ryukyu Islands, Japan (Takaoka 1977).

### 1.2.2 General morphology



Simuliid eggs are ovoid or elliptical and somewhat triangular in general shape, with a length of 0.1-0.5 mm. Their surface is a smooth unsculptured shell covered by a sticky, gelatinous matrix with few conspicuous external details. The eggs are cream colored when first deposited, but darken as the embryo matures (Adler 2005). Examples of *Simulium aureohirtum* egg mass on leaves are shown in Figure 1.1.

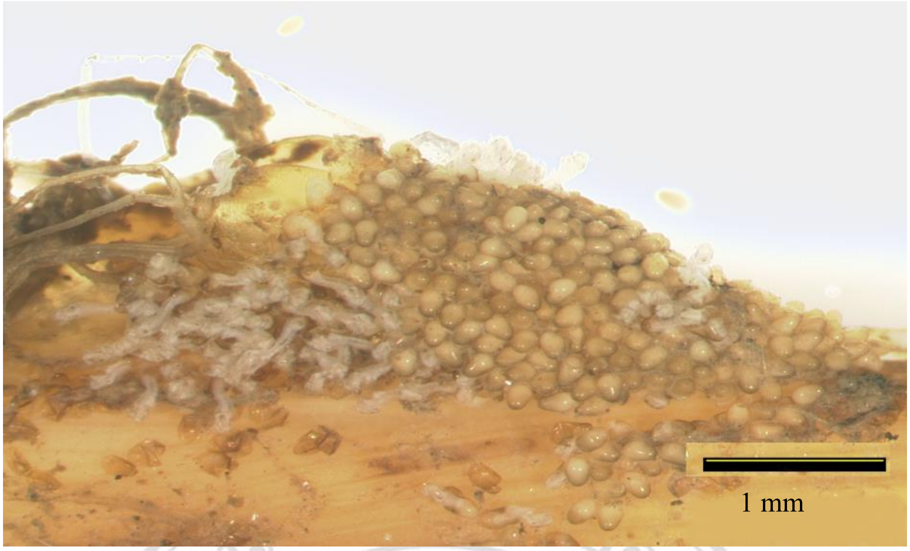


**Figure 1.1** Cluster of *Simulium aureohirtum* eggs (Srisuka 2007)

Adult black flies are quite small, at about 1.5-4 mm long, with stout bodies and a humped thorax. The adult is recognized by its wings, antennae and abdominal base. The wings are short and broad, and have tubular veins only toward the leading edge (Figure 1.2D). Eye facets on the upper half of the head are larger than those on the lower half in the male, but they are the same in females. The antennae are short and cylindrical, consisting of the scape, pedicel and 7-9 flagellomeres (Figure 1.2A). The first abdominal segment has been modified to form a prominent flange (called the basal scale), fringed with fine hairs (Figure 1.2B) (Takaoka and Davies 1995; Takaoka 2004).



The pupa is enclosed in a case (cocoon), with a pair of spiracular gills on the thorax (Figure 1.3G), and a unique arrangement of hooks on the abdomen (Figure 1.3I, J). The pattern of hooks comprises a transverse row of four hooks per side positioned dorsally on segments 3 and 4, and two hooks per side placed ventrally on segments 5-7. One hook per side on segments 6 and 7 is a rarity. The larva possesses two pseudopods (called the thoracic proleg and posterior circlet) (Figure 1.3K) and a pair of large cephalic (or labral) fans on the head (Figure 1.3L), although the latter characteristic is absent or reduced in some species (Takaoka and Davies 1995; Takaoka 2004). The larva, pupa and adult stage of *Simulium atipornae* are shown in Figure 1.4.

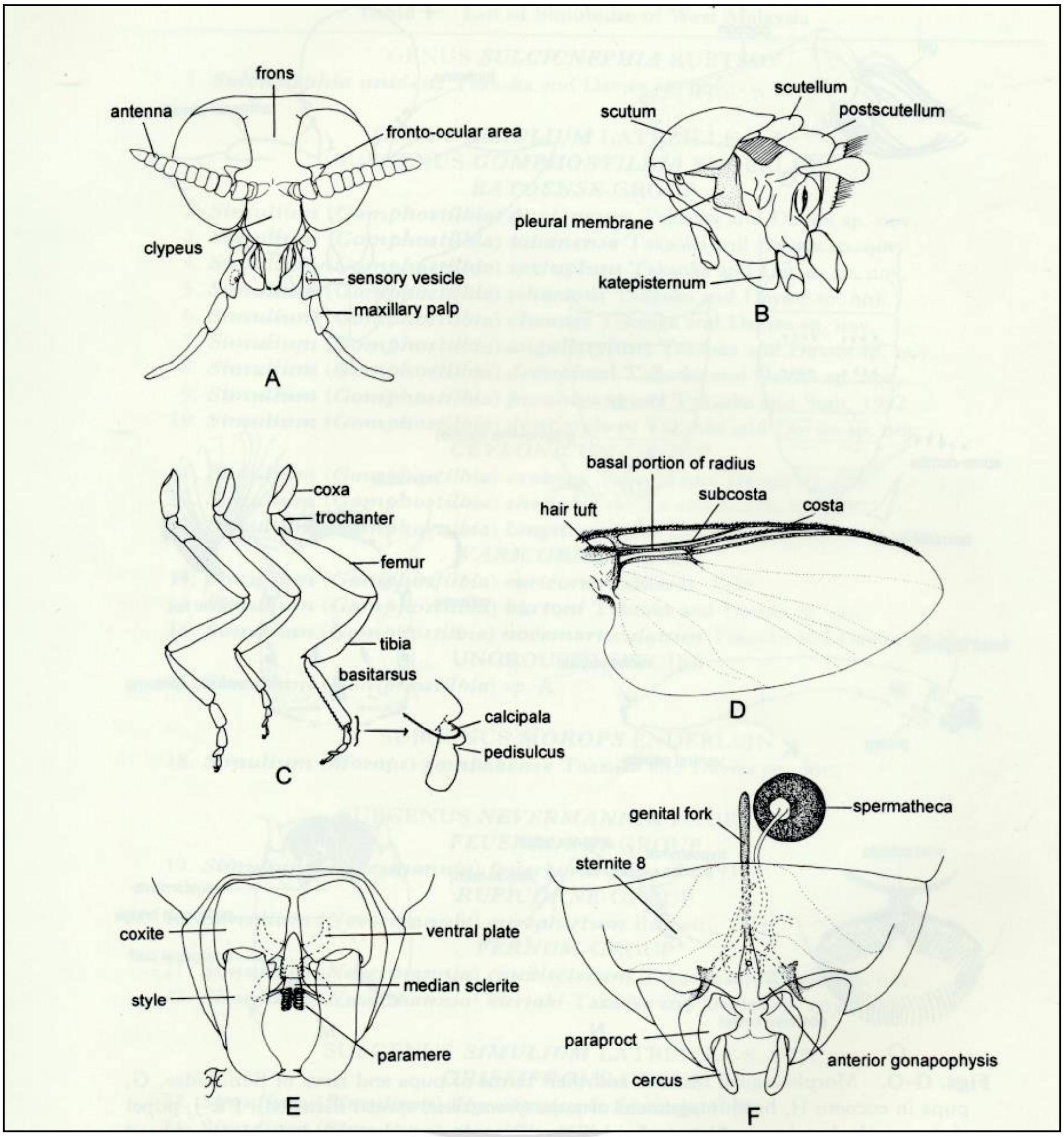


**Figure 1.2** Morphological features and their terms of adult *Simulium*. A, female head (front view); B, thorax (side view); C, female legs (fore, mid and hind legs from left); D, Wing; E, male genitalia (ventral view); F, female (ventral view) (Takaoka and Davies 1995)

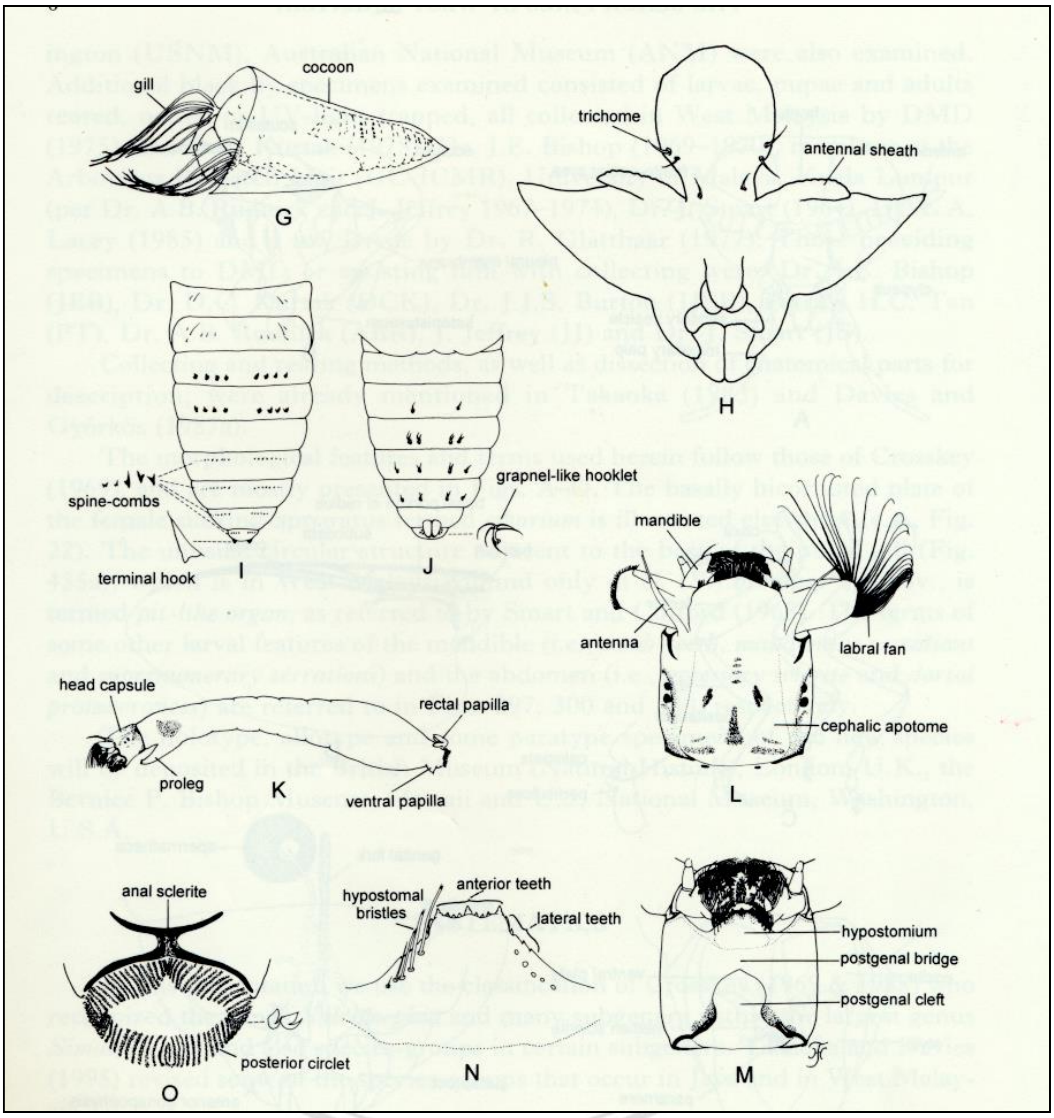


**Figure 1.3** Morphological features of Simuliidae and their terms of pupa and larva. G, pupa in cocoon; H, head integument of pupa (front view, spread flattened); I, pupal abdomen (dorsal view): J, pupal abdomen (ventral view); K, mature larva (side view); L, laval head capsule (dorsal view); M, larval head capsule (ventral view); N, hypostomium; O, posterior tip of the larval abdomen (posterodorsal view) (Takaoka and Davies 1995)

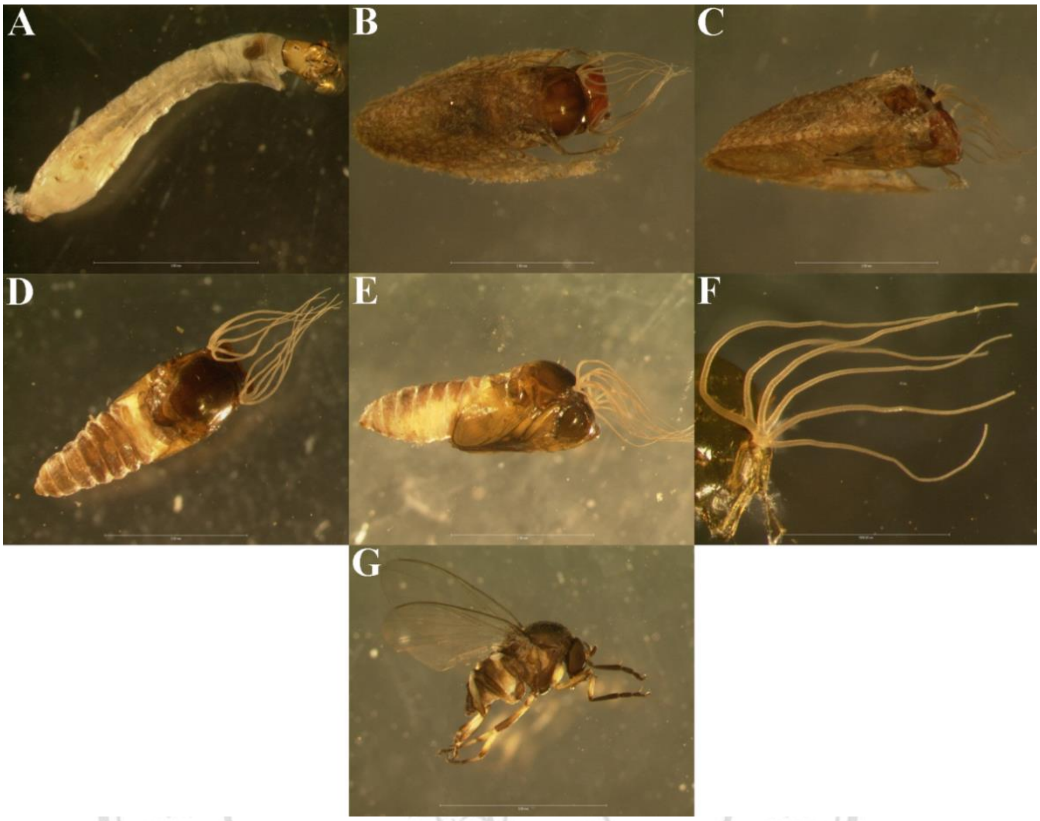

**Figure 1.4** Morphological features of *Simulium atipornae*. A mature larva (lateral view); B, pupa in cocoon (dorsal view); C, pupa in cocoon (lateral view); D, pupa (dorsal view); E, pupa (lateral view); F, gill filaments (right side, outer view); G, adult (lateral view). Scale bars: 1 mm for F; 2 mm for B-E; 3 mm for A and G (Takaoka et al. 2014c)

### 1.2.3 Life cycle

The length of life cycle in black flies varies with species and environmental conditions. Their life cycle is a complete metamorphosis or holometabolous (Figure 1.5). Females lay their eggs after mating in a variety of lotic environments ranging from trickles of water and pond outlets to large rivers. An average of 200-500 eggs is laid per oviposition. Various species oviposit in different ways, ranging from a free distribution of eggs, in which the female taps her abdomen on the water's surface during flight, or oviposits while landed on wet surfaces such as stones, rocks, or grasses trailing in the water, to crawling underwater to deposit her eggs. Incubation time differs from 2 days to 6 months depending on the species and water temperature. Larvae may remain at the site of hatching if the substrate and food supply are adequate, though they may move around in a looping action. This involves their posterior circlet, which is a single stout proleg (Figure 1.3K) on the first thoracic segment, and an expression of 'silken' threads is secreted from their silk glands onto the substrate. Alternatively, they may drift downstream on 'silken' threads in order to find suitable sites. Larvae pass through a series of 4-9 stages (average 7 stages) before reaching pupal stage. Growth rates fluctuate with water temperature and the quality and quantity of available food. Most larvae with labral fans are filter feeders. The mature larva is about 5-13 mm long, according to species, and can be recognized by a blackish mark termed the gill spot (or respiratory organ of the future pupa) on each side of the thorax (Figure 1.6). Larval development takes about 1 to 2 weeks, depending on species and temperature. They spin cocoons of various shapes according to species, which serve to anchor and protect the developing pupae. The duration of the pupal stage ranges from 2 to 7 days. At emergence the adult rises to the water's surface in a bubble of air, which prevents them from becoming wet, or they escape by crawling up partially submerged objects such as vegetation on rocks. On reaching the water's surface, the adults immediately take flight (Service 1980). After mating, and a suitable blood meal, if required, the life cycle begins again. Females of some species are non-bloodsuckers, with reduced mouthparts similar to those of males. *S. aureohirtum* is the species distributed most widely in the Oriental Region, and has been known to develop its first batch of eggs without a blood meal (autogenous), although its mouthparts are developed normally. The number of annual generations varies with species. For example, most black flies have one generation per year (univoltine), while continuously breeding in tropical areas for as many as 16 or more generations (multivoltine) (Takaoka 2004).

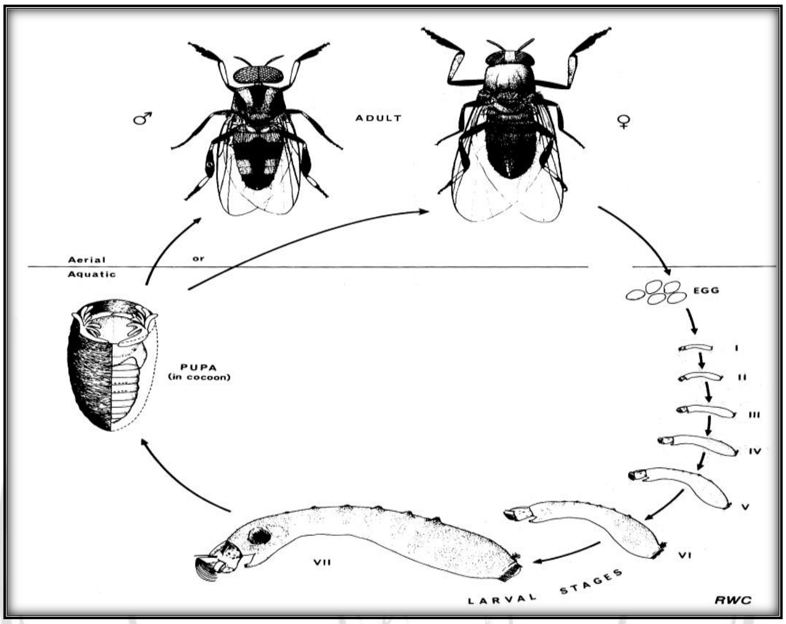


**Figure 1.5** Life cycle of black flies (Crosskey 1990)


**Figure 1.6** Gill spot of mature larva of *Simulium merga* (lateral view).
Scale bar: 3 mm (original photograph from Srisuka W)

### 1.2.4 General biology

#### 1) Breeding sites

The habitats of immature black flies vary across families, which occupy those ranging from temporary trickles of water to large rivers (McCreadie and Adler 2012), and they actually attach themselves to various substrates that are available in streams, such as fallen leaves, rock surfaces, trailing grasses, tree roots and mud. A large river supports colossal populations that yield more than a billion flies per day per kilometer of river (Adler 2005). Interestingly, few species have evolved to phoretically associate with decapod Crustacea (crabs, prawns) or Ephemeroptera (mayflies) in Africa and the Himalayan region. Larvae and pupae of *Simulium nyasalandicum* and *Simulium woodi* occur on the sides, chelipeds, and basal segments of walking legs of the crab, *Potamonautes pseudoperlatus*. They also occur on other species of crab. The most important of these phoretic simuliid species is *Simulium neavei*, which is a vector of onchocerciasis (http://www.blackfly.org.uk/simbiol2.htm). Many environmental factors including water temperature, pH, dissolved oxygen concentration, stream width and depth, stream substrates, and turbidity, have been known to affect black-fly diversity and distribution (McCreadie et al. 2006; Landeiro et al. 2009; Rabha et al. 2013). Black-fly distribution patterns may be explained by biotic factors, such as trophic relationships and food availability as well as abiotic factors (Figueiró et al. 2012). Other environmental changes that are associated with enrichment, i.e., alteration of the flow regime and changes in streamside vegetation can influence the distribution and abundance of black flies (Pachón and Walton 2011). Several studies on black-fly communities associated with environmental factors have been reported from various parts of the world, though, mostly from America, Europe and Africa (McCreadie et al. 2006; Landeiro et al. 2009; Pachón and Walton 2011; Figueiró et al. 2012; Rabha et al. 2013).

#### 2) Adult behavior

Black flies are essentially diurnal insects. Biting occurs outside at almost any time during daylight hours, but each species may prefer different times of biting. The circadian rhythm of biting activity varies with the age of the flies, with parous females feeding earlier in the day than nulliparous ones.

Mating occurs shortly after emergence, and some females are probably intercepted and inseminated by males before they reach a resting site (Adler 2005). Mating in a few species takes place on the ground in the vicinity of the breeding site, but a large majority mate on the wing, when males form small swarms in association with visual markers, which may be located 100 or 200 m from the breeding site. Male simuliids can recognize females from up to a distance of 50 cm away and pursue them in an attempt to couple.

Both male and female black flies feed on plant juices, but most female species take blood meals. Many species of black flies feed mostly from birds (ornithophilic) and other mammalian hosts (mammalophilic), and several species also bite humans (anthropophilic). Some anthropophilic species seem to prefer large animals, for example, donkeys or cattle and bite humans only as a poor alternative. Ornithophilic and mammalophilic species generally differ morphologically in the shape of their claws on the last segment of the legs, i.e., the claws are usually of simple shape in mammal feeders, whereas bird feeders have toothed claws. When feeding, the female simuliid retracts its labella and pushes against the skin, which is stretched tightly by tiny teeth and spines at the tips of the labrum and hypopharynx (Adler 2005). Black flies suck up blood pooling from lacerated blood vessels into the midgut by muscular pumps in the cibarium and pharynx. Blood-feeding takes about 3-6 minutes. After feeding, the blood-engorged females shelter and rest in vegetation, on trees and in other natural outdoor environments, until the blood-meal is digested (Service 1980). Oviposition habits vary greatly between species. *Simulium damnosum* females oviposit communally in the short period between late afternoon and early evening (Cupp 1996).



**3) Natural enemies**

Parasites and predators burden all life stages of black flies. Parasites include bacteria, fungi (blastocladiomycetes, hyphomycetes, trichomycetes and zygomycetes), helicosporidia, ichthyosporeans, mermithid and filarial nematodes, microsporidia, protists (ciliates and haplosporidia), stramenopiles, water mites, and viruses. Most parasites manifest themselves in the larval stage. Larvae with patent microsporidian infections have large, dense, white or red cysts in the abdomen (Adler 2005). Diptera with at least 12 families and other predaceous arthropods (Crustacea and insects: Coleoptera, Odonata, Plecoptera, Trichoptera), invertebrates (Turbellaria), and browsing fish are predators of black flies. Among Diptera families, the main predators of black flies in immature stages are Chironomidae (non-biting midges) and Empididae (dance flies) (Werner and Pont 2003).

### 1.2.5 Medical and veterinary importance

Black flies have been considered as vectors of many pathogens, e.g., filarioid nematodes of the genus *Onchocerca* in humans, cattle and deer, the genus *Dirofilaria* in bears, the genus *Splendidofilaria* in ducks, blood protozoan of the genus *Leucocytozoon* and *Trypanosoma* in birds, and viruses (cytoplasmic polyhedrosis, iridescent, rift valley fever, vesicular stomatitis) in horses and cattle, and chlamydial bacteria that cause blindness in sheep and abortion in cattle. Black flies can transmit the filarial nematode, *Onchocera volvulus,* which causes onchocerciasis or river blindness, and mansonellosis in humans caused by *Mansonella ozzardi*. Furthermore, black-fly bites can cause other very severe problems in humans, since they frequently inflict pain, localized swelling, chronic dermatitis and inflammation accompanied by intense irritation that lasts for several days or even weeks (Figure 1.7). Repeated biting by some species can cause headaches, fever, swollen lymph glands and aching joints.

**Figure 1.7** Dermatitis on the leg caused by black fly bites

Additionally, Takaoka et al. (2012a) documented 19 zoonotic onchocerciasis cases caused by the *Onchocerca* species of animal origin, as reported from several countries (prior to 1989 and from 1990 to 2012). These include one each from Canada (Ali-Khan 1977), Switzerland (Siegenthaler and Gubler 1965), Ukraine (Azarova et al. 1965), Albania (Pampiglione et al. 2001), Austria (Koehsler et al. 2007), Hungary (Sallo et al. 2005), Kuwait (Hira et al. 2008) and Turkey (Otranto et al. 2011), and eight from Japan (one unpublished and four published cases from Oita, one published case from Hiroshima, and two unpublished cases from Hiroshima and Shimane, respectively, in southwest Honshu) (Takaoka et al. 1996, 2001, 2004, 2005; Uni et al. 2010; Uni et al. unpublished data), and three from the U.S.A. (Beaver et al. 1974; Burr et al. 1998; Wright et al. 2002). Unlike human onchocerciasis, in which severe dermal and ocular lesions are caused by microfilariae produced from gravid female worms, zoonotic onchocerciasis is in general caused by a single immature adult female or male worm, and thus, no microfilariae are produced. Conjunctivitis and other ocular lesions caused by adult worms invading the ocular or periocular tissue regions (ocular zoonotic onchocerciasis), or subcutaneous nodules that form around the worm in various parts of the body, can be of clinical importance. The disease is diagnosed by detecting an *Onchocerca* worm or its parts in ocular tissue or a resected subcutaneous nodule. However, specific identification based on morphological characteristics is not easy because in most cases only small parts of a worm are available (Takaoka et al. 2012a). The black-fly vectors of zoonotic onchocerciasis in Japan are shown in Table 1.1.

**Table 1.1** Vector status of black-fly species for seven species of *Onchocerca* in Japan

| ***Onchocerca* species**[a] | **Host animals** | **Vector status of black fly species** | | |
|---|---|---|---|---|
| | | **Natural vectors**[b] | **Putative vectors**[c] | **Suspected vectors**[d] |
| *O. dewittei japonica* | *Sus scrofa* | *Simulium bidentatum* | *Simulium arakawae* | |
| | | | *Simulium japonicum* | |
| | | | *Simulium oitanum* | |
| | | | *Simulium quinquestriatum* | |
| | | | *Simulium rufibasis* | |
| *Onchocerca* sp. sensu | *Sus scrofa* | *Simulium bidentatum* | *Simulium arakawae* | |
| Fukuda et al. 2008 | | | *Simulium oitanum* | |
| *O. lienalis* | *Bos taurus* | *Simulium arakawae* | | |
| | | *Simulium daisense* | | |
| | | *Simulium kyushuense* | | |
| *Onchocerca* sp. sensu | *Bos taurus* | *Simulium arakawae* | | |
| Takaoka & Bain, 1990 | | *Simulium bidentatum* | | |
| | | *Simulium daisense* | | |
| | | *Simulium oitanum* | | |
| *O. eberhardi* | *Cervus nippon* | | *Simulium arakawae* | |
| | | | *Simulium bidentatum* | |
| | | | *Simulium oitanum* | |
| *O. skrjabini* | *Cervus nippon* | | *Simulium arakawae* | *Simulium japonicum*[e] |
| | *Capricornis crispus* | | *Simulium bidentatum* | *Prosimulium* sp. |
| | | | *Simulium oitanum* | *Twinnia japonensis* |
| *O. suzukii* | *Capricornis crispus* | | | *Simulium japonicum*[e] |
| | | | | *Prosimulium* sp. |

[a]*Onchocerca gibsoni* and *Onchocerca gutturosa*, parasites of cattle, and *Onchocerca cervicalis*, a parasite of horses, are not included because their vectors are unknown or dubious.

[b]Based on infective larvae found in wild-caught female black flies.

[c]Based on complete development of microfilariae to infective larvae through intrathoracic inoculated infection experiments.

[d]Based on detection of parasite species-specific DNA sequences from wild-caught female black flies.

[e]It is possible that this is *Simulium kawamurae* because female *Simulium japonicum* and *S. kawamurae* are morphologically indistinguishable (modified from Takaoka et al. 2012a).

**1.2.6 Black fly fauna in Thailand**

The progress of black-fly fauna surveys in Thailand are as follows: in 1984, Takaoka and Suzuki pioneered a survey on black flies in Thailand by discovering 7 new species (*S. barnesi*, *S. chamlongi*, *Simulium chiangmaiense*, *S. inthanonense*, *S. nakhonense*, *Simulium siamense* and *Simulium thailandicum*) and 5 new records (*S. aureohirtum*, *Simulium digrammicum*, *S. nigrogilvum*, *S. nodosum* and *S. rufibasis*). In 1996 or 12 years later, subsequent surveys by Takaoka and Saito brought about an additional new species (*Simulium siripoomense*) and 10 new records (*S. asakoae*, *Simulium brevipar*, *Simulium decuplum*, *Simulium duolongum*, *S. fenestratum*, *Simulium feuerborni*, *Simulium pahangense*, *Simulium parahiyangum*, *Simulium quinquestriatum* and *Simulium tani*). In 1997, 1 new subgenus (*Davisellum*) and 1 new species (*S. courtneyi*) were discovered by Takaoka and Adler. Continuous surveys between 1999 to 2000 by Takaoka and Kuvangkadilok found 5 new species (*Simulium baimaii*, *Simulium chainarongi*, *Simulium chaliowae*, *Simulium chumpornense* and *Simulium triglobus*) and 9 new records (*Simulium angulistylum*, *Simulium dentistylum*, *Simulium gombakense*, *Simulium grossifilum*, *Simulium malayense*, *Simulium nobile*, *Simulium rudnicki*, *Simulium sheilae* and *Simulium yongi*). Thus, during the period from 1984 to 2000 or 16-years of black-fly fauna surveys in Thailand, 38 black-fly species were discovered, of which 14 and 24 were new species and new records, respectively. Interestingly, an additional 8-year-survey between 2001 to 2009 by Takaoka and Choochote brought about the discovery of 1 new subgenus, *Asiosimulium* (Takaoka and Choochote 2005b), 2 new records (*Simulium burtoni* and *Simulium cheongi*) (Takaoka and Davies 1995) and 32 new species (*S. angkaense*, *Simulium bullatum*, *Simulium chanyae*, *Simulium chiangklangense*, *Simulium choochotei*, *Simulium crocinum*, *Simulium datfaense*, *S. doipuiense*, *S. fangense*, *Simulium fruticosum*, *Simulium lampangense*, *Simulium laoleense*, *S. maenoi*, *S. manooni*, *S. mediocoloratum*, *S. merga*, *Simulium oblongum*, *Simulium otsukai*, *Simulium phayaoense*, *Simulium phukaense*, *Simulium prayongi*, *Simulium pukaengense*, *Simulium setsukoae*, *Simulium suchariti*, *S. surachaii*, *Simulium takense*, *Simulium thongsahuani*, *Simulium udomi*, *Simulium visuti*, *Simulium wanchaii*, *Simulium weji* and *Simulium yuphae*) (Takaoka and Choochote 2004a,b 2005a-j, 2006a-e, 2007), thus, achieving the biggest discovery yet of new black-fly species in the genus *Simulium* s.l. in Thailand. In additional surveys between

2008 to 2012, other researchers discovered 14 new species (*Simulium adleri*, *S. chiangdaoense*, *Simulium chayamaritae*, *S. chomthongense*, *Simulium curtatum*, *Simulium doisaketense*, *Simulium kuvangkadilokae*, *S. maeaiense*, *Simulium nanense*, *S. phahompokense*, *Simulium trangense*, *Simulium vanellum*, *Simulium vessabutrae* and *Simulium wichaii*) (Jitklang and Kuvangkadilok 2007; Jitklang et al. 2008; Takaoka and Choochote 2009; Takaoka and Srisuka 2009, 2010a-e, 2011; Takaoka et al. 2009, 2010a,b, 2012b) and 2 new records (*Simulium ghoomense* and *Simulium novematiculatum*). Therefore, from1984 to 2012, 28 years of black-fly fauna surveys in Thailand have brought about a total of 88 black-fly species, comprising 60 new species and 28 new records in areas over 34 provinces of the Kingdom. All species are classified into 6 subgenera in the genus *Simulium* s.l., i.e., *Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Montisimulium*, *Nevermannia* and *Simulium* s. str., of which the first two were established as new. The genus *Simulium* s. l. can be distinguished from other genera by a combination of characteristics (Davies and Györkös 1987), i.e., the adult antenna has 7-9 flagellomeres; the anterior wing veins have spinules as well as hairs and the radial sector is not forked; the hind basitarsus has a well-developed calcipala and the second segment of the hind tarsus has distinct pedisulcus; the pupa has a wall pocket-, shoe-, or boot-shaped cocoon; the larva has rather low hypostomal teeth and the median tooth is not trifid; and the anal sclerite has posterior arms that are subequal to, or longer than, the anterior ones.

Interestingly, 5 new species (*Simulium atipornae*, *Simulium furvum*, *Simulium khunklangense*, *Simulium lomkaoense*, and *Simulium piroonae*) were discovered in this study (Takaoka et al. 2013a,b, 2014c-e). In addition, this study was the first to describe the male, pupa and last-instar larvae of *Simulium wanchaii*. Therefore, a total of 93 black-fly species have been reported in Thailand (listed below).



### List of 93 Simuliidae species in Thailand

**Genus *Simulium* Latreille s. l.**

**1. Subgenus *Asiosimulium*** Takaoka & Choochote

1. *furvum* Takaoka & Srisuka, 2013
2. *oblongum* Takaoka & Choochote, 2005

3. *wanchaii* Takaoka & Choochote, 2006

**2. Subgenus *Daviesellum*** Takaoka & Adler

4. *courtneyi* Takaoka & Adler, 1997

5. *pahangense* Takaoka & Davies, 1995

**3. Subgenus *Gomphostilbia*** Enderlein

**A. *asakoae* species-group**

6. *asakoae* Takaoka & Davies, 1995

7. *chiangdaoense* Takaoka & Srisuka, 2009

**B. *batoense* species-group**

**a) *decuplum* subgroup**

8. *decuplum* Takaoka & Davies, 1995

**b) *duolongum* subgroup**

9. *duolongum* Takaoka & Davies, 1995

10. *siamense* Takaoka & Suzuki, 1984 (complex)

**c) *parahiyangum* subgroup**

11. *dentistylum* Takaoka & Davies, 1995

12. *parahiyangum* Takaoka & Sigit, 1991

**C. *ceylonicum* species-group**

**a) *sheilae* subgroup**

13. *curtatum* Jitklang, Kuvangkadilok, Baimai, Takaoka & Adler, 2008

14. *inthanonense* Takaoka & Suzuki, 1984

15. *sheilae* Takaoka & Davies, 1995

16. *trangense* Jitklang, Kuvangkadilok, Baimai, Takaoka & Adler, 2008

**b) *rosemaryae* subgroup**

17. *udomi* Takaoka & Choochote, 2006

**D. *darjeelingense* species-group**

18. *chayamaritae* Takaoka & Srisuka, 2010

**E. *epistum* species-group**

**a) *zonatum* subgroup**

19. *adleri* Jitklang & Kuvangkadilok, 2008 [2007]

20. *cheongi* Takaoka & Davies, 1995

21. *datfaense* Takaoka & Otsuka, 2009

**b) *atratum* subgroup**

22. *angulistylum* Takaoka & Davies, 1995

23. *otsukai* Takaoka & Choochote, 2009

**F. *gombakense* species-group**

24. *gombakense* Takaoka & Davies, 1995

25. *prayongi* Takaoka & Choochote, 2005

**G. *varicorne* species-group**

**a) *chumpornense* subgroup**

26. *chumpornense* Takaoka & Kuvangkadilok, 2000

27. *kuvangkadilokae* Pramual & Tangkawanit, 2008

28. *piroonae* Takaoka & Srisuka, 2014

**b) *burtoni* subgroup**

29. *burtoni* Takaoka & Davies, 1995

**c) *novemarticulatum* subgroup**

30. *novemarticulatum* Takaoka & Davies, 1995

**H *Gomphostilbia*** species unplaced to species-group

31. *doisaketense* Jitklang, Kuvangkadilok, Baimai, Takaoka & Adler, 2008

**4. Subgenus *Montisimulium*** Rubtsov

32. *angkaense* Takaoka & Choochote, 2005

33. *ghoomense* Datta, 1975

34. *laoleense* Takaoka & Choochote, 2005

35. *merga* Takaoka & Choochote, 2005

36. *nanense* Takaoka & Srisuka, 2010

37. *phahompokense* Takaoka & Srisuka, 2010

38. *surachaii* Takaoka & Choochote, 2005

**5. Subgenus *Nevermannia*** Enderlein

**A. *feuerborni* species-group**

39. *chiangklangense* Takaoka & Choochote, 2005

40. *fangense* Takaoka & Choochote, 2006

41. *feuerborni* Edwards, 1934

42. *fruticosum* Takaoka & Choochote, 2005

43. *maeaiense* Takaoka & Srisuka, 2011

44. *vessabutrae* Takaoka & Srisuka, 2010

45. *wichaii* Takaoka, 2010

**B. *ruficorne* species-group**

46. *aureohirtum* Brunetti, 1911

**C. *vernum* species-group**

47. *chomthongense* Takaoka, Srisuka & Choochote, 2012

48. *khunklangense* Takaoka & Srisuka, 2013

**6. Subgenus *Simulium* Latreille s. str.**

**A. *christophersi* species-group**

49. *atipornae* Takaoka, Srisuka & Choochote, 2014

**B. *griseifrons* species-group**

50. *choochotei* Takaoka, 2002

51. *crocinum* Takaoka & Choochote, 2004

52. *digrammicum* Edwards, 1928

53. *grossifilum* Takaoka & Davies, 1995

54. *maenoi* Takaoka & Choochote, 2002

55. *mediocoloratum* Takaoka & Choochote, 2004

56. *nigrogilvum* Summers, 1911

57. *phayaoense* Takaoka & Choochote, 2005

58. *phukaense* Takaoka & Choochote, 2005

59. *pukaengense* Takaoka & Choochote, 2005

60. *rudnicki* Takaoka & Davies, 1995

61. *suchariti* Takaoka & Choochote, 2004

62. *thongsahuani* Takaoka & Otsuka, 2009

63. *vanellum* Huang, Phasuk, Chanpaisaeng & Adler, 2010

64. *visuti* Takaoka & Choochote, 2006

65. *yongi* Takaoka & Davies, 1997

**C. *malyschevi* species-group**

66. *baimaii* Kuvangkadilok & Takaoka, 1999

67. *lomkaoense* Takaoka & Srisuka, 2014

68. *siripoomense* Takaoka & Saito, 1996

**D. *multistriatum* species-group**

69. *bullatum* Takaoka & Choochote, 2005

70. *chainarongi* Kuvangkadilok & Takaoka, 1999

71. *chaliowae* Takaoka & Boonkemtong, 1999

72. *chanyae* Takaoka & Choochote, 2007

73. *fenestratum* Edwards, 1934

74. *lampangense* Takaoka & Choochote, 2005

75. *malayense* Takaoka & Davies, 1995

76. *takense* Takaoka & Choochote, 2005

77. *triglobus* Takaoka & Kuvangkadilok, 1999

**E. *nobile* species-group**

78. *nobile* De Meijere, 1907

79. *nodosum* Puri, 1933

**F. *striatum* species-group**

80. *chiangmaiense* Takaoka & Suzuki, 1984

81. *nakhonense* Takaoka & Suzuki, 1984

82. *quinquestriatum* (Shiraki, 1935)

83. *thailandicum* Takaoka & Suzuki, 1984

**G. *tuberosum* species-group**

84. *brevipar* Takaoka & Davies, 1995

85. *doipuiense* Takaoka & Choochote, 2005

86. *manooni* Takaoka & Choochote, 2005

87. *rufibasis* Brunetti, 1911

88. *setsukoae* Takaoka & Choochote, 2004

89. *tani* Takaoka & Davies, 1995

90. *weji* Takaoka, 2001

91. *yuphae* Takaoka & Choochote, 2005

**H. *variegatum* species-group**

92. *barnesi* Takaoka & Suzuki, 1984

93. *chamlongi* Takaoka & Suzuki, 1984

According to the above list, the black-fly fauna of Thailand is very rich in species diversity, patterns of distribution and endemicity, as found through surveys conducted from 1984 to 2014. Furthermore, based on the results obtained from a one-year survey in Doi Inthanon and Doi Suthep-Pui National Parks, where natural environmental conditions are well preserved, it was remarkable how these two parks demonstrated similar, very rich fauna and species diversity, but markedly different species composition, due to a high percentage of species being endemic to each park, which were approximately 100 km away from each other (Choochote et al. 2005; Ittiponpanya 2006; Srisuka 2007). It is the first substantial evidence supporting that the more natural environments are preserved, the richer black-fly fauna and its species diversity becomes.

Recently, the fact that *S. nodosum* bites humans was first confirmed at Ban Pang Faen, Doi Saket district, Chiang Mai province, northern Thailand (Takaoka et al. 2003). Also, an additional anthropophilic black-fly species, *S. nigrogilvum*, was reported for the first time at this location. The one-year study in Doi Inthanon National Park, Chiang Mai province, northern Thailand, revealed that at least 23 black-fly species belonging to 4 subgenera (*Gomphostilbia*, *Montisimulium*, *Nevermannia* and *Simulium* s. str.) of the genus *Simulium* s. l. were attracted to humans (Choochote et al. 2005; Srisuka 2007). Anthropophily was confirmed in 5 species, i.e., *S. rufibasis*, *S. nigrogilvum*, *S. chamlongi*, *S. nodosum* and *S. asakoae*, in order of avidity. On the other hand, a one-year study in Doi Suthep-Pui National Park, Chiang Mai province, northern Thailand, demonstrated that at least 25 black-fly species belonging to 5 subgenera (*Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Nevermannia* and *Simulium* s. str.) of the genus *Simulium* s.l. were attracted to humans (Ittiponpanya 2006). Eight species with confirmed anthropophily, i.e., *S. doipuiense*, *S. fenestratum*, *S. nakhonense*, *S. manooni*, *S. mediocoloratum*, *S. chamlongi*, *S. barnesi* and *S. maenoi*, were incriminated as man-biting, of which only 4 ranked as main human-biters that seemed to exist in species-specific localities. Four species were determined by seasonal abundance and biting intensity related to altitude, i.e., *S. rufibasis*; *S. doipuiense*, *S. nigrogilvum* and *S. nodosum* at altitudes of 1,800-2,565 m; 1,300-1,800 m; 600-1,300 m; and < 600 m, respectively. Based on the recovery of infective stages ($L_3$ larvae) from wild-caught females, 2 species, i.e., *S. nigrogilvum* and *S. nodosum*, were incriminated recently as natural vectors of *Onchocerca* spp., which can probably cause zoonotic filarial infection in the Thai population, and this was reported for the first time in Thailand and/or the Southeast Asian region (Takaoka et al. 2003; Fukuda et al. 2003). The third stage larvae recovered from *S. nodosum* resembled *Onchocerca suzukii*, which is a parasite from a wild Japanese bovid, suggesting that an unknown *Onchocerca* species from ruminants was transmitted in Thailand.

Throughout a subsequent year, Ishi et al. (2008) studied the vectorial roles of the two predominant man-biting species, *S. asakoae* and *S. nodosum*, in zoonotic filarial transmission, as well as their seasonal and daily biting activity patterns at Ban Pang Faen, Chiang Mai province, northern Thailand. The results revealed that the filarial larvae found in *S. nodosum* and *S. asakoae* were morphologically different from each other. The short and thick infective larvae found in *S. asakoae* differed from all known filarial larvae; which suggested that they might be a bird parasite (Splendidofilariinae or Lemdaninae). Infection of mammophilic *S. nodosum* with large *Onchocerca* type infective larvae was confirmed in this area. Natural filarial infections were found each month (except December) in either *S. nodosum* or *S. asakoae* or both. It is suggested that people in this village are likely exposed throughout the year to the risk of infection with zoonotic filariae.

Besides these reports, significant progress has been made on black flies in Thailand regarding population cytogenetics (Kuvangkadilok et al. 1998; Pramual et al. 2009) and molecular genetics (Otsuka et al. 2003; Pramual et al. 2005; Thanwisai et al. 2006; Phayuhasena et al. 2010; Pramual et al. 2011a,b, 2012; Pramual and Adler 2014; Sriphirom et al. 2014; Thaijarern et al. 2014), ecological studies (Pramual and Kuvangkadilok 2009; Pramual and Wongpakam 2010) and parasites of larval black flies (Jitklang et al. 2012).

Remarkably, Choochote (2012) stated that although black flies are vicious blood-feeders and transmit many diseases to humans and animals, they are still beneficial to human life as shown in the folk-law of indigenous people in northern Thailand. Black flies are found mostly in mountainous areas associated with the richness of waterfalls and running streams and are called commonly “Khun”, while the Thai-Karen named adults with a shiny yellow body-color “Paboh” (or Khun Lueang), whereas those of a brownish black or entirely black body-color are known as “Kasue” (or Khun Dum). The larvae of black flies attached to bed-rock beneath waterfalls and streams are called “Kokob” or “Kleau”, and the Thai-Karen harvests them for a special food called “Kokob Salad”, which is a source of protein (Figure 1.8).




**Figure 1.8** Harvesting black fly larvae (A and B) and the special food, Kokob Salad (C and D) (Choochote 2012)

## 1.3 Purpose of This Study

1.3.1 To determine diversity of black-fly species from different ecological environments in Thailand.

1.3.2 To evaluate ecological factors influencing black-fly species diversity in Doi Phahompok National Park.

## 1.4 Usefulness of the Study

All the results obtained during the 2-year-study period were necessary for understanding factors that explain black-fly distribution, by using the tenet that species distribution patterns can provide insight into the mechanism of species structure assemblages. Understanding these factors gave new insights into the crucial question of whether global or regional ecological determinants affect the general distribution of species diversity. The substantial data of this study provide insight emphasizing on the importance of protecting and managing the natural environment of Doi Phahompok National Park, which is currently securing high biodiversity of various living organisms. Additionally, new species, new records and unknown stages of known species discovered in this study are necessary in forming a robust monograph or pictorial key, and species diversity-map of black-fly fauna in Thailand.