Classification and Characters

The order Diptera contains two suborders: Nematocera (long horned flies) and Brachycera (short horned flies). The latter includes taxa, formerly referred to as a third suborder, Cyclorrhapha, but now included in the infraorder Muscomorpha. The Nematocera are divided into seven infraorders, the Tipulomorpha, Blephariceromorpha, Axymyiomorpha, Bibionomorpha, Psychodomorpha, Ptychopteromorpha, and Culicomorpha. The suborder Brachycera is divided into three infraorders, the Tabanomorpha, Asilomorpha, and Muscomorpha, the first two corresponding to the Orthorrhapha while the third is the former Cyclorrhapha. Within the Muscomorpha, there are two easily defined but unequally sized sections, the Aschiza and the Schizophora. The latter has two subdivisions, Acalyptratae and Calyptratae (Borror *et al*., 1989; Gillott, 1995).

In the primitive families (Nematocera) larvae have a completely sclerotized head capsule (except in most Tipulidae). The larval mandibles usually move in horizontal and bear subapical teeth. There are several families of Nematocera in which all or almost all species have both aquatic larvae and pupae. In the higher families (Brachycera) larvae either lack a head capsule entirely or have one only partially formed. Mandibles in the Brachycera rotate vertically and have no subapical teeth. Most families with aquatic larvae also have large numbers of terrestrial or semiaquatic species that pupate on land (Borror *et al*., 1989; Hilsenhoff, 1991; Williams and Feltmate, 1992; McCafferty, 1998; Dudgeon, 1999).

Suborder Nematocera

Larvae have a head that is usually well developed and conspicuous, with the mandibles toothed or brushlike and moving horizontally. In some groups, the head may be incomplete or retracted into the thorax. The body often tapers anteriorly and has various lobelike processes posteriorly.

Pupae are obtect, and developing adult structures are apparent. Developing antennae usually extend over the eyes and reach the bases of developing wings. Thoracic respiratory horns or spiracular gills are usually well developed.

Adults usually have slender antennae with 6 or more segments, plumose in some males. Body is often small and mosquito-like or midgelike. The medium- and large-sized adults usually have very long legs. Small flies may not have long legs. Wing venation varies from complete to greatly reduced. This suborder only has the radius (R) 5-branched. R_{2+3} often forked.

Suborder Brachycera: Tabanomorpha and Asilomorpha

Larvae have a body that is more-or-less tapered and pointed anteriorly. The head is usually incomplete and indistinct. If the head capsule is distinct, as seen in dorsal view, then body lacks prolegs. Mandibles are usually sickle-shaped and move parallel to each other in a vertical plane.

Pupae are obtect except soldier flies (Stratiomyidae). Puparium of soldier flies is not capsulelike and is very little changed from the larva. Developing antennae do not reach bases of developing wings and do not lie over the eyes.

Adults are usually stout-bodied and medium-sized. Antennae have less than five, usually three segments, although segment 3 may have numerous annulations and a terminal bristle. Wing venation has Rs 3-branched, with R_{4+5} forked. No frontal suture.

Suborder Brachycera: Cyclorrhapha

Larvae either taper or are blunt anteriorly, and are wrinkled or maggotlike. The head is completely reduced. Mouthparts are hooklike.

Pupae are encased in a capsulelike puparium formed from the last larval skin and lacking a distinct head.

Adults are typically flylike, and stout-bodied. Antennae are three-segmented, aristate and segment 3 has no annulation. Wing venation has Rs 2-branched. Frontal suture absent (Aschiza) or present (Schizophora).

Cytotaxonomy

Studies of larval salivary gland chromosomes have been of major importance in the taxonomy of a number of Diptera (Rothfels, 1979). In addition, Baimai (1986) reported that cytological differences are generally useful in studies of taxonomy and evolutionary relationships of many groups of insects. There are many examples of closely related species groups of Diptera that were recognized by using detailed investigation of chromosomal rearrangements.

The larval silk-gland chromosomes provide important taxonomic characters in the family Simuliidae, especially for revealing and identifying morphologically similar species known as sibling species or cryptic species (Rothfels, 1988). Sibling species can be a problem for taxonomists who use external features as the sole criteria for identification (Baimai, 1986). Many black fly morphospecies are complexes of isomorphic, ecologically unique sibling species (Adler *et al*. 2004). Study of larval salivary gland chromosomes of black flies frequently reveals the existence of taxonomically unresolved sibling species but it may be restricted to homosequential siblings (Rothfels, 1979).

Family Simuliidae

The Simuliidae or black flies in Thailand are not known to be a vector of any diseases of man. However, at Doi Inthanon National Park, Chiang Mai province, northern Thailand, these flies can cause irritation to the local people, domestic animals, livestock, and tourists (Kuvankadilok *et al*., 1999a). In some regions, such as Africa, Mexico, and Central America, certain species of black flies act as vectors of onchocerciasis or river blindness, a disease caused by a filarial worm (*Onchocerca volvulus*) and characterized by large subcutaneous swellings. In some patients the worms may get into the eye and cause partial or complete blindness (Borror *et al*., 1989). A recent study of female black flies at Ban Pang Fan, Chaing Mai province reported that three simuliid species, *Simulium nodosum* Puri, 1933, *S. asakoae* Takaoka and Davies, 1995, and *S. nigrogilvum* Summers, 1911 were attracted to human, and the first of these was naturally infected with *Onchocera* larvae (Takaoka *et al*., 2003). Furthermore, at Tambol Ban Laung, Doi Inthanon National Park, nine simuliid species were attracted to a human, with natural infections of two species, *S. nigrogilvum* and *S. asakoae* by a filarial larva (Fukuda *et al*., 2003).

External morphology

The recognition and classification of many flies must be done on adults. Black flies are unusual among Diptera because almost all stage can be used for species identification, and larvae and pupae are sometimes easier to identify than adults. Morphological features of immature stages provide good taxonomic characters for constructing the keys. Black fly eggs are so simple and uniform, they provide no characters useful in identification (Crosskey, 1990). The general morphology and life cycle of black flies has been reviewed by various authors (Crosskey, 1990; Peterson, 1996; Service, 1996; Bass, 1998) as below.

Adult

They are small, about 1.2-6 mm long, dark, stout-bodied and hump-backed (Figure 1). As their vernacular name indicates they are usually black but may be reddish brown, gray, orange, or yellow in ground color. The female is dichoptic with frons visible between the separated eyes above the antennae (Figure 2A). In the male is holoptic, almost always without frons, eyes meeting in midline of head above antennal bases (Figure 2B). However, there are a few species in which the male eyes nearly meet along the midline below the antennae, or are similar those of the female.

Eye facets on the upper half of the head are larger than those on the lower half in the male but are the same in the female. There are no ocelli. The short horn-like antennae are the same in both sexes and distinctly segmented with 9-11 (usually 11 segments) but without long hairs. The proboscis is shorter than head. Both the mandibles and the laciniae of the maxillae are serrated or toothed at the tips, and are used for cutting and piercing. In males and in a few species in which females do not bite, the mandibles and maxillae are not toothed. The five-segmented maxillary palps hang downwardly and carry on the third segment a large sensory pit (Lutz's organ). The thorax is usually high and strongly arched. The wings are broad, about 1.4-6 mm long, colorless and transparent, with a large anal lobe. The venation is characteristic, with only the veins near the anterior margin well-developed, and the rest of the wing with an indistinct venation. The legs are short and moderately stout with elongated basitarsus. The hind leg basitarsus usually with an anterior process (calcipala) on the inner apical surface and second tarsal segment usually with a variably distinct notch (pedisulcus) near its base. The tarsal claws are short and simple or have a basal or subbasal tooth. The abdomen is elongate with ten segments. The first tergite is modified in both sexes to form a projecting collar (basal scale) that bears a fringe of long setae. The female terminalia has a Y-shaped genital fork and paired terminal ventral lobes (paraproct and cercus) (Figure 3B and D) and the male terminalia has paired gonopod claspers (coxite and style) (Figure 3A and C).

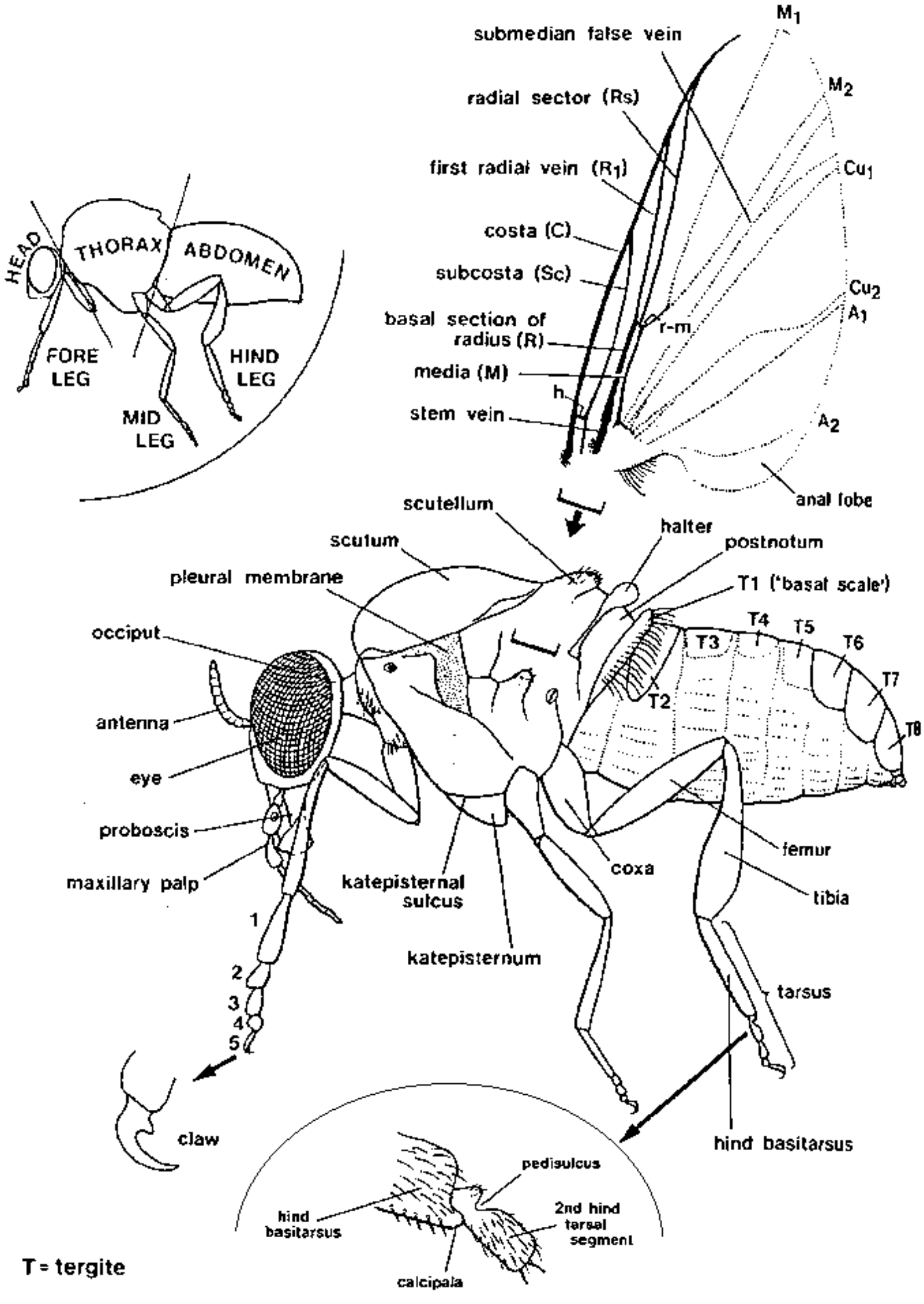
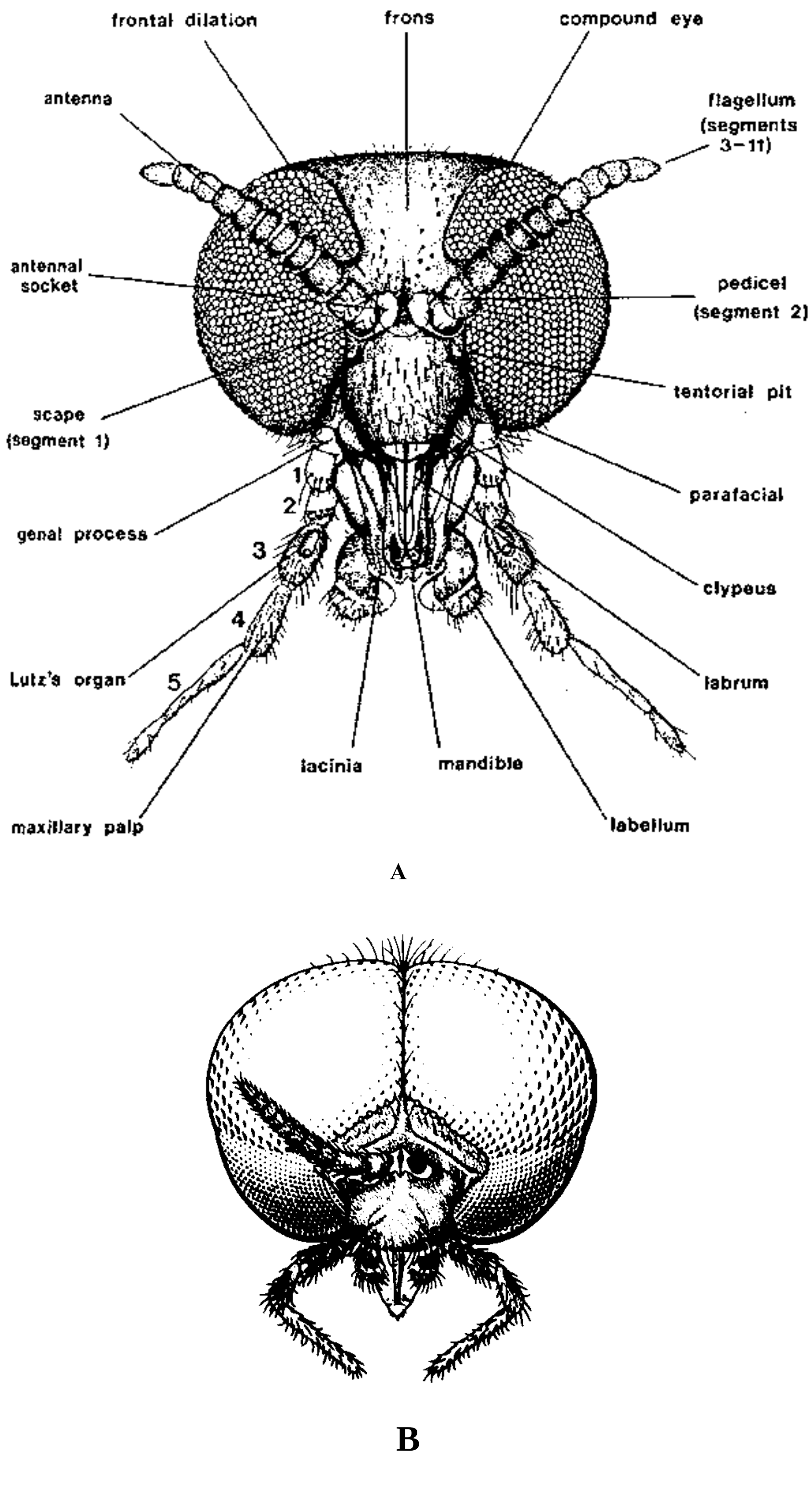


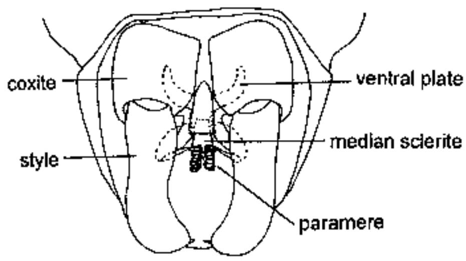
Figure 1 Morphological features of female black fly

Source: Crosskey (1990)

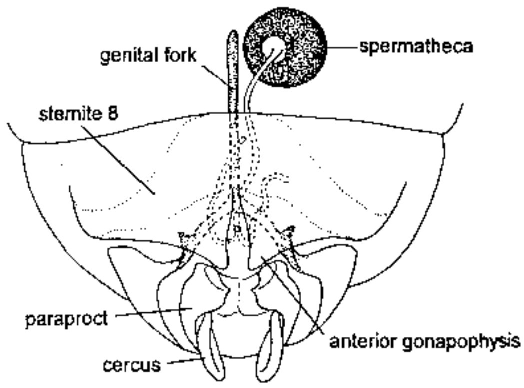


<u>Figure 2</u> Front view of adult heads: A, *Simulium* female; B, *Simulium* (*Simulium*) *decorum* male

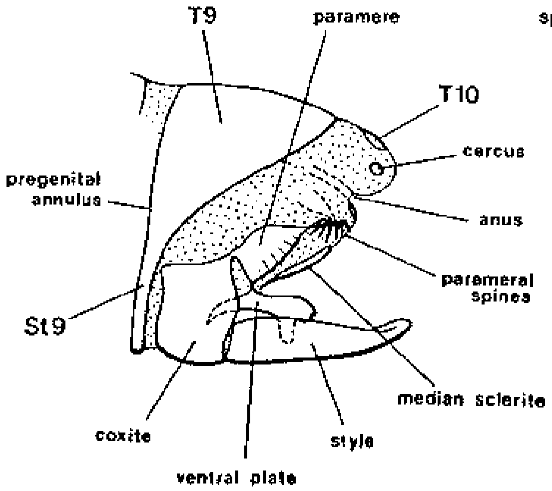
Source: Crosskey (1990); Peterson (1996)



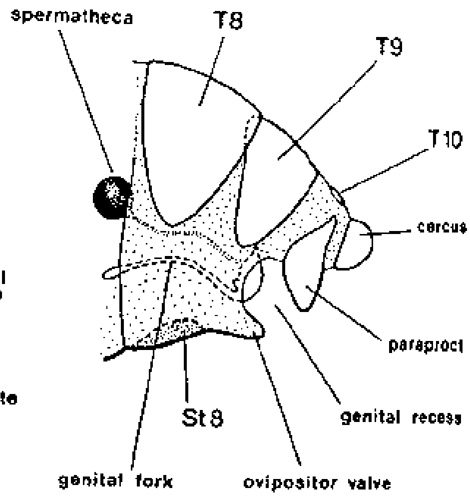
A



B



C



D

<u>Figure 3</u> A, B: basic morphology of the terminalia in ventral view. A, male; B, female. C, D: basic morphology of the terminalia in lateral view. C, male; D, female

Source: Crosskey (1990); Takaoka and Davies (1995)

Pupa

They are about 2-7 mm long (Figure 4C). The head is anteroventral to and flexed beneath the thorax. Antennal sheaths of the female usually reach the posterior of head, while in male they extend about one-half to three-quarter of that distance (Figure 4B). The thorax is enlarged, strongly arched dorsally, and flattened ventrally. The anterior half of the thoracic mesonotum usually have 2-5 dorsal and 1-2 lateral pairs of specialized setae (trichomes). A respiratory gill arises on each anterolateral corner of thorax and usually consists of one to many short or long slender filaments. The abdomen has nine segments and with a series of setae and small hooks. The last segment often bears a dorsal pair of terminal hooks or tubercles (Figure 4D). The pupa is covered by a cocoon that is attached to the substrate (Figure 4A). The cocoon may be constructed with differing proportions and patterns of coarse and fine silk strands. There are two basic forms of cocoons, which are usually described as shoe and slipper shaped.

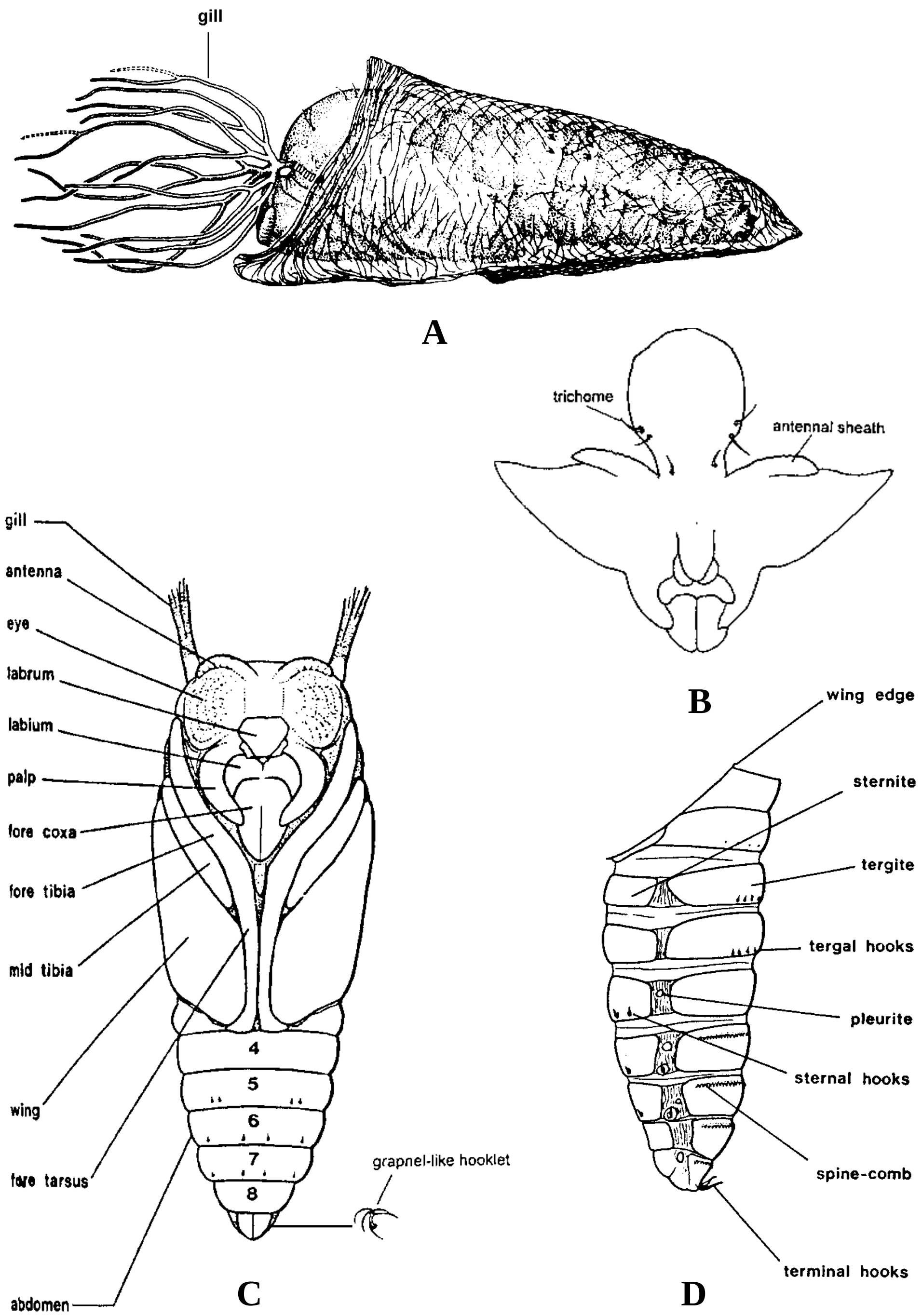


Figure 4 Morphological features of pupa. A, *Simulium* (*Psilozia*) *vittatum* in lateral view; B, head integument in front view; C, obtect condition; D, abdomen in lateral view

Source: Crosskey (1990); Peterson (1996); Takaoka and Davies (1995)

Larva

Mature larvae are about 3.5-15 mm long (Figure 5A). They have a characteristic dumb-bell shape, slender and somewhat cylindrical. The body is pale gray to pale yellowish brown to nearly black in color. Some may be tinged with irregular splotches of reddish, orange, greenish or purplish color. The head is prognathous with a pair of labral fan (cephalic fan) used in filter-feeding (rarely reduced or absent). The antennae are slender near the base of each head fan. Ventrally, the mouthparts include a pair of mandibles with complex teeth and brushes. An anteroventral toothed plate or subtriangular, hypostomium bears a series of heavily sclerotized teeth along the anterior margin, and has smooth or variously serrated lateral margins (Figure 5C and D). The posteroventral margin of the head usually is indented by the postgenal cleft, which varies in size and shape. Dorsally, the head capsule is well-developed and heavily sclerotized, with a variable pattern of pale or dark spots (Figure 5B). Two longitudinal sutures form the edge of a plate-like area known as the cephalic apotome. The thorax has a single proleg having a terminal circlet of minute hooks arranged in rows, and a lateral sclerite on each side. Developing gill histoblasts are visible in the thorax of last instar. The abdomen has anterior segments that are slender and posterior segments that are variously enlarged. A pair of small ventral papillae or a single midventral bulge is present on segment 8. The posterior end of the abdomen bears a ring of many rows of minute hooks and an X-shaped (sometimes subquadrate, Y-shaped or absent) anal sclerite anterodorsal to the ring of hooks (Figure 5E). The colorless rectal organ is extrusible. This consists of three lobes that sometimes bear varying numbers of secondary lobules. The cuticle of the abdomen is thin, transparent and smooth, and may have fine setae or scales, especially posterodorsally.

Egg

The eggs are small and range from 0.1-0.54 mm long. They are oval to triangular in shape. The egg surface is smooth but in some species shows distinctive sculpturing and pitting when examined under a scanning electron microscope.

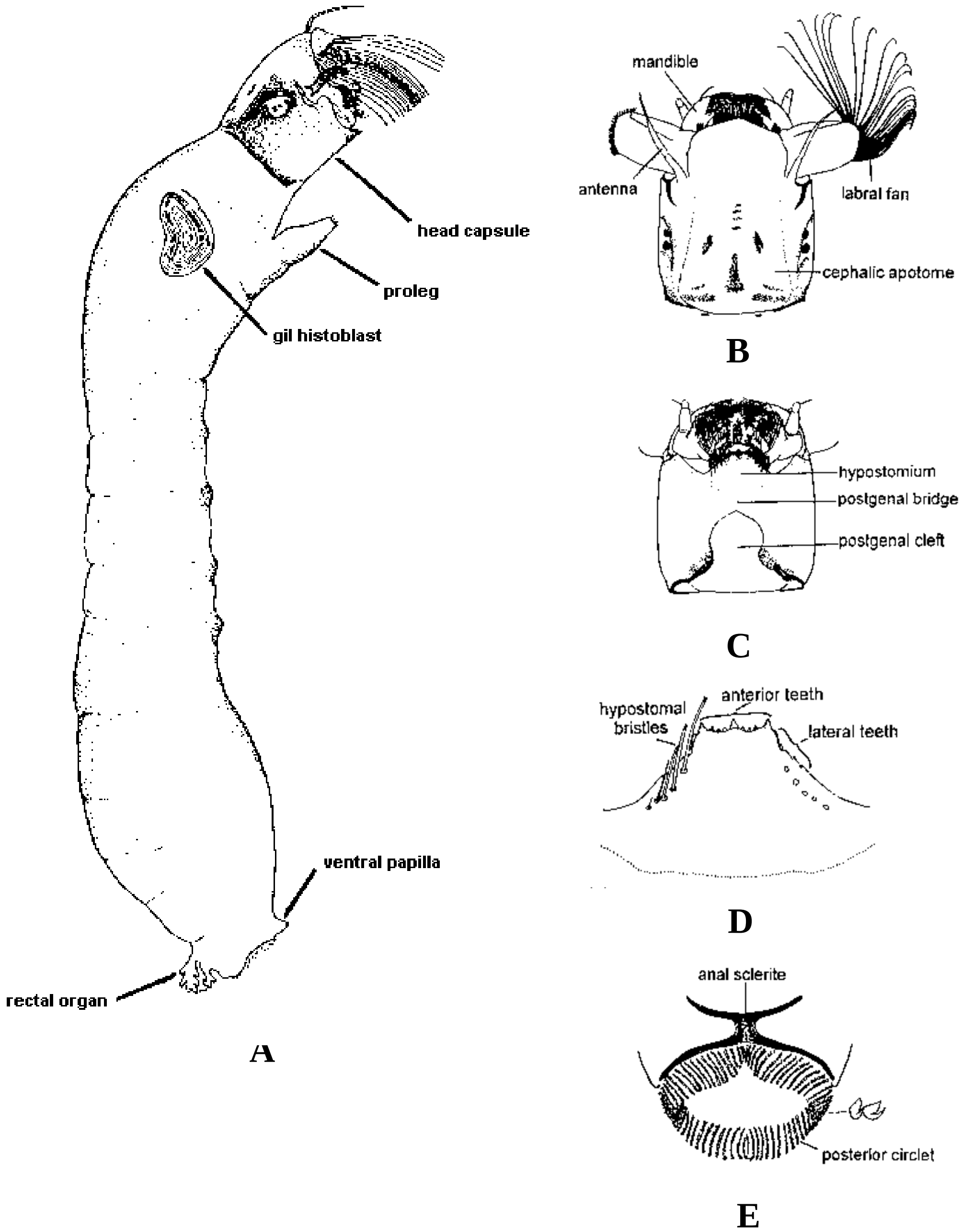


Figure 5 Morphological features of larva. A, lateral view; B, C: head capsule. B, dorsal view; C, ventral view; D, hypostomium; E, posterior tip of larval abdomen in posterodorsal view

Source: Bass (1998); Takaoka and Davies (1995)

Life cycle

The life cycle of black flies is one of complete metamorphosis. After mating, females lay their eggs in a wide range of flowing waters. Most females average 200-500 eggs, which are deposited in masses on objects near water or under water. Some species land at the water's edge, then crawl down a rock or plant to deposit eggs under water. The time required for hatching varies from 2-30 days, depending on species and water temperature. Larvae remain at the site of hatching if substrate and food supply are adequate, or spin a silk thread and drift downstream until a suitable site is located. There are 4-9 (usually 7) larval instars, with development requiring 4 days to 6 or 7 months, depending on water temperature and food availability. Some species overwinter as larvae. Mature larvae spin variously shaped cocoons that serve to anchor and protect the developing pupae while it is attached to the substrate. The pupal stage lasts from 2 to 7 days or 2 to 3 weeks, depending on water temperature. At emergence, the adult pulls itself out of the pupal skin through a T-shaped slit in the pupal thorax and rises quickly to water surface in a bubble of air. Mating usually occurs shortly after the initial flight. After mating and if required, a suitable blood meal the life cycle begins again. The number of annual generations varies with the species and locality. There may be 16 or more generations in tropical areas. In temperate regions there may be one to six generations. There is usually only one or two generations in arctic habitats.

Ecology and Distribution

Aquatic insects have been a major focus of many ecological studies in aquatic habitats. A principal reason for this is that they are major food items of important commercial and sport fish species (Cummins and Merritt, 1996). Aquatic insects serve as the link between their food and fish, as well as other higher trophic level vertebrates. Their distribution is worldwide. They usually are abundant, hence they have been used widely to evaluate water quality and the food base for fisheries. The distribution pattern of aquatic insect populations is the result of habitat selection and physical environmental conditions (Cummins and Merritt, 1996).

Diptera are diverse not in only species number, trophic importance, and numerical abundance but in terms of morphological and ecological characteristics (Courtney *et al*., 1996). Although several workers have studied the ecology and geographical distribution of aquatic Diptera, there are many species for which details are unknown. Herein detailed information are being confined to Simuliidae, partly because they are important from ecological and human health prospectively.

Worldwide, many studies had been done to determine the environment factors associated with black flies, such as the distribution of immature black flies related to stream size (McCreadie *et al*., 1995), water temperature, water velocity, food materials, substrate types (Ladle *et al*., 1977; Kuvangkadilok *et al*., 1999a). Moreover, species distributions were predictably correlated with spatial scale, geographic location and time (McCreadie and Adler, 1998). Corkum and Currie (1987) reported that sites with no black flies had significantly higher conductivity, greater depth, shallower slope and were farther from the Pacific Ocean than sites with black flies.

Black fly abundance has been associated with stream size, velocity and seasonal variation (Grillet and Barrera, 1997), whereas species richness was greater in larger, cooler, faster, more shaded streams with larger substrates (Hamada *et al*., 2002). Furthermore, seasonal changes can influence black flies density, species richness and assemblage composition (Burgherr *et al*., 2001), even though Malmqvist *et al*. (1999) found that neither species richness nor abundance of larvae was related to environmental factors.

Black Flies in Thailand

In Thailand, few details on black fly ecology and biology are available. This lack of data may influence future decisions on public health and economic impact in regard to both humans and animals. Worldwide, the Simuliidae contains approximately 1,720 described species (Crosskey and Howard, 1997), but the fauna of tropical Asia is incompletely known. The Oriental Region contains about 246 described species (Takaoka and Davies, 1995). Recent interest in the fauna of Thailand has led to the discovery of many new species (Takaoka and Suzuki, 1984; Takaoka and Saito, 1996; Takaoka and Adler, 1997; Takaoka and Kuvangkadilok, 1999; Kuvangkadilok and Takaoka, 2000; Takaoka, 2000; Takaoka and Mulla, 2000; Takaoka, 2001; Takaoka and Choochote, 2002; Takaoka and Choochote, 2004a, 2004b, 2004c). At present, at least 40 species are known from Thailand, but new species continue to be discovered. Prior to our investigation, only three species of black fly had been recorded from Khao Yai National Park (Kuvangkadilok and Takaoka, 2000; Kuvangkadilok *et al.*, 2003; Kuvangkadilok, personal communication). Takaoka and Choochote (2004b) provided the most recent list of the species of Simuliidae in Thailand, and later added two additional species (Takaoka and Choochote, 2004c), see below.

A checklist of black flies of Thailand

Genus *Simulium* Latreille s.l.

Subgenus *Daviesellum* Takaoka and Adler

1. *Simulium courtneyi* Takaoka and Adler, 1997
2. *Simulium pahangense* Takaoka and Davies, 1995

Subgenus *Gomphostilbia* Enderlein

(A) *batoense* species-group

3. *Simulium angulistylum* Takaoka and Davies, 1995
4. *Simulium decuplum* Takaoka and Davies, 1995
5. *Simulium dentistylum* Takaoka and Davies, 1995
6. *Simulium gombakense* Takaoka and Davies, 1995
7. *Simulium parahiyangum* Takaoka and Sigit, 1992
8. *Simulium siamense* Takaoka and Suzuki, 1984

(B) *ceylonicum* species-group

9. *Simulium asakoae* Takaoka and Davies, 1995
10. *Simulium inthanonense* Takaoka and Suzuki, 1984

11. *Simulium sheilae* Takaoka and Davies, 1995

(C) *varicorne* species-group

12. *Simulium burtoni* Takaoka and Davies, 1995
13. *Simulium chumpornense* Takaoka and Kuvangkadilok, 2000

Subgenus *Montisimulium* Rubtsov

14. *Simulium* sp. G

Subgenus *Nevermannia* Enderlein

(A) *feuerborni* species-group

15. *Simulium feuerborni* Edwards, 1934

(B) *ruficorne* species-group

16. *Simulium aureohirtum* Brunetti, 1911

(C) *vernum* species-group

17. *Simulium caudisclerum* Takaoka and Davies, 1995

Subgenus *Simulium* Latreille s. str.

(A) *griseifrons* species-group

18. *Simulium choochotei* Takaoka, 2002
19. *Simulium crocinum* Takaoka and Choochote, 2004
20. *Simulium digrammicum* Edwards, 1928
21. *Simulium grossifilum* Takaoka and Davies, 1995
22. *Simulium maenai* Takaoka and Choochote, 2002
23. *Simulium mediocoloratum* Takaoka and Choochote, 2004
24. *Simulium nigrogilvum* Summers, 1911
25. *Simulium rudnicki* Takaoka and Davies, 1995
26. *Simulium sachariti* Takaoka and Choochote, 2004
27. *Simulium yongi* Takaoka and Davies, 1997

(B) *malyschevi* species-group

28. *Simulium siripoomense* Takaoka and Saito, 1996

(C) *multistriatum* species-group

29. *Simulium chainarongi* Kuvangkadilok and Takaoka, 1999
30. *Simulium chaliowae* Takaoka and Boonkemtong, 1999
31. *Simulium fenestratun* Edwards, 1934
32. *Simulium malayense* Takaoka and Davies, 1995

33. *Simulium triglobus* Takaoka and Kuvangkadilok, 1999

(D) *nobile* species-group

34. *Simulium nobile* De Meijere, 1907

35. *Simulium nodosum* Puri, 1933

(E) *striatum* species-group

36. *Simulium chiangmaiense* Takaoka and Suzuki, 1984

37. *Simulium nakhonense* Takaoka and Suzuki, 1984

38. *Simulium quinquestriatum* (Shiraki, 1935)

39. *Simulium thailandicum* Takaoka and Suzuki, 1984

(F) *tuberosum* species-group

40. *Simulium brevipar* Takaoka and Davies, 1995

41. *Simulium rufibasis* Brunetti, 1911

42. *Simulium setsukoae* Takaoka and Choochote, 2004

43. *Simulium tani* Takaoka and Davies, 1995

44. *Simulium weji* Takaoka, 2001

(G) *variegatum* species-group

45. *Simulium barnesi* Takaoka and Suzuki, 1984

46. *Simulium chamlongi* Takaoka and Suzuki, 1984

(H) *Simulium* s. str. unplaced to group

47. *Simulium baimaii* Kuvangkadilok and Takaoka, 1999

In Thailand, only one report has presented data on the distribution and abundance of larvae of black flies (Kuvangkadilok *et al*., 1999a). However, information on polytene chromosomes of simuliid species can be found in the papers by Kuvangkadilok *et al.* (1998, 1999b, 1999c, 2003).

MATERIALS AND METHODS

Materials

Field equipment needed to sample the immature stages of lotic Diptera

1. D-frame net
2. sample containers, white plastic or enamel pan
3. forceps, waterproof pen, scissor, turkey baster, notebook
4. small vials
5. ruler and measuring tape
6. map of Khao Yai National Park
7. pH-meter (Hanna instruments, model HI 8424 microcomputer pH meter)
8. flow meter (Global water GEM, model E U.S. PAT.2.618.986)
9. 70% ethanol and Carnoy's solution (1 part glacial acid: 3 parts 95% ethanol)
10. GPS technology (GARMIN GPS 12XL)
11. cameras

Laboratory equipment needed to process samples

1. forceps, petri dish, slides, pins, and insect boxes
2. specimen vials with caps or stoppers
3. sample labels
4. Olympus SZX-12 stereomicroscope and Nikon E-800 compound microscope
5. SPOT RT© color digital camera and Adobe Photoshop© 7.0 program
6. 50, 60, 70, 80, 90 and 95% ethanol
7. 10% KOH
8. 50% acetic acid
9. 1N HCl
10. xylene

11. n-butylalcohol
12. permount
13. feulgen stain
14. sulfur water
15. general references and published keys on aquatic Diptera (Johannsen, 1934; Takaoka, 1979; Takaoka and Suzuki, 1984; Takaoka and Davies, 1995, 1996; Byers, 1996; Courtney *et al*., 1996; Dudgeon, 1999; Takaoka and Kuvangkadilok, 1999; Courtney, 2000; Takaoka and Choochote, 2004b, 2004c).

Methods

Sample collection and processing

Each site was sampled for 30-35 minutes by holding a D-frame net downstream while the substrate immediately upstream was agitated. Larvae were also hand collected from stones, bedrock, trailing vegetation, twigs, leaves, and assorted debris. All larvae were preserved in 70% ethanol except black fly larvae which were preserved in Carnoy's solution (1 part glacial acetic acid: 3 parts 95% ethanol) in the field. Three to five changes of Carnoy's were used to ensure removal of water and proper fixation of chromosomes. Samples were then stored at $4^{\circ}C$ until they were processed. Black fly pupae were removed from the substrate, placed on damp tissue in small vials, and returned to the laboratory for rearing. Larvae, pupae and emerged adults were identified to genus and species using published keys and keys constructed through a combination of these keys. The concept of genera and species groups follows primarily the classification of Crosskey and Howard (1997). Morphological identifications were based on descriptions by Takaoka and Suzuki (1984) and Takaoka and Davies (1995). Morphological features of black fly stages were photographed. Procedures for slide mounting are detailed in Appendix 1.

Cytotaxonomic study

Larvae for chromosomal analysis were dissected and stained with the Feulgen method (Rothfels and Dunbar, 1953), a standard technique used in black fly cytogenetics. Chromosomal terminology follows that of Rothfels *et al*. (1978), Rothfels (1988), and Adler *et al*. (2004). Most black flies have three chromosomes, labeled I, II, and III in decreasing order of length, with S (short) and L (long) designating chromosome arms, each of which can be identified by major landmarks. Chromosome I has no universal landmarks for the family, but the banding patterns of the IS base and end are highly conserved. The IIS arm is characterized by the Ring of Balbiani, the "shoestring" usually central in the "bulge," and the basal "trapezoid." The IIL arm is universally characterized by "3 sharp" bands near the centromere and the parabalbiani. The IIIS arm often has a splayed end and a "blister" with two heavy bands on one side. There is no universal marker in IIIL. The location of the nucleolar organizer can be a useful diagnostic aid for some taxa. In the present study, landmarks in the IIS arm were emphasized. Chromosomes were photographed under oil immersion. Procedures and directions for preparing and staining larval polytene chromosomes are given in Appendix 2.

This study examined the chromosomes of the subgenus *Gomphostilbia* in Khao Yai National Park in order evaluate cytological evidence for sibling species among morphologically identical species. Of particular interest was the *ceylonicum* species-group, which demonstrate similar characters of the pupal gill filaments and the shape of postgenal cleft in larval stages (Kuvangkadilok *et al.*, 2003). The species in this problematic group need to be sorted out chromosomally and then linked to morphospecies.

Voucher specimens and detailed collection data are deposited in the Department of Entomology, Faculty of Agriculture, Kasetsart University.

Data analysis

Relationship between species occurrence and physical factors were determined by using with further manipulations using the R system (R Development Core Team, 2005).

The sampling effort was examined with plots of species accumulation curves (Colwell and Coddington, 1994) singleton and doubleton curves (Scharff *et al*., 2003), calculation of sampling intensity (Coddington *et al*., 1996) and inventory completion index (Sørensen *et al*., 2002).

The Chao 1 estimator of species richness was chosen to examine from among the many proposed estimators reviewed in Colwell and Coddington, 1994.

Fisher's α index of diversity was chosen and adopted following the recommendations of Southwood and Henderson (2000).

Canonical Correspondence Analysis (CCA) was used to examine community composition patterns related to species among collection sites and physical variables (Ter Braak, 1986).

Place

Location

Khao Yai National Park is the oldest national park in Thailand (established in 1962) and one of the largest (2,168 km^2). The park includes parts of four provinces: Nakhon Ratchasima, Saraburi, Nakhon Nayok, and Prachinburi (Figure 6) and is known for its biodiversity, especially its vertebrates, invertebrates, and vegetation. Much of Khao Yai is covered by tropical and submontane broad-leaved evergreen forests (Gray *et al*., 1994). The rich biota results, in part, from the park's altitudinal range (60-1,351 m). The park is dominated by a large sandstone plateau with many streams and waterfalls.

Climate

The climate is under direct influence of the southwest and northeast monsoons (Tourism Authority of Thailand, 2000). The wet season extends from May to October. The dry season occurs from November to February and the hot season from March to April (National Park Division, n.d.). The air temperature, relative humidity and rainfall were recorded from July 2000 to June 2001 at Khao Yai National Park Headquarters Meteorological Station (Table 2). The average maximum air temperature was 35.14°C in April and average minimum of 7.94°C in January. Annual rainfall was 2,030.9 mm. Highest monthly rainfall in May was 347.2 mm, with the lowest of 0 mm between November 2000 to February 2001. Relative humidity varied between 98.41 to 99.29 %.

Vegetation

There are five main forest types in Khao Yai National Park (Soontornpitakkool, 1996; Chaikuad, 2000; Tourism Authority of Thailand, 2000).

1. Mixed deciduous forest: This forest type is shady but sunlight can still reach the ground below. This forest type occurs in the altitude range 400-600 m above mean sea level (MASL).

2. Dry evergreen forest: This type occurs along the lowland in the east between 100-400 MASL.

3. Tropical evergreen forest: Most of park is covered with this forest type which is found between 400-1,000 MASL. This forest type maintains high humidity in a dense cover of perennial trees. Little sunlight can reach no much to the ground below. Vegetation in the lower elevations is the same as in the dry evergreen forest.

4. Hill evergreen forest: This forest occurs at altitudes over 1,000 MASL. Most vegetation consists of gymnosperms. The ground flora is typically shrubs, ferns and orchids.

5. Grassland and secondary forest: This vegetation is a result of human activities such as shifting cultivation, road construction, and fires.

Wildlife

Khao Yai National Park is covered with a variety of forest types and very suitable as wildlife habitat. The number of wildlife (i.e., vertebrate) species includes 71 species of mammals, 315 or more species of birds, and 70 species of reptiles and amphibians. Data on the number of insect species in the park is scarce. The species of butterflies recorded during 1961-1966 was 182, but recent data suggest more should be present (Tourism Authority of Thailand, 2000).

Collection sites.

The study area included a range of lotic habitats, from temporary roadside creeks to permanent waterfalls. Monthly collections were made at 20 sites; half of the sites were sampled alternate week. Most sampling sites were relatively undisturbed by human activity, though three sites sampled that were disturbed by tourist activity. Levels of disturbance were assessed following Damrak (2004). A site description conducted qualitative assessment of canopy cover and primary substrate, the latter including the categories bedrock (B), rock (R), gravel (G), and sand (S). Based on relative abundance of different substrates, five substrate classes were recognized: R-G-S, B-R-G, G-R-S, G-S-R and S-R-G. Substrate assessments here differ slightly from those of Damrak (2004) because individual assessments were made with particular attention to the different target taxa. Collecting site descriptions and photographs are shown in Table 3. Stream width, depth, velocity, temperature, pH, and site location (latitude, longitude and altitude) were measured at the time of each sample.

Duration

Sites were sampled every month from June 2000 to July 2001, with sporadic sampling also being carried out from 2003 to 2004, in order to obtain additional specimens or life stages that were rare during the year of intensive sampling. Specimen identification and data analysis were conducted over 3 years in the laboratory.

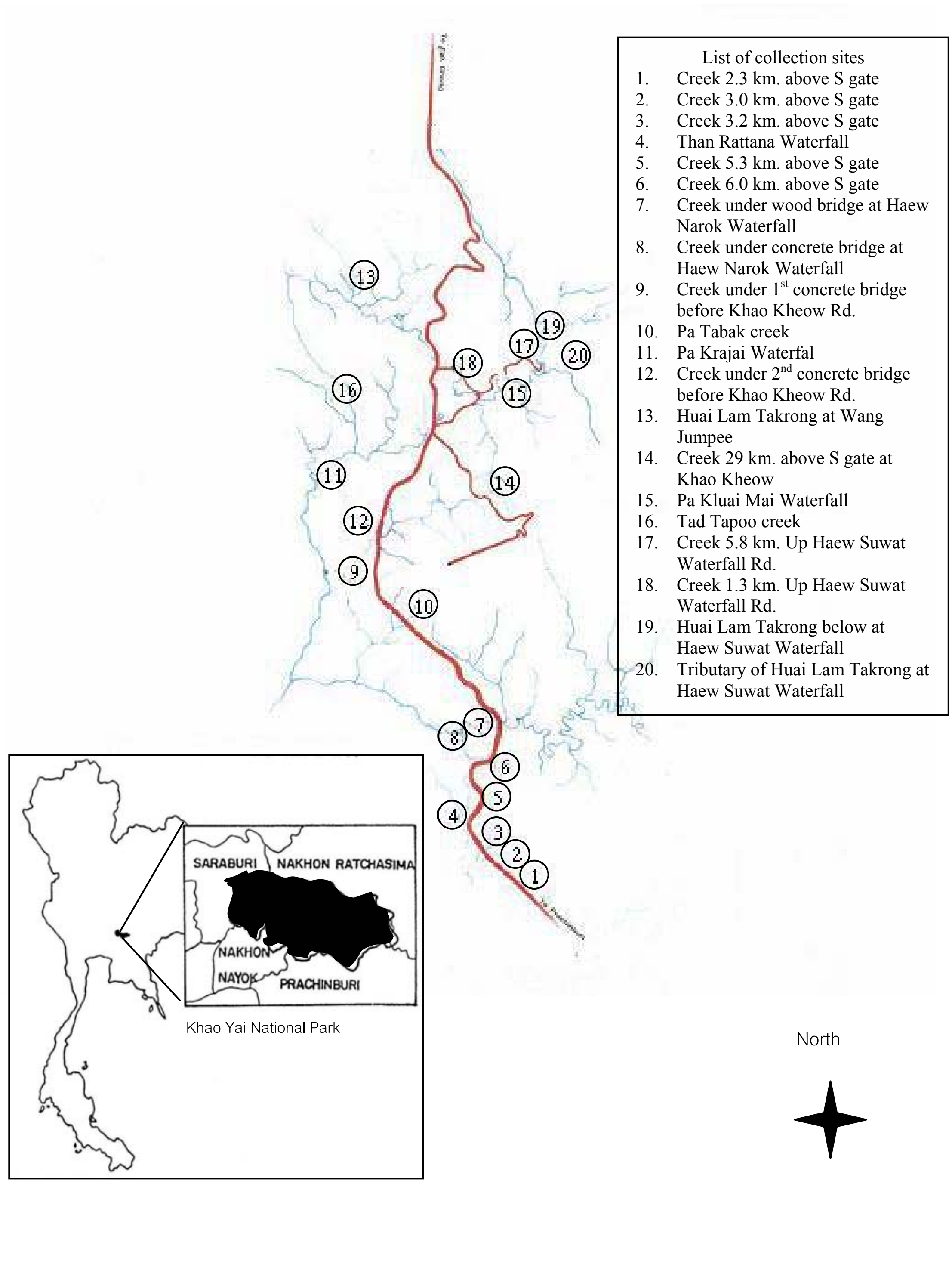


Figure 6. Khao Yai National Park, showing black fly collection sites

Source: National Park Division, n.d.

Table 2 Air temperature (°C), relative humidity (%) and rainfall (mm) at Khao Yai National Park between July 2000 and June 2001

Month		Air temperature (°C)		Average relative humidity (%)	Rainfall (mm)
		Average minimum	Average maximum		
July	2000	-	29.70	99.09	297.3
August		-	29.47	99.12	319.2
September		10.48	31.69	99.1	327.8
October		10.10	31.45	99	318.6
November		10.29	31.36	99	0
December		8.63	30.00	98.54	0
January	2001	7.94	30.42	98.41	0
February		9.40	31.17	99.14	0
March		11.16	31.25	99.29	36.4
April		11.26	35.14	98.66	114.9
May		11.01	32.98	98.87	347.2
June		11.05	32.82	98.93	269.5
Total					2,030.9

Table 3 Collection site descriptions at Khao Yai National Park and photographs

Site	Image of site	Canopy	Primary substrata	Disturbance
1. Creek 2.3 km. above S gate		Closed	Rock Gravel Sand	Undisturbed
2. Creek 3.0 km. above S gate		Closed	Rock Gravel Sand	Undisturbed
3. Creek 3.2 km. above S gate		Open	Rock Gravel Sand	Undisturbed
4. Than Rattana Waterfall		Open	Bedrock Rock Gravel	Disturbed
5. Creek 5.3 km. above S gate		Closed	Gravel Rock Sand	Undisturbed

Table 3 (Continued)

Site	Image of site	Canopy	Primary Substrata	Disturbance
6. Creek 6.0 km. above S gate		Closed	Gravel Rock Sand	Undisturbed
7. Creek under wood bridge at Haew Narok Waterfall		Closed	Gravel Sand Rock	Undisturbed
8. Creek under concrete bridge at Haew Narok Waterfall		Open	Rock Gravel Sand	Undisturbed
9. Creek under 1st concrete bridge before Khao Kheow Rd.		Open	Rock Gravel Sand	Undisturbed
10. Pa Tabak creek		Closed	Rock Gravel Sand	Undisturbed

Table 3 (Continued)

Site	Image of site	Canopy	Primary Substrata	Disturbance
11. Pa Krajai Waterfal		Open	Bedrock Rock Gravel	Undisturbed
12. Creek under 2nd concrete bridge before Khao Kheow Rd.		Open	Rock Gravel Sand	Undisturbed
13. Huai Lam Takrong at Wang Jumpee		Open	Rock Gravel Sand	Undisturbed
14. Creek 29 km. above S gate at Khao Kheow		Closed	Rock Gravel Sand	Undisturbed
15. Pa Kluai Mai Waterfall		Open	Rock Gravel Sand	Disturbed

Table 3 (Continued)

Site	Image of site	Canopy	Primary Substrata	Disturbance
16. Tad Tapoo creek		Closed	Sand Rock Gravel	Undisturbed
17. Creek 5.8 km. Up Haew Suwat Waterfall Rd.		Open	Gravel Sand Rock	Undisturbed
18. Creek 1.3 km. Up Haew Suwat Waterfall Rd.		Closed	Gravel Sand Rock	Undisturbed
19. Huai Lam Takrong below at Haew Suwat Waterfall		Open	Bedrock Rock Gravel	Disturbed
20. Tributary of Huai Lam Takrong at Haew Suwat Waterfall		Open	Rock Gravel Sand	Undisturbed

RESULTS AND DISCCUSION

1. Physical variables

1.1 Altitude

The twenty sample sites ranged over 764 m, from the lowest site at 188 m (site 1) to the highest at 952 m (site 14). The 952 m was considered an outlier, with no other sites near this altitude, with most of the high altitude sites being in the 650-750 m range (Table 4).

1.2 Drought

Not all streams were perennial, with most of the lower altitude streams experiencing drought of more than four months during the dry season (Table 4). Most of the higher altitude streams did not experience drought although the highest altitude site (site 14) was an exception experiencing 4 months of drought.

All streams were impacted by the diminishing rains of the dry season, some to the extent that all surface water disappears from the streambed. However, the onset and lifting of drought periods varied between sites. Most streams (six of the ten streams experiencing drought) first experienced drought during December-January although site 6 experienced drought as early as late November. Drought had lifted at site 1 (lowest altitude), the last to receive drought relief in late June, while site 14 was relieved as early as mid April.

1.3 Water temperature

Annual median water temperatures ranged from a low of 21.5°C (site 4) to 24.7°C (site 14) (Table 4). Generally water temperature and altitude showed a monotonic inverse relationship (ρ = -0.6326, $p < 0.01$) which was quite linear (r^2 = 0.5696, $p < 0.001$). However, sites 17 and 18 were much warmer than expected for their altitude, without these sites the relationship is even stronger (ρ = -0.7778, $p < 0.001$; $r^2 = 0.7662$, $p < 0.001$).

There was considerable seasonal variation in water temperature with most sites having variation greater than 4°C while site 13 had a range of 8°C (Figure 7).

1.4 pH

Median site pH ranged from 5.69 (site 14) to as high as 7.91 (site 6), though most sites were in the range 6.69-7.32 with a median of 6.84 (Table 4).

There was little seasonal variation in pH (Figure 8) and no clear pattern although there was trend for "drying" sites to experience decreasing pH-presumably as a result of increasing concentrations of organic acids.

1.5 Stream size

"Stream size" *per se* was not measured. However, stream width (maximum and minimum), stream depth (maximum and minimum) and current velocity (maximum and minimum) were all measured as separate parameters. However, all six of these parameters were found to be highly multicollinear (Table 5) and so collectively can be treated as indicators of "stream size", the high collinearity of all the parameters meaning that only one parameter need be examined as a stream size measure. Maximum stream width was chosen.

Median maximum stream width ranged from the smallest stream at site 18 with a stream width of only 1 m to the largest stream at site 19 with a maximum width of 20 m (Table 4).

There was a good deal of seasonal variation in maximum stream width with different sites also having different levels of variation (Figure 9). The greatest variation was seen at site 8, which had a spate width of 35 m during July 2000 but was reduced to only 1 m during the dry season (March-April 2001). Of course, those sites that experienced drought (stream width maximal of 0 m) could be regarded as having even greater relative variation in stream width.