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A 23kD MOLECULE OF *PLASMODIUM FALCIPARUM* BINDS SPECIFIC IgG FROM SPLENOMEGALIC, PARASITEMIC, BUT ASYMPTOMATIC CHILDREN

——A PILOT STUDY IN A MALARIOUS COMMUNITY IN PALAWAN, THE PHILIPPINES——

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Abstract: A pilot sero-epidemiological study was performed in Palawan, The Philippines, in a community within which annual malarial transmission takes place. Sera obtained from splenomegalic and asymptomatic children with current falciparum infection (Group I) were examined using a Western blotting technique to determine the reactivity to electrophoresed antigenic molecules of *Plasmodium falciparum* (*P.f.*). Group I sera consistently exhibited specific reactivity of IgG to a 23kD molecule, to which sera obtained from another group of asymptomatic children with neither parasitemia nor splenomegaly (Group II) exhibited no distinct reactivity. A 58kD molecule was also exhibited by the Group I sera, while several Group II sera reacted with a 52 kD molecule in association with the 47kD which was reported to be indicative of present and/or recent past clinical episode of falciparum infection. Determination of the immunological features of the antigenic molecules of parasites by this type of sero-epidemiological study will provide a new assay system for evaluation of immune status in communities endemic for malaria.

Key words: *Plasmodium falciparum*, malaria, epidemiology, antigenic polypeptides

INTRODUCTION

It is generally accepted that responses to malarial infection vary considerably depending on the immune status of the affected individual (Marsh, 1992). Although *P.f.* infection can be fatal to those without immunity, the same parasites cause asymptomatic malaria in highly immune individuals who have experienced repeated infections. Trials to determine the immune status of inhabitants of a malarious region have been made using a Western blotting technique, with examination of sera obtained from individuals with various histories of *P.f.* infection . Thelu *et al.*(1991) carried out a longitudinal study in Burkina Faso to examine the development of immune responses to *P.f.* malaria. Three bleedings were carried out before, during, and after the seasonal peak of transmission, and the predominance of reactivities to 115 and 103 kD antigen molecules was noted. Kano *et al.* (1990a) specified the 47kD *P.f.* antigen in a study on sera obtained from Japanese patients with acute naive falciparum infections. This molecule also specifically reacted with sera collected from parasitemic and symptomatic patients in endemic areas in The Sudan. It was thus shown that the 47kD band was indicative of present and/or recent past *P.f.* infection with clinical manifestations.

The present study was carried out in a village endemic for malarial infection in Palawan, The Philippines, with special focus on splenomegalic children who had developed parasitemia but manifested no significant clinical signs of malaria. This group of children was thought to be resistant to malaria, and their sera were tested with a Western blotting technique. The results were compared with *P.f.* bands exhibited by sera of another group of children who manifested neither splenomegaly nor parasitemia. Certain immunologic features of the defined antigenic molecules and their usefulness in immuno-epidemiologic studies of malaria

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are to be discussed.

SUBJECTS, MATERIALS AND METHODS

Subjects studied

This study was conducted in the southern part of Puerto Princesa City, the capital of Palawan province, at the end of the rainy season, December 1992. In a previous study, it was found that the forest fringe was the focus of transmission of malaria within the study area (Rivera *et al.*, 1993). Both falciparum and vivax malaria were prevailing in this community. Many children residing in this part of the community were found to exhibit splenomegaly. In the 0-9 year age group, 19 of 38 children examined had palpable spleens. Interviews with mothers revealed that splenomegalic children had suffered repeated febrile and/or malaria attacks; some cases of malaria were confirmed by examination of peripheral smears. However, there were no cases of severe malaria or death due to malaria in previous years. After explaining the study and obtaining permission, 5ml of blood was collected from each of 11 asymptomatic children (2 - 9 years old) with splenomegaly (Group I). Children who resided near the seashore within the study area did not have palpable spleens. As a non-splenomegalic group (Group II), 10 of these children, 4-12 years of age, were included in the study. Giemsa-stained thin and thick smears were also prepared for each donor. The serum from each donor was separated in a laboratory of the local hospital, and was kept at -20 °C. The frozen serum specimens were brought to Japan for immunofluorescence testing and Western blotting studies.

Parasitological study

Both thin and thick blood films were microscopically studied. The examinees were considered parasitologically negative when no parasites were detected in any microscopic field of either type of blood smear.

Serological study

An indirect immunofluorescent antibody test (IFAT) was performed on all serum specimens using the method reported by Kano *et al.* (1990b). The secondary antibody employed was an FITC-conjugated rabbit immunoglobulins to human IgG (γ-chain specific; Dako, Denmark).

Parasites used in the study

An established strain of *P.f.* of Gambian origin and adapted to *in vitro* culture was used in this study. This strain, SGE1, was donated by Professor Ambroise-Thomas in 1979, and was maintained in our laboratory by continual *in vitro* culture with occasional freezing . The parasite had been cultured in an ordinary CO_2 incubator (Miyagami and Waki, 1985) using RPMI 1640 with a 10% volume of human O type RBC with the addition of 10% human sera (Trager and Jensen, 1976). The high pathogenicity of this strain for *Aotus trivirgatus* was unchanged, and the parasites, even after continuation of *in vitro* culture for more than 10 years, caused fatal infections when introduced into monkeys. However, knob formation on infected RBCs had been lost after the culture.

Western blotting study

Asynchronous cultured parasites, in which were included no gametocytes, were harvested when parasitemia reached >20%, and the infected RBCs were hemolyzed with 0.05% saponin. The lysate was centrifuged with a refrigerating centrifuge at 3,600 rpm for 15 minutes, and then washed 3 times with phosphate buffered saline (PBS). After the final wash, the sediment was dissolved in the antigen solution (2.3% SDS, 5% 2-ME, 1 mM PMSF, 0.0625 M Tris-HCl, *pH*6.8). The resulting material was stored at -35°C until use, and was adjusted to a concentration of 30μg/20μl when applied to one-dimensional electrophoresis in 10% acrylamide gels, following the method of Laemmli (1970). The reference markers used were SDS-PAGE Standards (BioRad, CA). Western blotting was performed by electrotransfer to polyvinylidene difluoride filter (PVDF) (Clear Blot-P, ATTO, Tokyo) using Horizeblot (ATTO, Tokyo) with absorbent papers soaked in blotting buffer (0.1M Tris-HCl, 0.192M Glycine, 20% methanol). Non-specific reactions to the PVDF were blocked by soaking the membrane in 10% skimmed milk in PBS at 4°C overnight and then washed three times with PBS. The blotted PVDF strips were incubated with each serum sample at 37°C for 2 hours. After washing, the strips were then incubated with peroxidase-labelled goat anti-human IgG (Dako, Denmark) at 37°C for 1 hour. The substrate for the enzyme reaction was from a Konica immunostain kit (Konica, Tokyo, Japan) and the molecular weights (M.W.) of fractionated antigen bands were determined using the curves obtained with the Standard markers.

RESULTS

Interviews, spleen examination and parasitological and serological testing were performed for the children

Table 1 *Profiles of children, with schematic representation of results of electrophoresis, slide examination and IFAT.*

	No.	Age	Sex	Symptoms	Splenomegaly	Slide	IFA titer *P.f.*	IFA titer *P.v.*	Antigenic molecules (kD) 23	31	37	47	52	54	58	70	74	83	94	100	102	105	120	160
Group I	1	9	F	−	+	*P.f.* & *P.v.*	4096	64	■	·	·	·	·	·	■	·	·	·	·	·	■	·	·	·
	2	6	M	−	+	*P.f.*	4096	64	■	·	·	·	·	·	■	·	·	·	·	·	■	·	·	·
	3	4	F	−	+	*P.f.*	1024	16	■	■	·	■	·	·	·	·	·	·	·	·	·	·	·	·
	4	2	F	−	+	*P.f.*	256	16	■	·	·	·	·	·	■	·	·	·	·	·	·	·	·	·
	5	3	M	−	+	*P.f.*	1024	16	■	■	·	·	·	·	·	·	·	·	·	·	·	·	■	·
	6	4	F	−	+	−	256	64	■	■	·	·	·	■	·	·	·	·	·	·	·	·	■	■
	7	8	F	−	+	*P.f.*	64	16	■	·	·	■	·	·	■	·	·	·	·	·	·	·	·	·
	8	8	M	−	+	*P.f.*	1024	64	■	·	·	·	·	·	·	·	·	·	■	·	·	·	·	·
	9	5	F	−	+	*P.f.*	1024	16	■	·	·	·	·	·	·	·	·	■	■	·	·	·	·	·
	10	5	M	−	+	*P.f.*	1024	16	■	·	■	■	■	·	·	·	·	·	·	·	·	■	·	·
	11	8	M	−	+	−	1024	16	■	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Group II	12	8	F	−	−	−	16	<4	·	·	·	■	■	·	·	·	·	·	·	·	·	·	·	·
	13	4	F	−	−	−	<4	<4	■**	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
	14	11	F	−	−	−	64	16	■**	■	·	■	■	·	·	·	·	·	·	·	·	·	·	·
	15	6	F	−	−	−	16	4	·	·	·	■	■	·	·	·	·	·	·	·	·	·	·	·
	16	6	F	+*	−	*P.v.*	64	256	·	·	·	■	·	·	·	·	·	·	·	·	·	·	·	·
	17	8	M	−	−	−	16	16	·	·	·	·	·	·	·	·	■	·	·	·	·	·	·	·
	18	12	F	−	−	−	64	16	·	·	■	■	■	·	·	·	·	·	·	·	·	·	·	·
	19	9	F	−	−	−	64	4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
	20	9	M	−	−	−	16	4	·	·	·	·	·	■	·	·	·	·	·	■	·	·	·	·
	21	11	M	−	−	−	256	16	■**	·	·	·	·	■	·	■	·	·	·	■	·	·	·	·

* fever and chills, ** faint

participating in the study. Both parasitological and serological findings, shown in Table 1, demonstrated predominance of falciparum malaria over vivax malaria within the study area. All children studied had *P.f.* titers higher than *P.v.* titers by the IFAT, but one who had *P. v.* parasites in the blood smear showed *P.f.* titer at 1:64 versus *P.v.* titer at 1:256. This epidemiological finding was also obtained in the previous study performed in the same area (Rivera *et al.*, 1993). In the present study, two distinctive groups of children could be differentiated using the lists in Table 1. All children in Group I exhibited splenomegaly and high *P.f.* titers (1:64 - 1:4096). Nine of these 11 children was parasitemic. Regardless of apparent infection, however, none of them manifested clinical signs of malaria. On the other hand in Group II, 9 out of 10 children examined manifested neither splenomegaly nor *P.f.* parasitemia. One child (No. 16) in this group had contracted vivax infection. *P.f.* titers in Group II ranged from 1:16 - 1:256, with one negative case. Again, clinical signs were not found in this group, except in the case of the girl with vivax malaria. These findings suggest that the children in Group I were splenomegalic and resistant to *P.f.* malaria and had high serum titers to *P.f.* parasite, and appeared to support the hypothesis that the children in Group I had been attacked by repeated *P.f.* infections and eventually had developed resistance to clinical malaria. On the other hand, individuals in Group II seemed to have had fewer episodes of *P.f.* infection than those in Group I, as indicated by lower titers of the specific antibodies.

In the next part of the study, particular parasite molecules reactive to specific IgG in the collected sera of the two groups were studied by Western blotting analysis. A notable difference between sera from the two groups was evident in the blotted maps (Table 1). A 23kD molecule was consistently reactive with IgGs of Group I children who were splenomegalic and parasitemic but asymptomatic. The same molecule, however, was not distinctly reactive with IgGs of Group II children who were asymptomatic, not parasitemic and had a non-palpable spleen. The pattern of the other bands present varied considerably by serum specimen, and thus consistent findings concerning the immunological features of the corresponding molecules could not be obtained. However, many more bands were present for Group I sera than for Group II sera. In particular, bands equal to or higher than 102kD were reactive with several Group I sera. In addition, 4 of the 11 children in Group I exhibited IgG reactivity to a 58kD molecule, while none of the 10 sera from Group II exhibited this band. On the other hand, 4 of 5 sera reactive to the 47kD molecule in Group II also exhibited a 52kD molecule.

DISCUSSION

Using a Western blotting technique, we have shown that the 23kD *P.f.* molecule binds specific IgGs from splenomegalic but asymptomatic children with parasitemia. The children of this group (Group I) showed higher IFAT titers than those of non-splenomegalic and non-parasitemic children (Group II). Indeed, many more bands were exhibited by sera from Group I than by the sera from Group II, which had lower IFAT titers. It might be suggested that the 23kD molecule was exhibited by sera because antibodies to the parasite components were simply present at high concentrations in Group I. However, this possibility could be ruled out, since some of the sera in Group II also exhibited high IFAT titers (eg. No.14=1:64, No.18=1:64, No.21=1:256), while the reactivities of these sera to the 23kD band were null or faint. In contrast, some antigen bands, such as the 47kD and 52kD bands, were exhibited by low-titer (1:16) sera in Group II and not by high-titer sera in Group I. These findings support the view that reactivity to the 23kD molecule is specific to asymptomatic children with splenomegaly and parasitemia, and suggest that reactivity of serum to the 23kD molecule is a reflection of resistance to symptomatic malaria. Further work should still be required to determine whether the reactivity was a unique feature of the strain used or whether this phenomenon could be substantiated with other isolates and sera taken from other geographical locations.

One of the present authors previously reported that the 47kD molecule was strongly exhibited by sera of non-immune Japanese patients who had contracted falciparum malaria (Kano *et al.*, 1990a). This was also true of Sudanese children who lived in an endemic locality and developed fever and parasitemia. It therefore appeared that presentation of the 47kD molecule indicated a present or recent symptomatic episode of malaria developed in susceptible individuals. This finding is consistent with those of the present study. In Group II, the 47kD molecule was exhibited by sera of 5 of 10 non-splenomegalic children who were considered less resistant to clinical malaria. In fact, results of a questionnaire obtained for those 5 children supported their recent history of clinical malarial infection. On the other hand, in Group I, only 3 children exhibited the 47kD band, suggesting that the 47kD molecule may be more closely related to the presence of severe symptoms in patients. Although the number of sera studied was small, association of the 47kD with the 52kD bands was also noted in the Group II, suggesting the presence of an immunogenic relationship between these two molecules. The other antigenic molecules detected in this study require further investigation to determine their immuno-epidemiological significance.

The objective of the present pilot study is to provide a new malariometric index using defined molecules. Using presently available methods, it is difficult to differentiate those who are susceptible and those who are protected from clinical disease irrespective of apparent parasitemia. Determination of these criteria is indispensable for establishment of a rational malaria program, especially for children who are the major target of the control operation. In addition, this kind of study will provide suggestions for determination of parasite molecules as candidates for vaccine development using an immuno-epidemiological approach.

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A SURVEY OF INTESTINAL PARASITIC INFECTIONS IN SAN NARCISO, VICTORIA, ORIENTAL MINDORO, PHILIPPINES

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Key words : Parasitological survey, San Narciso, Victoria, Oriental Mindoro, Philippines,

Parasitism is usually considered as a common condition in rural populations in developing countries. Intestinal parasitic infections are not unusual in these populations. Here we describe the prevalence of intestinal helminth and protozoan infections among the inhabitants in San Narciso, Victoria, Oriental Mindoro, Philippines where malaria and schistosomiasis are endemic (Saniel, 1951).

The study area San Narciso, Victoria is on Mindoro Island, the seventh largest island of the Philippines. The mountains lie at almost full length of the island from the north to the south, and the island is divided into two provinces; Oriental Mindoro, the east of the mountain ridge and Occidental Mindoro, the west of the mountain ridge. The economy of this island is based on agriculture, especially rice farming. The barrio, San Narciso is situated at the west shore of Lake Naujan in Oriental Mindoro.

In determing the prevalence of intestinal parasitic infections, the samples of 242 individuals aged 1 to 88 (142 males and 100 females) screened in the barrio were allocated proportionately in the age-stratified population. The sample included approximately 24% of the population. In August 1994, each inhabitant was given a stool container and instructed to return it the following day with stool specimen. Instructions were given to ensure procurement of freshly passed stool samples in their local dialect. Stool examination was made with formalin - ether concentration technique.

Of 242 persons examined, 190 (78.5%) were found infected with one or more intestinal parasites (Table 1). Seventy two (29.8%) had one parasite infection, 57 (23.6%) two different parasites, 42 (17.4%) three parasites, 12 (5.0%) four parasites, 5 (2.1%) five parasites and 2 (0.8%) six parasites.

The eggs of helminths and cysts of protozoa found are *Ascaris lumbricoides, Trichuris trichiura,* hookworm, *Schistosoma japonicum, Fasciola* sp., *Entamoeba histolytica, E. coli, Endolimax nana, Giardia lamblia* and *Iodamoeba buetschlii. T. trichiura* (48.8%) was the predominant helminth in the barrio, followed by *A. lumbricoides* (43.8%). Hookworm prevalence was relatively low (11.6%). Eggs of *S. japonicum* and *Fasciola* sp. were found in 5.4% and 0.8%, respectively. No tapeworm eggs were detected throughout the survey. The most prevalent protozoan was *E. coli* (20.7%) in this study area, followed by *E. nana* (12.0%) and *G. lamblia* (10.3%). Both *E. histolytica* and *I. buetschlii* were detected in 5.4%. No attempt was made to identify the species of hookworm although previous studies suggested that all infections were with *Necator americanus* (Carney *et al.*, 1981). The prevalence of hookworm infection was significantly higher in males than in females ($p<0.01$) while *Ascaris* and *Trichuris* infection were not different between sexes. The protozoan prevalence showed no clear difference between sexes.

Age-prevalence profiles for three major soil-transmitted helminths, *Ascaris, Trichuris* and hookworm infections are shown in Fig. 1. *Trichuris* infection attained an early peak of over 60% in the age-group 0-10 years, then it showed a definite downward trend with increasing age. A similar profile was observed in *Ascaris* infection. Studies of the relationship between age and the prevalence of ascariasis in a range of

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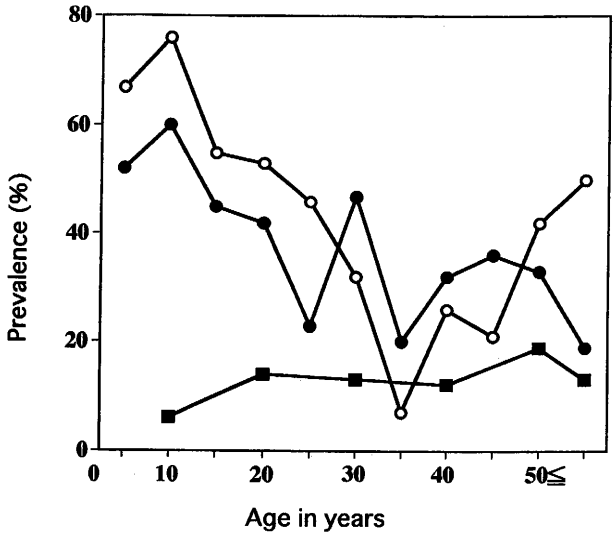

Figure 1 Age prevalence profiles for *A. lumbricoides*, *T. trichiura* and hookworm infections in San Narciso, Oriental Mindoro, Philippines in August 1994

LEGEND : —●— ; *Ascaris lumbricoides*
—○— ; *Trichuris trichiura*
—■— ; Hookworm

Table 1 Prevalence of intestinal protozoan and helminthic infections by sex in San Narciso, Victoria, Oriental Mindoro, Philippines in August 1994.

PARASITE	No. of examined		
	MALE	FEMALE	TOTAL
	142	100	242
Protozoan			
Entamoeba histolytica	7†(4.9)	6(6.0)	13(5.4)
E. coli	29(20.4)	21(21.0)	50(20.7)
Endolimax nana	17(12.0)	12(12.0)	29(12.0)
Giardia lamblia	11(7.7)	14(14.0)	25(10.3)
Iodamoeba buetschlii	7(4.9)	6(6.0)	13(5.4)
Helminth			
Ascaris lumbricoides (fer.)	48(33.8)	35(35.0)	83(34.3)
A. lumbricoides (unf.)	10(7.0)	13(13.0)	23(9.5)
Trichuris trichiura	71(50.0)	47(47.0)	118(48.8)
Hookworm	23(16.2)	5(5.0)	28(11.6)
Schistosoma japonicum	9(6.3)	4(4.0)	13(5.4)
Fasciola sp.	1(0.7)	1(1.0)	2(0.8)

LEGEND: † : No. of positives
fer. = fertilized egg
unf. = unfertilized egg
Number in parentheses indicates the percentage

communities reveals the existence of two patterns (Crompton, 1989). In one the prevalence value remains high regardless of the age of the subjects and in the other the value declines once the subjects have attained adulthood as seen in Fig. 1. It remains unclear as to why the prevalence declines markedly with age in some communities and not with others. Obvious explanations suggest that some degree of immunity may have developed (Jones, 1977) or that behavioural changes that accompany adulthood result in reduced exposure to infective eggs. There is, however, still no solid evidence for either of these propositions (Crompton, 1989).

In contrast, the prevalence of hookworm infection in the age-group 0-10 years was less than 10%, then reached a peak at age of 20 and remained to be plateau of 12-19% throughout adulthood. Hookworm infection is acquired farther from the immediate vicinity of the house and the mates, being more active, run a greater risk of acquiring the infection than do young children (Pesigan *et al.*, 1958). Due to the low prevalence of *S. japonicum* and *Fasciola* sp. infections, the sample of infected cases within each age class was too small for meaningful age-stratified analysis.

The prevalence of *E. coli* and *E. nana* infections was not remarkably age-dependent while *G. lamblia* prevalence was already 19.4% in the youngest age class (0-10 years) and then decreased gradually with age.

In 1978, Carney *et al.* (1981) examined fecal samples in San Narciso and found that *Ascaris* (78.5%) infection was commonest and being followed by *Trichuris* (76.1%), hookworm (27.8%) and *Schistosoma* (15.9%) infections. The most prevalent protozoan was *E. coli* (17.1%), being followed by *E. nana* (3.9%), *G. lamblia* (3.2%) and *E. histolytica* (2.2%).

One year later, in 1979, Cabrera and Cruz (1983) examined 364 fecal samples for geohelminths in San Narciso and found that *Trichuris* infection was most prevalent (84.9%), being followed by *Ascaris* (74.7%) and hookworm (14.6%). Although data of their studies cannot be directly compared with those of our studies, it seems likely that in San Narciso, Victoria, Oriental Mindoro, geohelminth infections have decreased to some extent in the last one and a half decades, while protozoan infections have remained almost unchanged or showed an upward trend. At present time, no reasonable explanation on the cause of this phenomenon is possible.

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SIMULIUM (*SIMULIUM*) *YONGI* SP. NOV. (DIPTERA: SIMULIIDAE) FROM PENINSULAR MALAYSIA

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Abstract: *Simulium* (*Simulium*) *yongi* sp. nov. is described based on reared females, reared males, pupae and mature larvae collected from Peninsular Malaysia. The immatures of this species were previously misassociated with an adult female holotype of *S.* (*S.*) *pahangense* Takaoka and Davies. This new species is characterized by the combination of the following features: scutum unpatterned, tarsal claws simple and anterior gonapophysis enlarged and widely bare along posterior margin in the female; ventral plate transverse without toothed margins and style with a prominent basal protuberance pointed dorsally in the male; and gill with six filaments per side and cocoon wall-pocket-shaped, with very thin and almost transparent wall in the pupa. This species seems to be related to *S.* (*S.*) *rudnicki* Takaoka and Davies from Langkawi Island, Peninsular Malaysia, *S.* (*S.*) *ufengense* Takaoka from Taiwan, *S.* (*S.*) *fuzhouense* Zhang and Wang from Fujian, south China, and *S.* (*S.*) *taipokauense* Takaoka, Davies and Dudgeon from Hong Kong, from which it differs readily by the darker legs.

Key words: black fly, Simuliidae, *Simulium*, Malaysia, new species

Our recent survey of black flies in Peninsular Malaysia has found a new black fly species belonging to the subgenus *Simulium* s. str. The pupal and larval stages of this species are similar to those misassociated with the adult female holotype of *Simulium* (*Simulium*) *pahangense* Takaoka and Davies, 1995.

This new species is here described based on the reared females, reared males, pupae and mature larvae.

Holotype and most paratype specimens will be deposited at the Natural History Museum (BMNH), London, U.K.

Simulium (*Simulium*) *yongi sp. nov.*

[*Simulium* (*Simulium*) *pahangense* Takaoka and Davies, 1995: part (pupa and larva misassociated with adult holotype)]

DESCRIPTION. **Female.** Body length 3.0-3.5 mm. ***Head***. Narrower than width of thorax. Frons brownish black, shiny, not pruinose, with several dark stout hairs along lateral margins; frontal ratio 1.2:1.0:1.1; frons-head ratio 1.0:4.2. Fronto-ocular area (Fig. 1) well developed. Clypeus brownish black, shiny, with silvery iridescent pruinosity, moderately covered with dark stout hairs. Antenna composed of 2+9 segments, brownish black except scape, pedicel and base of lst flagellar segment yellow when viewed from dorsally, or brownish black except scape, pedicel and a few basal flagellar segments yellow to dark yellow when viewed from front; each flagellar segment with a distinct pit distally on each lateral surface, gradually becoming smaller in size toward apical tip, except those on segment 9 located medially (Figs. 2 & 3); each pit provided densely with minute sensilla (Fig. 4); each flagellar segment also with a small pit distally on dorsal surface, except that on segment 9 located medially. Maxillary palp brownish black, with 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.9; 3rd segment not enlarged; sensory vesicle of moderate size, elliptical, with rugged surface, 0.3 × length of 3rd segment, with a moderate round opening distally. Maxillary lacinia with 13 inner and 13 outer teeth. Mandible with ca. 30 inner and 11 or 12 outer teeth. Cibarium (Fig. 7) with a cluster of ca. 50 conical tubercles medially. ***Thorax***. Scutum brownish black, shiny, thinly whitish grey pruinose, moderately covered with yellow pubescences as well as dark brown ones, inter-

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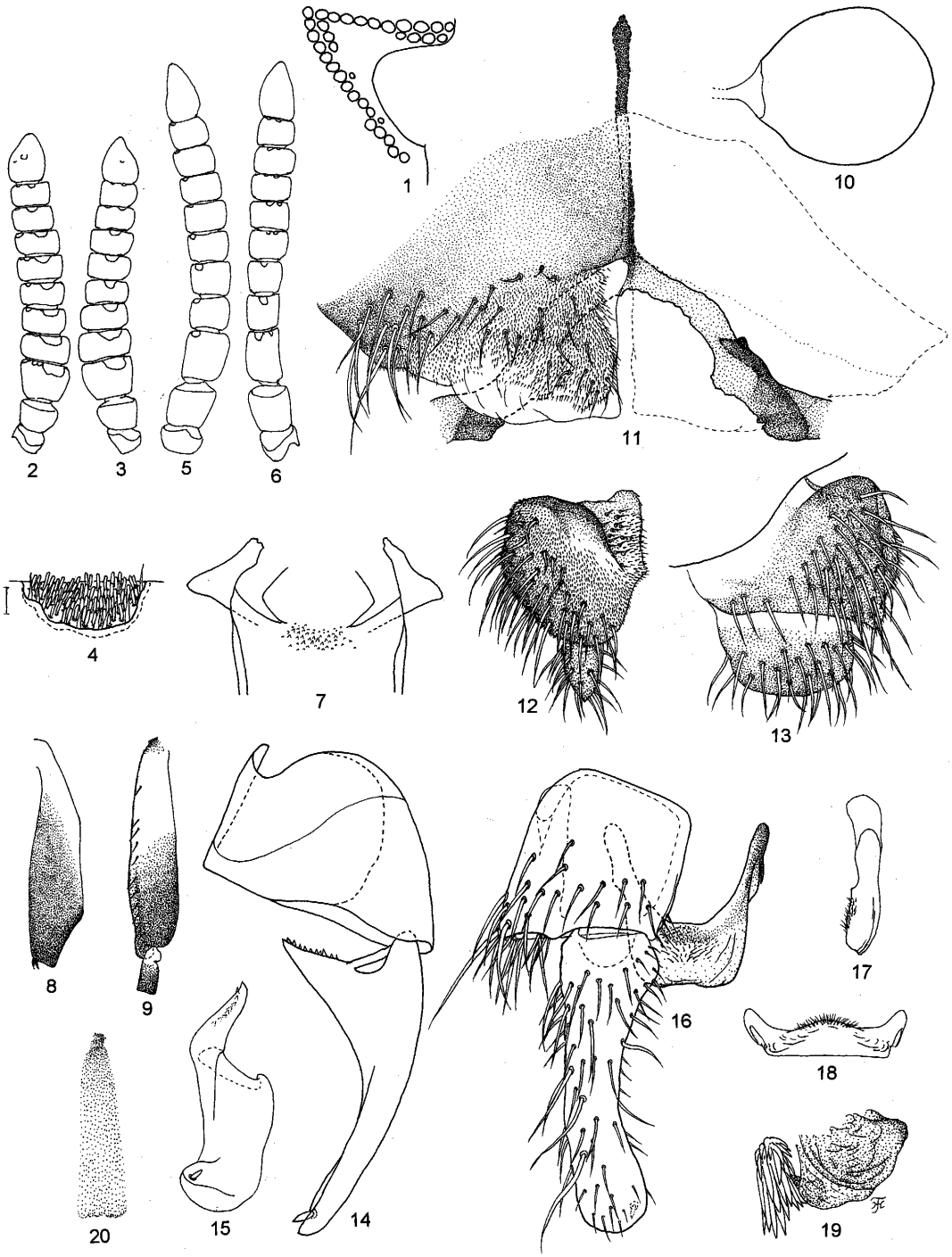
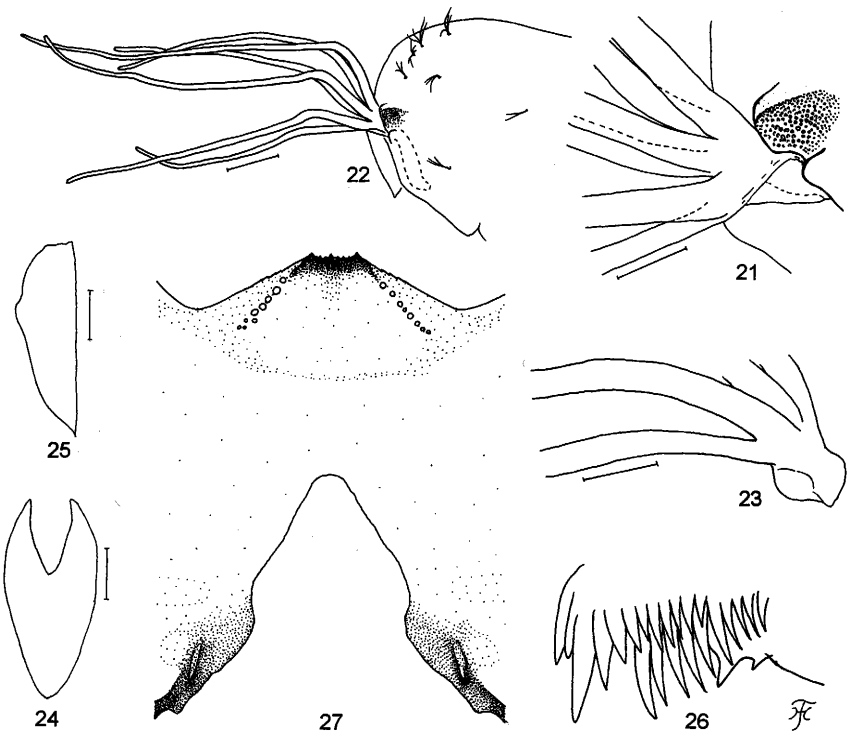
spersed with long, upright dark hairs on prescutellar area. Scutellum brownish black, with long dark hairs as well as short hairs. Postscutellum brownish black, shiny, whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, and bare. ***Legs***. Foreleg: coxa pale yellow; trochanter yellowish brown; femur dark brown; tibia largely white except distal 1/5 brownish black and inner surface of distal 1/2 brown to brownish black, with a large white sheen on outer surface in light; tarsus brownish black; basitarsus dilated, ca. 4.8 × as long as its greatest width. Midleg: coxa dark brown; trochanter dark brown with base yellow; femur dark brown except outer surface of base somewhat yellowish; tibia white except distal 1/5 dark brown, with a large white sheen on posterior surface in light; tarsus brownish black except basal 1/2 of basitarsus or a little more whitish. Hind leg: coxa dark brown; trochanter pale yellow; femur brown to dark brown with base pale yellow; tibia (Fig. 8) brown to brownish black with basal 1/5 and posterior surface of basal 3/4 white, and with a large white sheen on posterior surface in light; tarsus brownish black except a little more than basal 1/2 of basitarsus white and basal 1/2 of 2nd tarsal segment yellow; basitarsus parallel-sided (W:L = 1.0:5.3), ca. 0.64 × and 0.74 × as wide as hind tibia and femur respectively; calcipala short, ca. 0.7 × as long as its basal width; pedisulcus distinct at basal 1/3 of 2nd tarsal segment. Tarsal claws simple without any tooth. ***Wing***. Length ca. 2.6 mm; costa with spinules and hairs; subcosta fully haired; basal section of vein R bare except distal 1/4 to 2/3 with hairs; hair tuft at base of stem vein dark brown; basal cell absent. ***Abdomen***. Basal scale brownish black with a fringe of dark hairs; 2nd segment dark brown except basal 1/2 pale, with a dorsolateral pair of silvery iridescent spots broadly connected in middle; all other tergites dark brown to brownish black, with dark hairs, tergites 6-8 shiny. ***Genitalia*** (Figs. 10-13). Ventral surface of abdominal segment 7 with a weakly developed sternal plate medially. Sternite 8 well sclerotized, bare medially, with ca. 6 pale short setae submedially near posterior border, and with ca. 14 dark long hairs laterally on each side; anterior gonapophyses triangular in shape, membranous, expanded ventrally, each covered with ca. 16 short setae as well as numerous microsetae except narrow portion along inner margin and rather wide portion along posterior margin bare and transparent; inner borders narrowly separated from each other. Genital fork of inverted-Y form, with well sclerotized stem; arms rather wide, each with strongly sclerotized distal ridge having a distinct projection directed anterodorsally. Paraproct nearly as long as wide, rounded posterolaterally, strongly sclerotized and largely concave anteromedially in ventral view; paraproct slightly produced ventrally along medial margin, covered with numerous short hairs laterally and ventrally. Cercus rounded posteriorly, ca. 0.5 × as long as wide, covered with numerous short hairs. Spermatheca globular, well sclerotized, with minute internal setae; tube and small adjacent area of spermatheca unsclerotized.

Male. Body length 3.5 mm. ***Head***. Slightly wider than thorax. Upper eye consisting of large facets in 20 horizontal and 20 vertical rows. Clypeus black, white pruinose, iridescent when illuminated, sparsely covered with dark brown hairs. Antenna composed of 2+9 segments, dark brown except base of 1st flagellar segment pale; 1st flagellomere somewhat elongate, 1.6 × as long as 2nd flagellomere; flagellar segments 1-8 each with 1 or 2 small pits distally on each lateral surface (Figs. 5 & 6), which are not so developed as compared to those of ♀. Maxillary palp composed of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.6; 3rd segment of normal size, with a small, globular or elliptical sensory vesicle, 0.2 × length of 3rd segment, and with a small opening distally. ***Thorax***. Scutum brownish black (3 narrow longitudinal black vittae visible in alcoholic specimen), with silvery iridescent pattern differing with angles of light: when illuminated anteriorly and viewed dorsally scutum shows subanteriorly a transverse pair of narrow silvery iridescent spots widely spaced in middle (distance between spots ca. 1/3 × that of scutum); when illuminated laterally or posterolaterally and viewed dorsolaterally subanterior pair of spots fade and are replaced by an anterior pair of narrow iridescent spots on shoulders which extend posteriorly along lateral margins in a wide band and connect to a large transverse posterior spot on prescutellar area; when illuminated anterolaterally and viewed dorsolaterally scutum on each shoulder has a large anterior iridescent spot including anterior and subanterior narrow spots mentioned above, which is also contiguous to a broad lateral iridescent band along lateral border; scutum uniformly covered with dark brown recumbent pubescence, interspersed with long upright hairs on prescutellar area. Scutellum brownish black, silvery iridescent when illuminated, with several upright dark hairs as well as dark short hairs. Postscutellum brownish black, silvery iridescent when illuminated, and bare. Pleural membrane bare. Katepisternum longer than deep, and bare. ***Legs***. Foreleg: coxa pale yellow; trochanter and femur dark brown; tibia brownish black except median large portion on outer surface white and



Figures 1-20. Adult female and male of *Simulium* (*Simulium*) *yongi* sp. nov. 1, fronto-ocular area of ♀; 2 & 3, right antenna of ♀ showing a well developed pit on each flagellar segment (2, outside view; 3, inside view); 4, pit on flagellar segment 2 showing numerous sensilla (scale 0.01 mm); 5 & 6, left antenna of ♂ showing a small pit on each flagellar segment (5, inside view; 6, outside view); 7, cibarium of ♀; 8, hind tibia of ♀; 9, hind basitarsus and 2nd tarsal segment of ♂; 10, spermatheca; 11, 8th sternite, anterior gonapophyses and genital fork in situ (ventral view); 12 & 13, paraproct and cercus (12, ventral view; 13, lateral view); 14, coxite and style showing a prominent basal protuberance (lateral view); 15, style showing a prominent basal protuberance (end view); 16, coxite, style and ventral plate in situ (ventral view); 17 & 18, ventral plate (17, lateral view; 18, end view); 19, paramere (end view); 20, median sclerite (end view)



Figures 21-27. Pupa and larva of *Simulium* (*Simulium*) *yongi* sp. nov. 21, pupal thoracic integument near gill base showing a small area densely covered with tubercles (lateral view; scale 0.1 mm); 22, anterior 1/2 of pupal thoracic integument with branched trichomes and gill filaments (lateral view; scale 0.2 mm); 23, basal portion of a ventral pair of gill filaments showing inner filament with narrowed base (ventral view; scale 0.1 mm); 24 & 25, cocoon (24, dorsal view; 25, lateral view; scale 1 mm); 26, apex of larval mandible; 27, larval head capsule showing hypostomium (hypostomal setae omitted) and postgenal cleft (ventral view)

with a white sheen in light; tarsus brownish black; basitarsus somewhat dilated, ca. 5.6 × as long as its greatest width. Midleg: coxa, trochanter and femur brownish black; tibia brown to brownish black except posterior surface of basal 3/5 white and with a white sheen in light; tarsus dark brown to brownish black except basal 1/2 or a little less yellowish, though its border not well defined. Hind leg: coxa brownish black; trochanter yellow; femur and tibia brownish black except base yellow; base of tibia whitish sheeny when illuminated; tarsus brownish black except a little less than basal 1/2 of basitarsus and basal 1/3 of 2nd tarsal segment yellowish; basitarsus (Fig. 9) enlarged (W:L = 1.0:4.4), ca. 0.78 × as wide as hind tibia and femur; calcipala small, W : L = 1.3 : 1.0; pedisulcus distinct. ***Wing***. Length ca. 2.4 mm; other features as in ♀ except subcosta and basal portion of radius completely bare. ***Abdomen***. Basal scale blackish with a fringe of long dark hairs. Terga dark brown to brownish black with dark hairs; segments 2, 5-7, each with a pair of silvery iridescent spots dorsolaterally, those on segment 2 broadly connected in middle. ***Genitalia*** (Figs. 14-20). Coxite in ventral view nearly quadrate, a little longer than its width. Style narrow and elongate, ca. 1.8 × as long as coxite, spatulate ventrodorsally, with a subterminal spine, and with a prominent basal protuberance which is pointed dorsally and furnished with 8-12 conical spines on anterior surface. Ventral plate in ventral view transverse, much wider than its length, rounded posterolaterally, with posterior margin untoothed and somewhat concave medially, and covered with fine appressed setae centrally; basal arms long, curved outwardly and forwardly. Parameres wide basally, each with numerous parameral hooks. Median sclerite narrow, slightly widened toward apex.

Pupa. Body length (excluding gill filaments) 3.0-3.5 mm. ***Head***. Integument yellowish brown, bare, with 1

facial and 2 frontal pairs of long, branched trichomes (split into 2-4); antennal sheath bare. ***Thorax***. Integument yellowish brown, bare on anterior 1/2 except small area at base of gill densely covered with tubercles of irregular shapes (Fig. 21), moderately covered with small, cone-shaped tubercles on posterior 1/2; thorax anteriorly with 3 dorsal and 2 lateral pairs of long, branched trichomes (split into 3-6), posteriorly with 1 lateral pair of branched trichomes (split into 2 or 3). Gill (Fig. 22) with 6 filaments arranged in sessile pairs; outer filaments of each pair brown, subequal in length (1.5-2.0 mm) and thickness, acutely bent at basal 1/5; inner filament of each pair somewhat paler and shorter than outer ones, subequal in thickness to outer filament except that of lower pair much narrower basally, ca. 0.6 × as wide as outer filament of the same pair; all filaments gradually tapered toward apical tip except inner filament of lower pair increasing its width from base to basal 1/5 of its length, then tapered toward apical tip (Fig. 23), with annular ridges and furrows throughout their length except near base, densely covered with minute tubercles. ***Abdomen***. Tergum 1 yellowish, with 1 bifid slender hair on each side. Tergum 2 on each side with 1 long bifid seta, 1 short spinous seta, and 4 short simple hooked spines of equal size. Terga 3 and 4 each with 4 hooked spines along posterior margin on each side. Tergum 8 with a transverse row of spine-combs on each side. Terga 6, 7 and 9 each lacking spine-combs, with a transverse row of comb-like groups of minute spines on each side. Tergum 9 lacking terminal hooks. Sternum 4 with 1 simple hook and a few short setae on each side. Sterna 5-7 each with a pair of simple hooks on each side. Grapnel-like hooklets absent. ***Cocoon***. Wall-pocket-shaped, very thin, transparent film-like, anterior margin irregular in form, usually deeply concave dorsoposteriorly as shown in Fig. 24; anterolateral portions rather high up to anterior end, appearing as a flap when viewed from side (Fig. 25); cocoon usually somewhat extending ventrolaterally.

Mature larva. Body length 6.5-8.0 mm. Body color greyish. Cephalic apotome pale yellow with a small dark area in middle along posterior border, with faint positive head spots. Antenna composed of 3 segments and apical sensillum, much longer than stem of labral fan; length ratio of segments (from base to tip) 1.0:1.4:0.5. Labral fan with 50-54 main rays. Mandible (Fig. 26) with usual mandibular serration of 1 medium tooth and 1 small one (sometimes posterior small tooth forked apically), without supernumerary serrations; comb-teeth decreased in length from 1st to 3rd. Hypostomal teeth 9 in number, median tooth and each corner tooth longer than others; lateral margins moderately serrate apically; 8 or 9 hypostomal bristles diverging posteriorly from lateral border on each side. Postgenal cleft (Fig. 27) medium in depth, 1.8-2.0 × as long as postgenal bridge; lateral margins on posterior 1/2 nearly parallel-sided or slightly converged at base. Thoracic cuticle almost bare. Abdominal cuticle bare except last segment moderately covered with short, colorless setae on each side of anal sclerite. Rectal papilla of 3 lobes, each with 15-17 finger-like secondary lobules. Anal sclerite X-shaped, with broadened anterior arms ca. 0.65 × as long as posterior ones. Ventral papillae absent. Posterior circlet with 154-170 rows of hooklets with up to 22 hooklets per row.

TYPE SPECIMENS. Holotype ♀ (BMNH), slide-mounted, reared from pupa collected from a cascading stream, crossing the Hulu Langat-Semenyih Road, near Sungai Tekala, 18th mile from Kuala Lumpur, Selangor State, Malaysia, 25.III.1996, by H.Takaoka and Y.Hoi-Sen. Paratypes: 4 ♀, 2 ♂, reared from pupae, 5 pupae, 5 mature larvae (BMNH), in alcohol, same data as holotype; 6 ♀, 1 ♂, reared from pupae, 5 pupae, 5 mature larvae, collected from a cascading stream, crossing the road from Tapah to Tanah Rata, 33 km to Tana Rata, Tapah, Perak State, 13.III.1996, by H.Takaoka; 4 pupae, 1 pupal exuvia and 4 mature larvae, collected from a tributary of Selangor R., at farm, W Fraser's Hill, Pahang State, 2.XII.1975, by D.M.Davies, J.J.S. Burton and P.Tan; 1 pupa and 1 mature larva, 21 Km NE of Kuala Lumpur, 1.1 Km N of Gombak Hospital, Selangor State, 26.XI.1975, by D.M.Davies and P.Tan; 1 pupal exuvia and 2 mature larvae, tributary of Kenyoi R., 24 Km SE of Bentong, Pahang State, 4.XII.1975, by D.M.Davies, J.J.S.Burton and P.Tan.

ECOLOGICAL NOTES. Pupae and larvae of this species were found on rock suface of jetting water of cascading streams, 1.5-5.0 m wide, exposed to the sun or partially shaded. A few pupae were also collected on trailing grasses in the fast flowing water. Water temperature ranged from 20 to 26°C. Altitude varied from 230 to 1,010 m.

This species was collected together with *S.* (*S.*) *pahangense* and *S.* (*S.*) *grossifilum* Takaoka and Davies

In the stream of Tapah, 8 of 74 larvae of this species examined were infected with mermithid larva(e).

Three reared female flies were kept alive with sugar solution for five days but no development of ovaries was seen beyond stage II, suggesting anautogeny in ovarian development.

ETYMOLOGY. The specific name, *yongi*, is given in honor of Prof. Yong Hoi-Sen, Department of Zoology, University of Malaya.

DISTRIBUTION. Peninsular Malaysia.

REMARKS. This new species is characterized by the combination of the following features: antenna with a well developed pit on each lateral surface of flagellar segments 1-9, scutum unpatterned, tarsal claws simple, anterior gonapophysis enlarged and widely bare and transparent in the female; ventral plate transverse, without toothed margins, and style with a prominent basal protuberance in the male; and gill with six filaments per side, and cocoon wall-pocket-shaped with very thin, transparent wall in the pupa.

The female of this species resembles that of *S.* (*S.*) *rudnicki* described based on female specimens collected from Langkawi Island, Kedah State, Peninsular Malaysia (Takaoka and Davies, 1995), of which the scutum and the genitalia are very similar to those of *S.* (*S.*) *yongi*, although leg colorings are different from each other. The femora of *S.* (*S.*) *rudnicki* are mostly yellowish.

This species is closely related to *S.* (*S.*) *ufengense* described from Taiwan (Takaoka, 1979), and *S.* (*S.*) *fuzhouense* from Fujian, south China (Zhang and Wang, 1991), by having similar male genitalia. However, there are differences in the leg colorings, the arrangement of the pupal gill filaments, and the cocoon. In the latter two species, the femora of adult legs are largely yellowish, four of six pupal gill filaments are distinctly slenderer than the remaining two filaments, and the cocoon has a thick anterodorsal margin.

S. (*S.*) *taipokauense* (only the male adult is known) from Hong Kong (Takaoka *et al*, 1995) shows some similarities to this new species but differs by having the much longer basal protuberance of the style and the saddle-shaped ventral plate with a nipple-like median process.

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A NEW SUBGENUS, *SIMULIUM (DAVIESELLUM)*, AND A NEW SPECIES, *S. (D.) COURTNEYI*, (DIPTERA: SIMULIIDAE) FROM THAILAND AND PENINSULAR MALAYSIA

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Abstract. A new subgenus, *Daviesellum*, in the genus *Simulium* is established to accommodate two species from Thailand and Peninsular Malaysia. One of these species is *Simulium pahangense*, for which the male and larval silk-gland chromosomes are described for the first time. A new species, *S. courtneyi*, is illustrated and described from larvae, pupae and pharate males and females. The immature stages of both species inhabit torrential habitats, and larvae of both species contain the trichomycete fungus *Harpella melusinae*, representing the first record of this species from Thailand. *Simulium (Daviesellum)* is related perhaps most closely to the subgenera *Gomphostilbia* and *Morops*. *Sulcicnephia unidens* is synonymized with *S. (D.) pahangense*.

Keywords: *Simulium*, black fly, Simuliidae, *Daviesellum*, Thailand, Malaysia, new subgenus

INTRODUCTION

In a study of Simuliidae from Peninsular Malaysia, Takaoka and Davies (1995) described *Simulium (Simulium) pahangense* from a single adult female swept from over a stream in Fraser's Hill. In the same stream, pupae and larvae of two undescribed species were collected from a rock surface in jetting water and one of these two species was tentatively associated with this species and the other was described as *Sulcicnephia unidens* (Takaoka and Davies, 1995). However, our recent collections made in Thailand and Malaysia show that the female of *S. pahangense* and pupa and larva of *Su. unidens* belong to the same species, and the pupa and larva originally associated with *S. pahangense* consist of a distinct new species (= *S. (S.) yongi* Takaoka and Davies, 1997).

In this paper, *S. pahangense* is held as a valid name and *Su. unidens* is sunk as a junior synonym of the former species; and the male and larval silk-gland chromosomes of *S. pahangense* are described for the first time. Further, one new species related to *S. pahangense* is described from pharate females, pharate males, pupae and mature larvae collected from northern Thailand. To accommodate these two rare species, a new subgenus is described within the genus *Simulium*.

MATERIALS AND METHODS

Taxonomic procedures used are the same as those described by Takaoka (1983). Morphological terminology follows that of Crosskey (1969). One pharate male and female of the new species each was dissected partially from the pupal exuviae, dried with hexamethyldisilazane (Brown, 1993), and pinned. Peritrophic membranes from larval midguts were examined for trichomycetes, according to the procedure of Adler *et al.* (1996). The holotype and some paratypes are deposited in the Natural History Museum (BMNH), London, U.K. Additional paratypes and slides of trichomycetes are deposited in the Clemson University Arthropod Collection (CUAC), Clemson, South Carolina, U.S.A.

Six larvae of *S. pahangense* were preserved in acetic ethanol (1 : 3) and their silk-gland chromosomes were stained according to the procedures of Rothfels and Dunbar (1953). Chromosomal terminology follows that of Rothfels *et al.* (1978). All photographic negatives and prints of chromosomes are housed in the Clemson University Arthropod Collection.

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Subgenus *Daviesellum* sgen. nov.

Type-species: *Simulium* (*Daviesellum*) *pahangense* Takaoka and Davies, 1995

DIAGNOSIS: **Female** and **Male.** Antenna composed of scape, pedicel and 9 flagellar segments. Basal section of radius bare. Pleural membrane and katepisternum bare. **Female**. Frons shiny, with several hairs along lateral margins. Cibarium with numerous minute tubercles. Scutum white pruinose but not patterned. Tarsal claw with a small subbasal tooth. Abdomen with dorsolateral pair of white pruinose spots on segment 2, and tergites of segments 6-8 shiny. Anterior gonapophysis produced posteriorly, tongue-like. Genital fork with well developed wide arms. Paraproct of unique form, with a cluster of numerous dark, stout spines. Spermatheca with internal setae. **Male**. Scutum white pruinose, with 3 faint longitudinal vittae. Abdomen with dorsolateral pair of white pruinose spots on segments 2, 4-8. Coxite longer than wide, gradually narrowed posteriorly. Style elongate, 1.5-1.7 × length of coxite, slender, gradually tapered toward apex, with an apical spine. Ventral plate in form of transverse, haired plate, with a narrow longitudinal median process. Paramere narrow with numerous parameral hooks. Median sclerite wide, plate-like. **Pupa**. Head with 3 pairs of trichomes. Gill composed of 6 filaments in pairs. Tergum 2 on each side with 4 stout hooks, equivalent in size to those on terga 3 and 4. Terga 5-9 without spine-combs, and terminal hooks absent. Sterna 5-7 each with pair of simple, stout hooks on each side. Cocoon boot-shaped. **Larva**. Cephalic apotome widest just before posterior margin. Hypostomium very wide with 13 apical teeth composed of 1 prominent median tooth accompanied at its base by 2 small teeth on each side, and 4 small individual teeth on each side; lateral teeth absent. Postgenal cleft deep. Mandible with well developed comb-teeth, mandibular serration composed of a single tooth, and supernumerary serrations absent. Rectal papilla of 3 lobes, each with numerous secondary lobules. Proleg with sclerotized rod on each side. Anal sclerite of usual X shape, posterior arms much longer than anterior ones, somewhat widened medially when viewed posteriorly. Posterior circlet with over 400 rows of up to over 40 hooklets per row. Last abdominal segment remarkably bulged laterally or somewhat ventrolaterally, forming a large and a small papillae, which are visible when viewed ventrally.

ETYMOLOGY. This subgenus is named in honor of simuliid authority Douglas M. Davies.

PHYLOGENETIC RELATIONSHIPS AND TAXONOMIC REMARKS. Although the sister species *S. pahangense* and *S. courtneyi* sp. nov. might represent highly derived members of an existing subgenus, the morphological gap separating them from all other subgenera is substantial. We, therefore, establish a new subgenus, *Daviesellum*, to accommodate these species.

Subgenus *Daviesellum* shares preimaginal features —— undoubtedly convergent —— with those of other subgenera, such as the Palearctic subgenus *Obuchovia* and the New World subgenera *Hemicnetha, Hearlea,* and *Shewellomyia.* These features include a densely woven, boot-shaped cocoon with a high neck; short, rather compact pupal gills with fairly stout filaments; an elongate, gradually expanded larval abdomen; an enormous number of hooklets in the posterior circlet and on the prothoracic proleg; labral fans with short stems and stout primary rays; and a hypostomium with smoothly curved anterolateral margins and dense rows of thick bristles. Nearly all of these characters are typical of simuliids that aggregate in clusters or mats in torrential habitats. Chromosomal similarities between *Daviesellum* and *Hemicnetha*, such as an expanded centromere region in chromosome I and a splayed end in IIIS, are judged to be convergent or plesiotypic.

Male genitalic features of *Daviesellum* suggest a relationship with some species-groups of the subgenera *Gomphostilbia* (e.g. *baisasae, batoense, ceylonicum* groups) and *Morops* (e.g. *banauense* and *sherwoodi* groups). All these share an elongate, gradually tapered style bearing one apical spine and a ventral plate that, in lateral view, has a large, strongly produced keel. The parameral spines in *Daviesellum* are smaller and more subequal in size than those typically found in *Gomphostilbia* and *Morops.*

Both *Gomphostilbia* and *Morops* are nearly endemic to the Australasian and Oriental Regions (Takaoka 1996), further suggesting a relationship with *Daviesellum. Gomphostilbia* has been found in Thailand and Peninsular Malaysia; however, the presence of *Morops* in these two areas is unconfirmed.

This new subgenus is readily distinguished from other subgenera by the following unique characters: in the female, paraproct with a cluster of spines; in the male, elongate coxite and style; in the pupa, stout hooks on the second tergum; and in the larva, proleg with a sclerotized rod on each side, postgenal cleft with anterolateral extensions, hypostomium very wide with 13 apical teeth, mandibular serration (sensillum) composed of a single tooth, and posterior circlet with over 400 rows of up to over 40 hooklets per row.

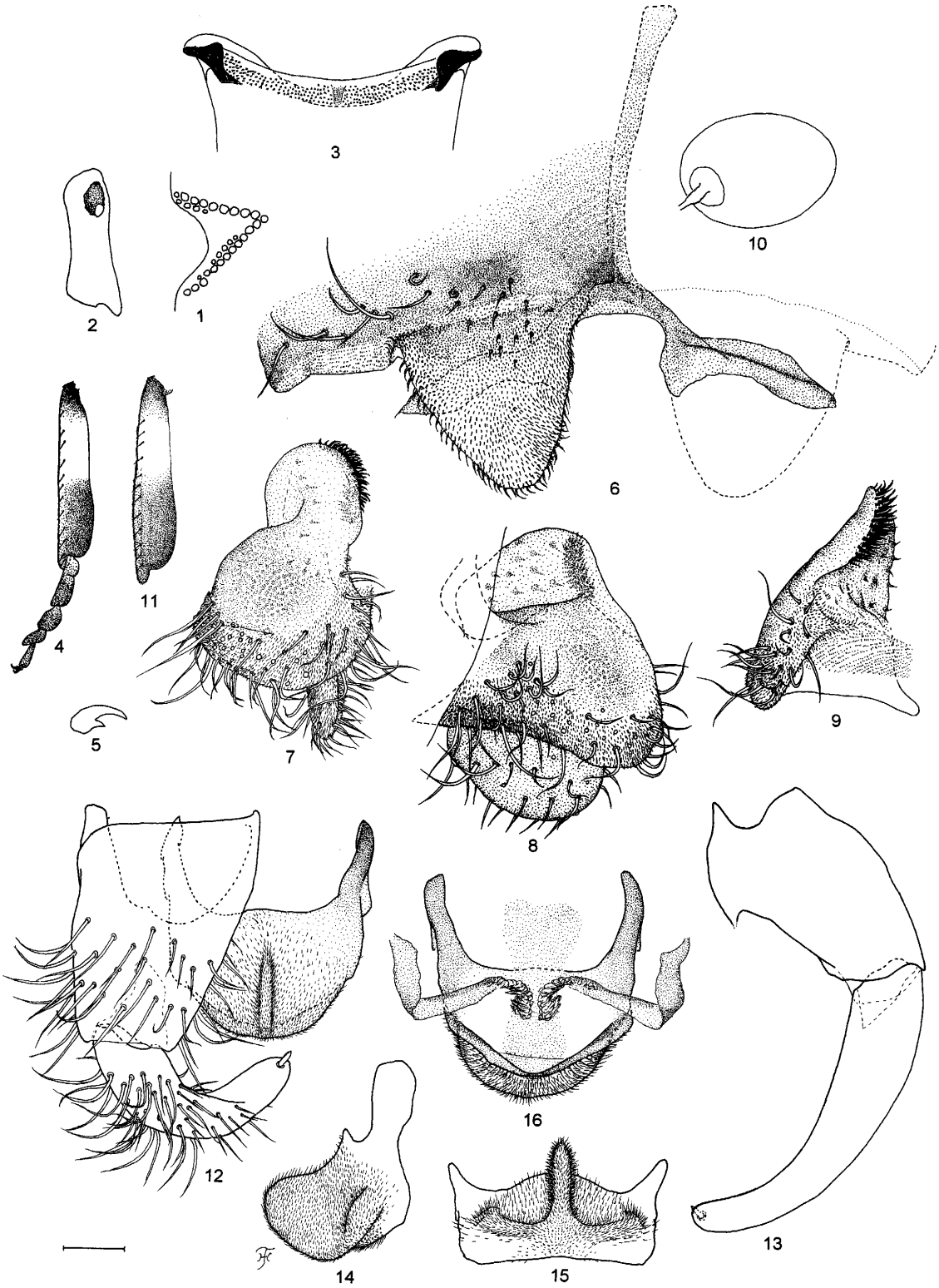
Simulium (*Daviesellum*) *pahangense* Takaoka and Davies, 1995

Simulium (*Simulium*) *pahangense* Takaoka and Davies, 1995: 150-155 (holotype female only and not pupa and larva).

Sulcicnephia unidens Takaoka and Davies, 1995: 10-13 (pupa and larva). NEW SYNONYMY.

DESCRIPTION. **Female**. Body length ca. 2.8 mm. ***Head***. Narrower than width of thorax. Frons black, shiny, thinly grayish pruinose, with several dark, stout hairs along lateral and lower margins; frontal ratio 1:1:1; frons-head ratio 1.0:3.3. Fronto-ocular area (Fig. 1) well developed. Clypeus black, shiny, thinly grayish pruinose, with dark stout hairs. Antenna composed of 2+9 segments, brownish black except scape, pedicel and 1st flagellar segment yellowish brown. Maxillary palp composed of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:0.8:1.7; 3rd segment not enlarged; sensory vesicle (Fig. 2) of medium size, oblong, with rugged surface, 0.2 × length of 3rd segment, with large round opening distally. Maxillary lacinia with 16 inner and 18 outer teeth. Mandible with ca. 42 inner and 22 outer teeth. Cibarium (Fig. 3) with numerous minute tubercles along posterior margin. ***Thorax***. Scutum black, shiny, whitish gray pruinose, moderately covered with short, dark, recumbent pubescence, interspersed with long, erect, dark hairs on prescutellar area. Scutellum dark brown with short, dark pubescence and long, dark hairs. Postscutellum dark brown, shiny, whitish gray pruinose and iridescent when illuminated, bare. Pleural membrane bare. Katepisternum longer than deep, bare. ***Legs***. Almost blackish except fore coxa, base of mid basitarsus and base of hind 2nd tarsomere pale brown, and basal 1/2 of hind basitarsus (Fig. 4) whitish. Base of mid and hind tibiae with slight whitish sheen when illuminated. Fore basitarsus somewhat dilated, 5.0 × as long as its greatest width. Hind tibia slightly narrower than femur; hind basitarsus (Fig. 4) nearly parallel-sided, width : length=1:5; calcipala and pedisulcus distinct; all tarsal claws with a small subbasal tooth (Fig. 5). ***Wing***. Length 2.9 mm; costa with spinules and hairs; subcosta bare; basal section of vein R bare; hairs at base of stem vein dark brown; basal cell absent. ***Abdomen***. Basal scale black with fringe of dark hairs; 2nd segment black, with pair of large, dorsolateral silvery iridescent spots broadly connected to each other medially; tergites 3-5 small, black; tergites 6-8 large, black, shiny, with dark hairs. ***Genitalia*** (Figs. 6-10). Ventral surface of abdominal segment 7 lacking sternal plate. Sternite 8 well sclerotized, with anterior expansion medially, bare medially but with several hairs and several short setae along posterior border on each side; anterior gonapophysis tongue-shaped, protruding ventroposteriorly, thin, membranous, covered with ca. 10 short setae as well as numerous microsetae. Genital fork of inverted-Y form; stem slender and well sclerotized; arms each with large, strongly sclerotized distal ridge. Paraproct of unique shape, well sclerotized, widely bare ventrolaterally, expanded anteriorly in form of round and compressed lobe with cluster of short, stout spines along ventral inner margin, with numerous stout hairs on setose area near posterior margin. Cercus nearly semicircular, covered with numerous stout hairs. Spermatheca nearly ovoid, well sclerotized except tube and small area of tubal base transparent, with reticulate pattern near tubal base; tube somewhat enlarged basally; minute internal setae present.

Male. Body length 2.8 mm. ***Head***. Slightly wider than thorax. Clypeus black, thinly whitish gray pruinose, with sparse dark hairs. Upper eye consisting of large facets in 19 horizontal rows and in 18 vertical columns. Antenna composed of 2+9 segments, brownish black except scape, pedicel and base of 1st flagellar segment yellow to dark yellow; 1st flagellomere elongate, 1.4 × as long as 2nd flagellomere. Maxillary palp with 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:2.5; sensory vesicle small, oblong, 0.19 × length of 3rd segment. ***Thorax***. Scutum black, white pruinose in certain angles of light, with 3 indistinct narrow longitudinal vittae (in alcohol these 3 longitudinal vittae distinct against dark brown ground color of scutum), densely covered with dark pubescence, intermingled with dark, long, upright hairs on prescutellar area. Scutellum black, covered with dark, long, upright marginal hairs as well as dark, short hairs. Postscutellum black, bare. Pleural membrane bare. Katepisternum longer than deep, bare. ***Wing***. Length not measurable; other features as in ♀, except subcosta bare. ***Legs***. Coloration nearly as in ♀. Fore basitarsus somewhat dilated, 5.3 × as long as its greatest width. Hind basitarsus (Fig. 11) gradually widened toward distal tip, 4.4 × as long as its greatest width, which is 0.67 × and 0.79 × as wide as hind tibia and femur, respectively; calcipala and pedisulcus well developed. ***Abdomen***. Basal scale black, with fringe of long, dark hairs. Dorsal surface of abdominal segments brownish black to black except anterior 1/2 yellow, with dorsolateral pair of white pruinose spots on segments 2 and 4-8, though those on segments 7 and 8 thinly pruinose; those on segments 2



Figures 1-16. Female and male of *S.* (*D.*) *pahangense*. 1, ♀ fronto-ocular area; 2, ♀ 3rd maxillary palpal segment; 3, ♀ cibarium; 4, ♀ hind tarsus; 5, ♀ tarsal claw; 6-10, ♀ genitalia —— 6, 8th sternite, anterior gonapophyses, and genital fork *in situ* (ventral view); 7 & 8, paraproct and cercus (7, ventral view; 8, lateral view); 9, paraproct (medial view); 10, spermatheca; 11, ♂ hind basitarsus; 12-16, ♂ genitalia —— 12, coxite, style and ventral plate *in situ* (left coxite and style omitted, ventral view); 13, coxite and style (lateral view); 14, ventral plate (lateral view); 15, ventral plate (end view); 16, ventral plate, parameres and median sclerite *in situ* (dorsal view). Scale bar 0.05 mm (only applying to figs. 6-10 & 12-16)

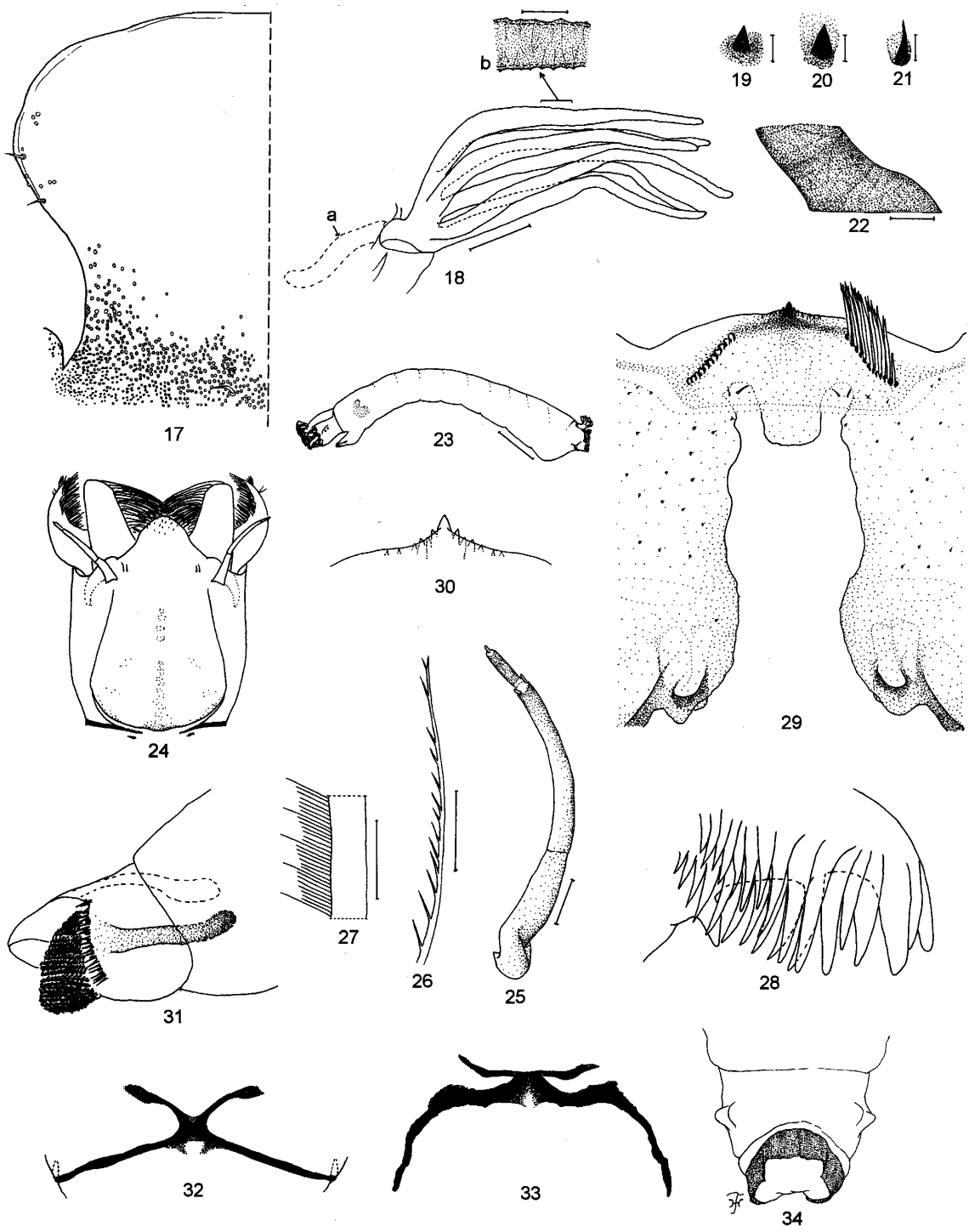
and 6 large and connected widely to each other in middle and those on segments 4 and 5 small and probably overlooked. ***Genitalia*** (Figs. 12-16). Coxite much longer than wide. Style elongate, ca. 1.5 × as long as coxite, gradually tapered toward apex, curved dorsally and with 1 apical spine. Ventral plate in ventral view transverse, shorter than wide, with posterior margin rounded and somewhat produced posteriorly like eaves, with median longitudinal process produced ventrally, and fully covered with minute setae. Paramere slender, with numerous hooks apically. Median sclerite plate-like, wide basally, somewhat narrowed medially and again widened apically.

Pupa. Body length (excluding gill filaments) ca. 3.5 mm. ***Head***. Integument pale yellow, face covered densely with small tubercles, frons almost bare; 1 facial and 2 frontal pairs of simple, short trichomes (Fig. 17). ***Thorax***. Integument pale yellow, bare except both sides and posterodorsal surface densely covered with small tubercles; 2 dorsal and 3 lateral pairs of simple, short trichomes, all subequal in length. Gill (Fig. 18) with 6 pale or yellowish, short filaments in pairs, almost sessile except dorsal pair short-stalked, tapering apically; all filaments subequal in length (1.0-1.4 mm) and thickness, their maximal diameter as thick as or a little thicker than interspiracular trunk; cuticle of filaments with numerous, sharp, transverse ridges forming well-defined reticulate patterns, uniformly covered with minute tubercles. ***Abdomen***. Tergum 1 pale, densely covered with minute tubercles, with 1 simple seta on each side. Tergum 2 pale, with 4 hooked spines (Fig. 19) directed forward and 2 simple, short setae of different length, on each side. Terga 3 and 4 each with 4 hooked spines (Fig. 20) and 1 simple, short seta on each side. Terga 5-9 without spine-combs. Tergum 9 without terminal hooks. Sternum 5 with pair of simple, stout hooks (Fig. 21) situated close together submedially on each side. Sterna 6 and 7 each with pair of simple, stout hooks similar to those on sternum 5, but widely spaced on each side. Grapnel-like hooklets absent. ***Cocoon*** (Fig. 22). Boot-shaped, with high neck, thickly woven, individual threads undetectable; anterior rim not thickened; surface smooth but with many bumps of silk in 2 Thailand specimens.

Mature larva. Body length ca. 6.5 mm. Body color reddish brown. Cephalic apotome (Fig. 24) widest somewhat before posterior border, pale yellowish, somewhat darkened medially just before posterior border, with faint positive head spots; cervical sclerites discrete and isolated from postocciput. Antenna (Fig. 25) composed of 3 articles and apical sensillum, a little longer than stem of labral fan; length ratio of articles (from base to tip) 1.0:1.3:0.4. Labral fan with ca. 52 main rays, of which pectination as in Figs. 26 & 27. Mandible (Fig. 28) characterized by well developed comb-teeth and inner teeth, with 1 mandibular serration (sensillum), without supernumerary serrations. Hypostomium (Fig. 29) very wide, lacking lateral serrations; anterior teeth (Fig. 30) 13 (12 in 1 specimen), with median tooth much longer and thicker than others; 11 or 12 hypostomal bristles lying close in row, diverging posteriorly from lateral border on each side. Postgenal cleft (Fig. 29) deep, anterior margin except middle portion extending forward, reaching posterior border of hypostomium, leaving incomplete postgenal bridge medially. Proleg with an elongate sclerite rod on each side (Fig. 31). Thoracic cuticle bare. Abdominal cuticle bare except dorsolateral areas on last segment sparsely covered with minute, uncolored setae. Rectal papilla of 3 lobes, each with 18-25 finger-like secondary lobules. Anal sclerite X-shaped, with short, slender anterior arms ca. 0.3 × length of posterior ones, which are narrow in dorsal view (Fig. 32) but somewhat widened medially in end view (Fig. 33). Posterior circlet with ca. 460 rows of hooklets with up to 53 hooklets per row. Last abdominal segment remarkably bulged ventrolaterally, forming double papillae, which are discernible when viewed ventrally (Fig. 34).

Larval silk-gland chromosomes (Fig. 35). 6 larvae (2 ♂ and 4 ♀) examined. Haploid number = 3. Arm associations standard. Homologues tightly paired. Chromocenter absent. Centromere bands thick, darkly staining. Centromere region of chromosome I large, expanded; slightly expanded in chromosome II; not expanded in chromosome III. Nucleolar organizer in middle of IIIS. IIL with symmetrical marker subbasal, followed by DNA puff; parabalbiani in approximately middle third of arm (diffuse edge distal), followed by gray band. IIS with trapezoidal marker basal, followed by Ring of Balbiani; bulge subterminal. IIIS with end splayed; blister subterminal with two heavy bands more terminal. IIIL with basal marker in basal third of arm. One ♂ with heterozygous inversion in base of IS.

SPECIMENS EXAMINED. Holotype ♀(BMNH), swept while flying over foam of a stream, one of tributaries of Selangor R., at farm, E101°44′20″/N3°40′, W Fraser's Hill, Pahang State, MALAYSIA, 2.XII.1975, by D.M. Davies, J.J.S. Burton & P. Tan. 1 pupal exuvia

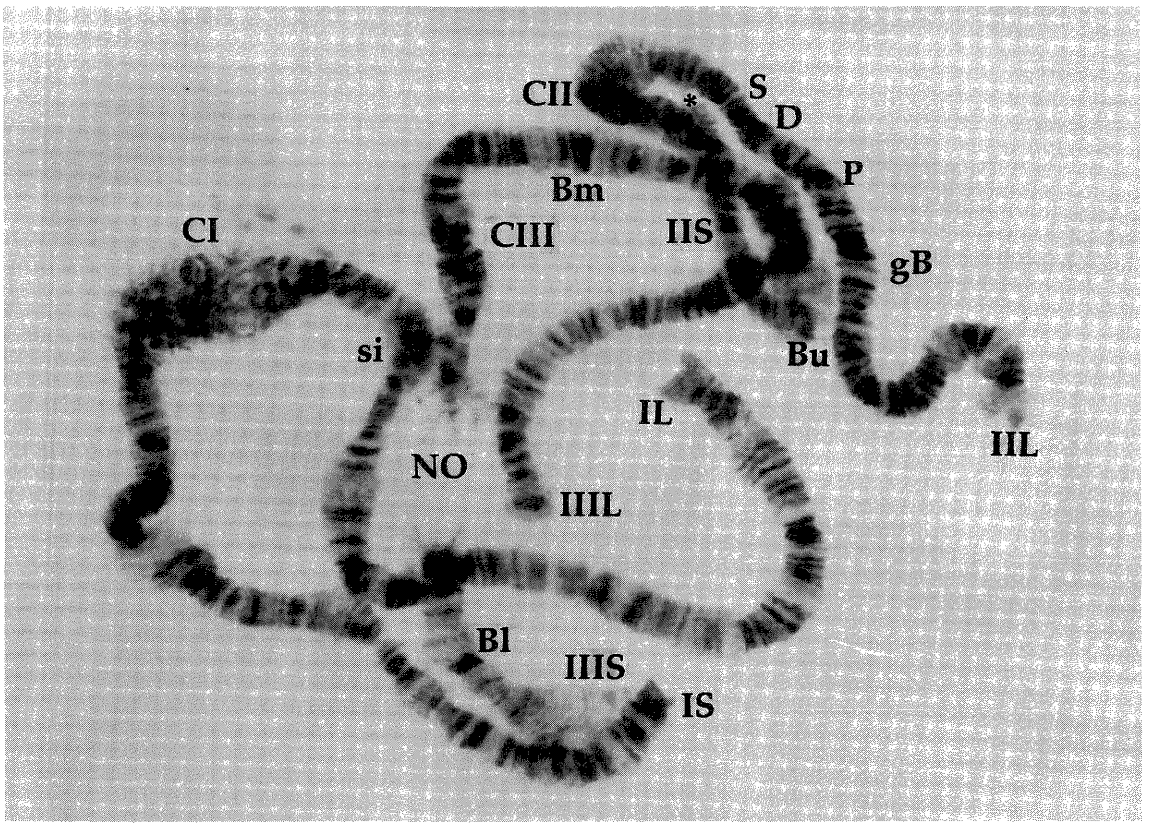


Figures 17-34. Pupa and larva of *S.* (*D.*) *pahangense*. 17-22, pupa —— 17, frons; 18, interspiracular trunk (a) and gill filaments showing enlagement of part of filament (b); 19-21, spinous hook (19, on 2nd tergum; 20, on 3rd tergum; 21, on 5th sternite); 22, cocoon (lateral view); 23-34, larva —— 23, whole body (lateral view); 24, head capsule (dorsal view); 25, antenna; 26, innermost ray of labral fan; 27, part of one of major rays of labral fan); 28, apex of mandible; 29, head capsule showing hypostomium and postgenal cleft (ventral view); 30, anterior teeth of hypostomium (ventral view); 31, proleg showing a lateral sclerotized rod; 32 & 33, anal sclerite (32, dorsal view; 33, end view); 34, posterior tip of larval abdomen showing ventrolateral papilla on each side (ventral view). Scale bars 0.03 mm for figs. 19, 20, 21, 26 & 27; 0.05 mm for figs. 18b & 25; 0.2 mm for fig. 18; 1.0 mm for figs. 22 & 23.

with cocoon (holotype of former *Sulcicnephia unidens*), 2 pupae, 2 mature larval head capsules, and 3 premature larvae (BMNH), same data as holotype; 1 ♂ reared from pupa, 2 mature larvae (BMNH), a cascading stream, crossing Hulu Langat-Semenyih Road, near Sungai Tekala, Selangor State, MALAYSIA, 25.III.1996, by H. Takaoka, A. Takaoka & H.S. Yong; 1 pharate ♀, 1 pupa, and 5 immature larvae (3 examined chromosomally, CUAC), and photographic negatives of larval silk-gland chromosomes (CUAC), Suai Mai waterfall, E98°27′/N9°20′, Phangna Province, THAILAND, 14.X.1994, by G.W. Courtney; 3 immature larvae (examined chromosomally, CUAC), Huai Tom Than Lod, 244 m in altitude, E99°17′/N14°39′, Kanchanaburi Province, THAILAND, 30.X.1994, by G.W. Courtney; 5 immature larvae (CUAC), Mai Yai waterfall, E98°30′/N8°53′, Surat Thani Province, THAILAND, 15.X.1994, by G.W. Courtney.

ECOLOGICAL NOTES. At the type locality (ca. 1,000 m in altitude) of Fraser's Hill, pupae and larvae were collected from a rock surface in jetting water of a stream 3-4 m wide, exposed to the sun. Water temperature was 21 °C. Collected with this species were *S.* (*Simulium*) *hirtinervis, S.* (*S.*) *tani, S.* (*S.*) *bishopi, S.* (*S.*) *yongi,* and *S.* (*Gomphostilbia*) sp. Our trial made in March 1996 to recollect this species at the type locality was disappointing. Neither simuliids nor other aquatic insects were breeding in this stream, which was muddy due to a golf course under construction upstream. In Selangor, Malaysia, a few immatures of this species were collected from a rock surface in a jetting, cascading stream 2-5 m wide, in a lowland area (ca. 230 m in altitude). Water temperature was 26 °C. Two other species, i.e., *S.* (*S.*) *grossifilum* and *S.* (*S.*) *yongi*, were also collected from the rock surface in the same stream.

Collections in Thailand were made from well-shaded waterfalls and torrential areas of streams, 18-20 °C,



Figures 35. Total chromosomal complement from larval silk glands of *S.* (*D.*) *pahangense* (male larva from Thailand, Phangna Province, Suai Mai waterfall, 14.X.1994), showing major landmarks. Chromosome arms are labeled IS, IL, IIS, IIL, IIIS, and IIIL. The end of chromosome arm IIS overlaps the middle portion of chromosome arm IIIL. CI, CII, CIII = centromeres of chromosomes I, II, and III, respectively. Bl = blister, Bm = basal marker, Bu = bulge, D =DNA puff, gB = gray band, NO = nucleolar organizer, P = parabalbiani, S = symmetrical group, si =heterozygous inversion, * = ring of Balbiani

flowing over bedrock. One stream (in Kanchanaburi Province) had heavy deposits of marl. None of the larvae in our collections had patent infections of parasites or pathogens. However, the guts of all six larvae of *S. pahangense* that we examined from Thailand (Phangna and Kanchanaburi Provinces) contained numerous thalli and trichospores of the trichomycete fungus *Harpella melusinae* Léger and Duboscq. This trichomycete has been recorded previously from the Oriental Region (Adler *et al.*, 1996), but this is the first record from Thailand.

DISTRIBUTION. Peninsular Malaysia and Thailand (new record).

Simulium (*Daviesellum*) *courtneyi* sp. nov.

DESCRIPTION. **Pharate female**. Body length 3.0 mm. ***Head***. As in ♀ of *S. pahangense* except the following characters: Frontal ratio 1.1:1.0:0.9; frons-head ratio 1.0: 3.8. Antenna dark brown except scape and pedicel yellow, and 1st flagellar segment yellow entirely when viewed ventrally but only basally when viewed dorsally. Proportional lengths of 3rd, 4th and 5th maxillary palpal segments 1.0:0.9:2.0; sensory vesicle 0.25 × length of 3rd segment. Maxillary lacinia with 14 or 15 inner and 16 outer teeth. Mandible with ca. 40 inner and 22 outer teeth. ***Thorax***. As in ♀ of *S. pahangense* except scutum dark brown to blackish brown, and scutellum not pigmented. ***Legs***. Coloration still incomplete appearing as follows: All coxae and trochanters brown to dark brown except fore coxa dark yellow. All femora pale except distal 1/5 or 1/6 blackish brown. All tibiae blackish brown with a large pale portion medially, and with base yellow. Fore tarsus blackish brown; mid and hind tarsi grayish brown except basal 1/2 of mid basitarsus grayish and basal 1/2 of hind basitarsus and 1/3 of hind 2nd tarsomere yellowish white. Fore basitarsus somewhat dilated, 5.1 × as long as its greatest width. Calcipala, pedisulcus and tarsal claw as in ♀ of *S. pahangense*. ***Wing***. Length not measurable; other characters as in ♀ of *S. pahangense*. ***Abdomen***. As in ♀ of *S. pahangense* except 6th to 8th tergites shiny grayish. ***Genitalia*** (Figs. 36-40). Nearly as in ♀ of *S. pahangense* except the following characters: Sternite 8 furnished with 12 long, stout hairs and a few short setae along posterior margin on each side. Anterior gonapophysis with ca. 20 short setae as well as numerous microsetae. Stem of genital fork slender with dilated anterior tip; each arm with large, strongly sclerotized distal ridge bearing a distinct projection directed anterodorsally. Paraproct largely concave anterolaterally when viewed ventrally. Spermatheca nearly globular in shape.

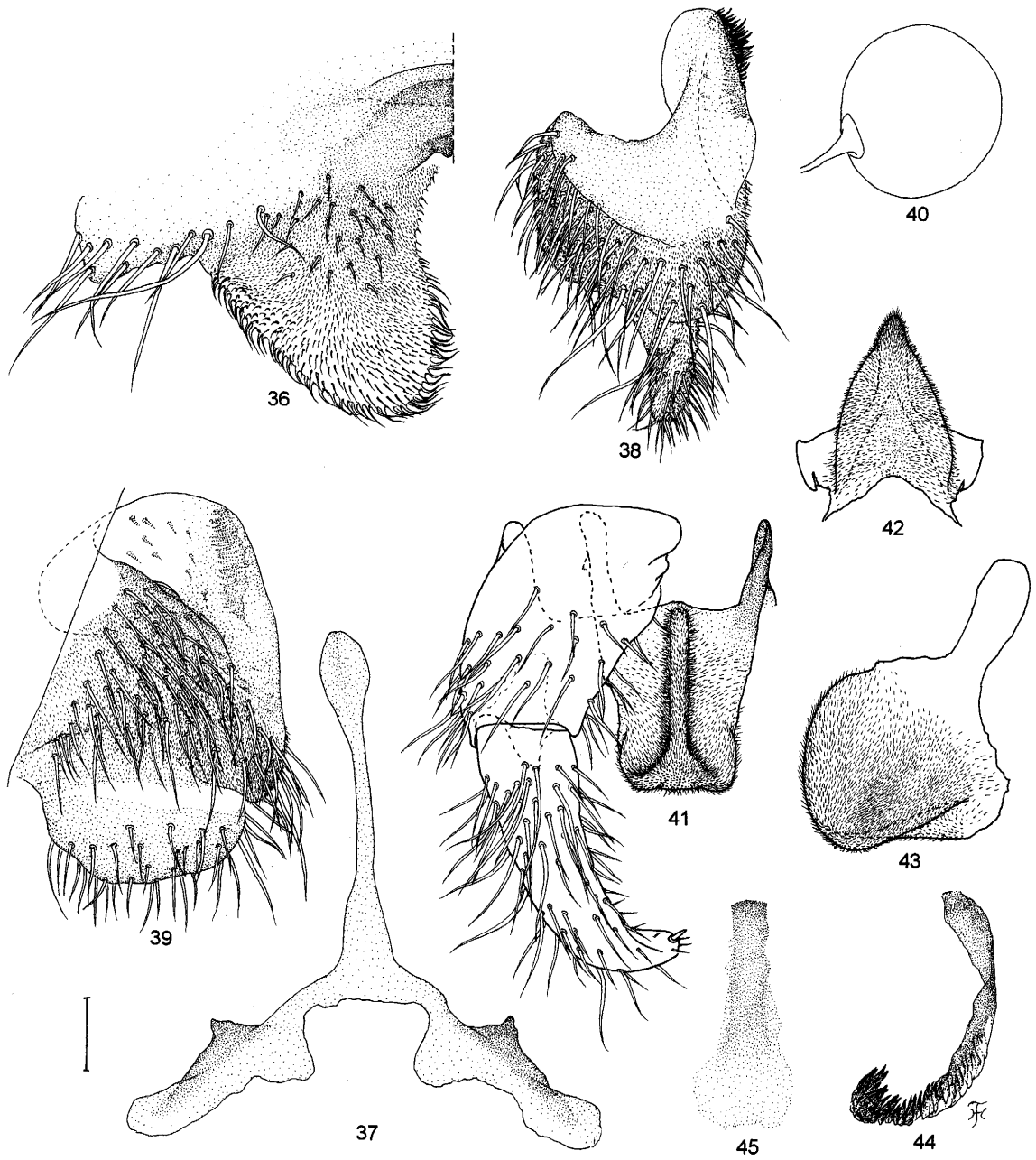
Pharate male. Body length 3.0 mm. ***Head***. As in ♂ of *S. pahangense* except the following characters: Upper eye consisting of large facets in 17 horizontal rows and in 16 vertical columns. In 1 ♂ antennae abnormal, 1st and 2nd flagellar segments completely fused becoming elongate 1st segment, and 3rd and 4th ones incompletely separated in right antenna, and 1st and 2nd flagellar segments and 7th and 8th ones incompletely separated respectively in left antenna. Maxillary palp with 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0 : 1.0 : 2.3; sensory vesicle small, oblong, ca. 0.16 × length of 3rd segment. ***Thorax***. As in ♂ of *S. pahangense* except scutum dark brown to blackish brown, and scutellum not pigmented. ***Wing***. Length not measurable; other features as in ♀, except subcosta bare. ***Legs***. Coloration nearly as in ♀. Fore basitarsus somewhat dilated, ca. 6. 2 × as long as its greatest width. Hind basitarsus gradually widened toward distal tip (length-width ratio not measurable). Calcipala and pedisulcus well developed. ***Abdomen***. As in ♂ of *S. pahangense* except dorsal surface dark brown. ***Genitalia*** (Figs. 41-45). Coxite longer than wide. Style elongate, ca. 1.8 × as long as coxite, curved dorsally, gradually tapered toward apex, with 1 apical spine. Ventral plate in ventral view transverse, longer than wide, gradually narrowed posteriorly, with median longitudinal process produced ventrally, covered densely with minute setae, with arms slightly diverging from each other; ventral plate in end view much produced ventrally along posterior margin, with lateral margins gently rounded and apically convergent, and fully covered with minute setae. Paramere slender, with numerous hooks. Median sclerite plate-like, narrow in basal 1/3, gradually widened toward apex, and rounded apically.

Pupa. As in *S.pahangense* except the following characters: Body length (excluding gill filaments) ca. 3.0 mm. ***Head***. Face and frons covered densely with small tubercles (Fig. 46). ***Thorax***. Gill filaments (Fig. 47) brown, subequal in length (0.6-1.0 mm) and thickness, their maximal diameter a little thinner than interspiracular trunk. ***Cocoon***. Surface smooth, shiny, without bumps of silk.

Mature larva. As in *S. pahangense* except the following characters: Body length 9.0-9.5 mm. Body color dark grayish brown. Antenna much longer than stem of labral fan; length ratio of articles (from base to tip) 1.00:1.57:

0.45. Labral fan with ca. 55 main rays. Hypostomium (Fig. 49) with 9 bristles on each side. Postgenal cleft (Fig. 49) deep but not reaching posterior border of hypostomium; anterior margin irregularly defined, usually with anterolateral extensions. Rectal papilla with 8 or 9 finger-like secondary lobules on each lobe. Anal sclerite (Fig. 52) with basal portion connecting anterior and posterior arms much narrower than that of *S. pahangense*. Posterior circlet with ca. 420 rows of hooklets with up to 44 hooklets per row.

TYPE SPECIMENS. Holotype pharate ♀ with pupal exuvia and cocoon (BMNH), Montathom waterfall, 730 m in altitude, E98°55′/N18°49′, Chiang Mai Province,



Figures 36-45. Female and male genitalia of *S.* (*D.*) *courtneyi* sp. nov. 36-40, ♀ genitalia —— 36, 8th sternite and anterior gonapophysis (right half only, ventral view); 37, genital fork; 38 & 39, paraproct and cercus (38, ventral view; 39, lateral view); 40, spermatheca; 41-45, ♂ genitalia —— 41, coxite, style and ventral plate (left coxite and style omitted, ventral view); 42 & 43, ventral plate (42, end view; 43, lateral view); 44, paramere (right side only, dorsal view); 45, median sclerite. Scale bar 0.05 mm (applying to all figures)

THAILAND, 4. XII. 1995, by M. Burgett & M. Titayavan. Paratypes: 1 pharate ♀, 1 pharate ♂, dried and pinned, 5 pharate ♂ (2 partially damaged), 6 pupae, 11 pupal exuviae (7 partially damaged), 2 mature larvae and 6 immature larvae (CUAC), all in alcohol, same data as holotype; 3 pharate ♂ (1 with abnormal antennae), 2 pupae and 2 pupal exuviae (BMNH), all in alcohol, same data as holotype except date 29.XII.1995; 1 immature larva (CUAC), same data as holotype except date 3. XI. 1994 and collector G.W. Courtney; 8 immature larvae (CUAC), Mai Yai waterfall, E98°30′/N8°53′, Surat Thani Province, THAILAND, 15. X. 1994, by G.W. Courtney.

ECOLOGICAL NOTES. This species was taken from two heavily shaded streams, 16-20 °C and 5-8 m wide, flowing over a series of bedrock cascades. *Simulium courtneyi* was collected with *S. pahangense* at only one site, Mai Yai waterfall in Surat Thani Province (15 October 1994). Although we examined the gut of only one larva of *S. courtneyi* (from Chiang Mai Province), it contained thalli of the trichomycete *H. melusinae*.

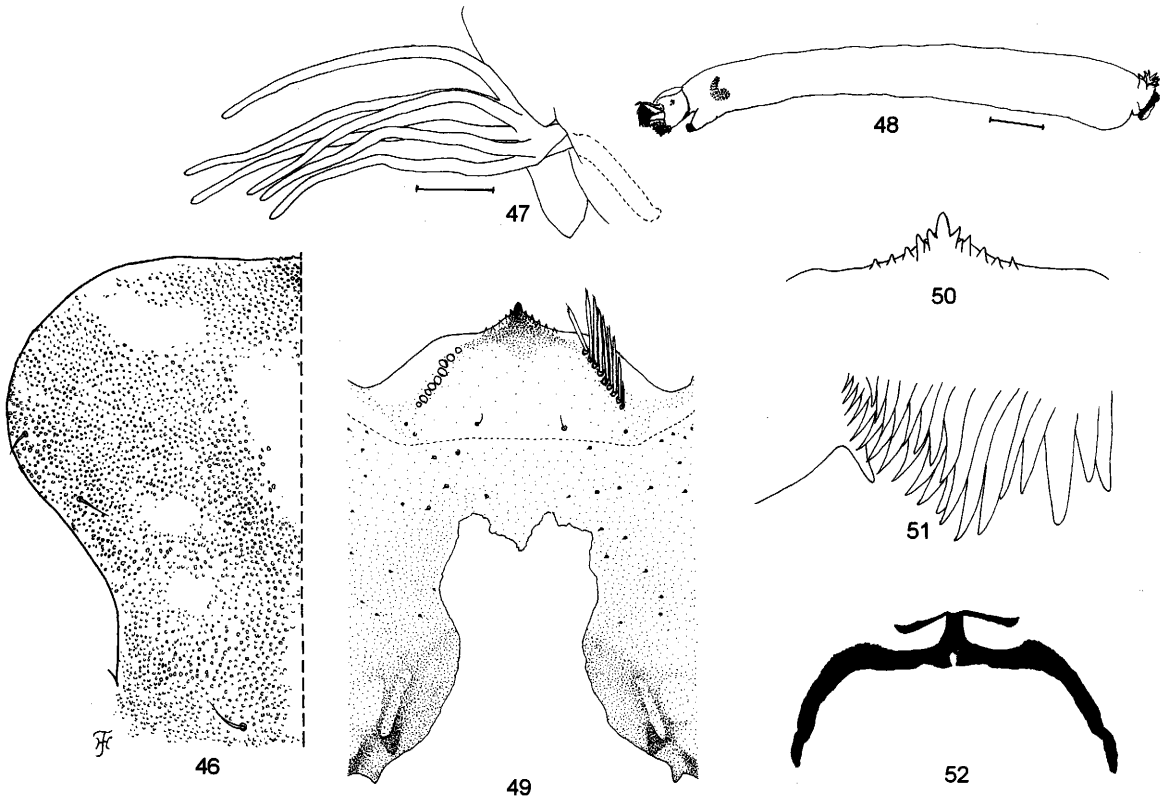
DISTRIBUTION. Thailand.

ETYMOLOGY. This species is named in honor of Gregory W. Courtney who provided us with the material from Thailand.

TAXONOMIC REMARKS. This new species shows close similarities to *S. pahangense* in all stages. However, it differs from the latter species chiefly by the following features: the shape of the genital fork and paraproct in the female; the shape of the ventral plate and median sclerite in the male; the size and coloring of the gill filaments, and the integument of the frons densely covered with tubercles in the pupa; and the shape of the postgenal cleft, as well as the length and coloring of the body in the larva.

ACKNOWLEGEMENTS

We are grateful to Dr. D. M. Davies, Professor Emeritus, McMaster University, Ontario, Canada, and Dr. R.W. Crosskey, the Natural History Museum, Lon-



Figures 46-52. Pupa and larva of *S.* (*D.*) *courtneyi* sp. nov. 46 & 47, pupa —— 46, frons (right half only); 47, gill filaments (left side, lateral view); 48-52, larva —— 48, whole body (lateral view); 49, head capsule showing hypostomium and postgenal cleft (ventral view); 50, anterior teeth of hypostomium (dorsal view); 51, apex of mandible; 52, anal sclerite (end view). Scale bars 0.2 mm for fig. 47; 1.0 mm for fig. 48

don, U.K., for their valuable suggestions. Thanks are due to Prof. Yong Hoi-Sen, University of Malaya, Kuala Lumpur, Malaysia, who helped HT in various ways during collection trips in Peninsular Malaysia. We thank Dr. G.W. Courtney, Grand Valley State University, Michigan, U.S.A., for providing material and habitat information, and Dr. D. C. Currie, Royal Ontario Museum, Toronto, Canada, for reviewing the phylogenetic portion of the paper. This study was in part supported by a Grant-in-Aid to HT from the Japan Society for the Promotion of Science.

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Research Note:

OCULAR TOXOPLASMOSIS IN AN AMAZONIAN JAPANESE SETTLEMENT

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Abstracts: Infection with *Toxoplasma gondii* is a frequent cause of retinal disease, and relatively high prevalence of the disease has been reported in Brazil. We experienced acquired and congenital forms of ocular toxoplasmosis in an Amazonian Japanese settlement, Tomé-Açu. Fundus photographs of the patients representing typical lesions of chronic retinitis are shown.

INTRODUCTION

The geographic distribution of toxoplasmosis is cosmopolitan, however the incidence varies from country to country. In Brazil, Lamb and Feldman (1968) reported that the prevalence of the infection was 52%, and particularly in the Amazonian area, it was as high as 56%. We also reported the distribution of anti-*Toxoplasma* antibodies for those living in an Amazonian Japanese settlement, Tomé-Açu (Sato *et al.*, 1989), in which we showed the overall prevalence of 75,2% without age or sex dependency. Our epidemiological study also revealed that some individuals were suffering from impaired vision. Thus in 1991, we tried to determine the prevalence of ocular toxoplasmosis in the same area by ophthalmic examinations and found out two cases with retinochoroidal lesions, which are described in the present report together with some epidemiological discussion.

SUBJECTS, MATERIALS AND METHODS

Study Area

The study area, Tomé-Açu (latitude 2°31' S, longitude 48°22' W), is located 125 km to the south of Belém, whose average temperature is 21-33°C and the rain fall in a year amounts 2,635 mm. The place, with natural water networks by Amazon branches, makes highly vegetated flat area at 11-30 m above sea level. The Japanese immigrants have been settling there since 1962, and now 70 families with about 350 people are supporting themselves mostly by agriculture. The number of Japanese Brazilian inhabitants subjected to the present study was 102, aged from 5 to 85.

Serologic and Ocular Examination

Antibodies against *Toxoplasma gondii* were assayed by micro-titer method with Latex agglutination test (Toxo-test MT, Eiken KK, Japan). Titers equal to or higher than 1 : 32 were considered positive.

Ophthalmic examinations were conducted for those who complained of impaired vision, in August, 1991. After dilation, individuals were screened with direct ophthalmoscopy for the presence of posterior uveitis by the identification of inflammatory retinal or retinochoroidal lesions. Fundus photography was performed by using portable retinal camera and Ektachrome RD135 film (Kodak).

RESULTS

Case 1

The patient was a 42-year-old male who immigrated into Tomé-Açu in 1972. He was fond of eating raw

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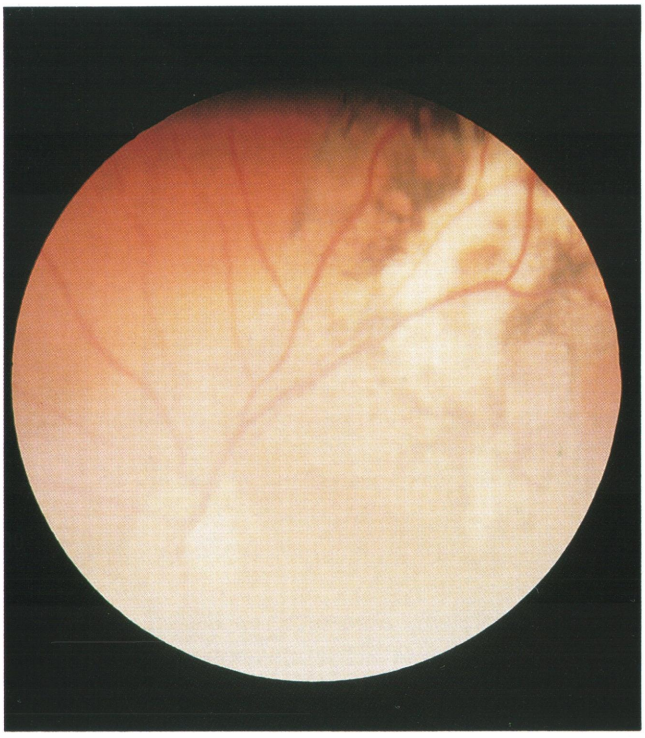

Photo 1. Fundus photograph of the left eye of the Case 1. Superior to the macula is a yellowish-white atrophic lesion with partially pigmented indistinct margin.

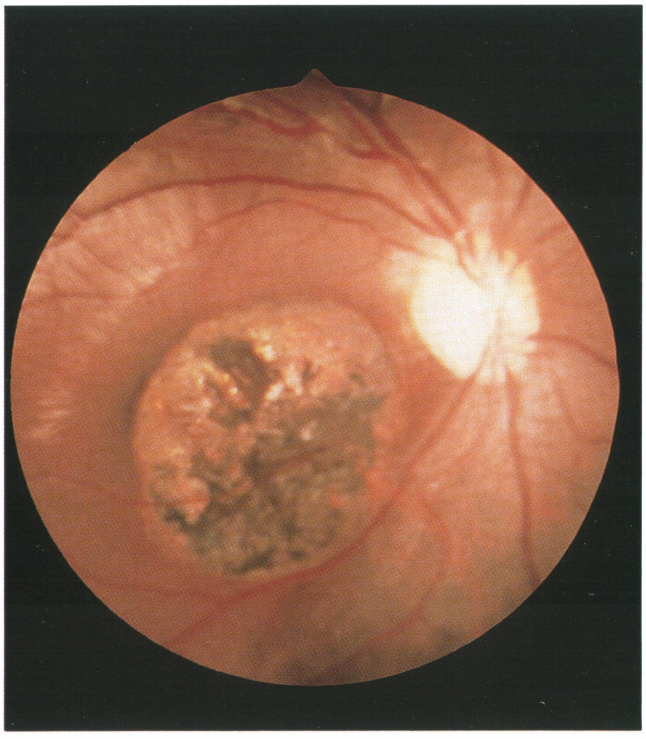

Photo 2A. Fundus photograph of the right eye of the Case 2, in which an atrophic lesion, whitish-gray with pigmentation, lies in the macular region with a well-delineated border.

meat and used to eat sliced pork uncooked. The last episode of eating raw pork was with his neighboring friend, one year before the onset of the disease. In 1974, he began to complain the impairment of vision in the inferior nasal field in the left eye, and was treated with a anti-malarial drug. A fundus photograph taken in 1991 is shown as Photo 1. An unilateral yellowish-white atrophic lesion with partially pigmented indistinct margin, was observed located superior to the macula in the left eye. Antibody titer against *Toxoplasma* was 1: 1024 in 1991. As a matter of fact, the friend of his who ate the raw meat together had become symptomatic of ocular toxoplasmosis 9 months prior to the Case 1's onset, and went back to Japan to be treated. (The detail of the history is unknown). Thus, the route of transmission to the Case 1 was considered to be from consumption of cyst-infected raw meat in the diet.

Case 2

The patient was a 13-year-old boy who had been blind from birth in the left eye, and was complaining of the defect of the superior nasal field of vision in the right eye. Strabismus was also observed in the left eye. Fundus photographs are shown as Photo. 2A and 2B.

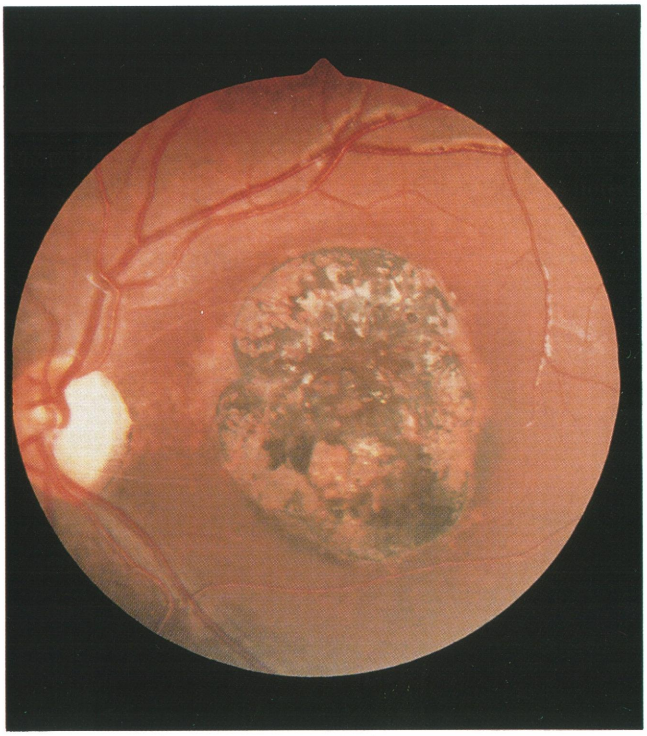

Photo 2B. Fundus photograph of the left eye of the Case 2. The same findings as in the right eye are noted.

Table 1 Anti-Toxoplasma antibody titers of the Case 2's family

Year	(Age*)	1988	1989	1990	1991
Mother	(35)	2,048**	4,096	2,048	
Father	(37)	512			
Case 2	(13)		32,768	2,048	2,048
Sister	(11)		32,768	8,192	
Brother	(9)		32,768	2,048	

*Ages in parentheses are those in August, 1991.
**Antibody titers are represented in reciprocal.

In both photographs, bilateral atrophic lesions, whitish-gray with pigmentation, were observed in the macular regions with well-delineated borders. Thus, the patient was thought to have been suffering from congenital ocular toxoplasmosis. Antibody titers against *Toxoplasma* are shown in Table 1. At the time of our survey, no other clinical manifestations of congenital toxoplasmosis were recognized on this patient.

Case 2's Family

Antibodies to *Toxoplasma* for the Case 2's parents and 2 siblings were also measured, and the titers were shown in Table 1. His mother manifested considerably high titers at 1 : 2048 (1988 and 1990) and 1 : 4096 (1989), and also his father showed positive titer at 1: 512 (1988). Although his sister and brother showed the titers as high as 1 : 32,768 in 1989, ocular toxoplasmosis was not recognized on either of them. The family was keeping several cats in the house and the children were making close contact with the cats, playing often with them or even sleeping with them.

DISCUSSION

Acquired toxoplasmic chorioretinitis was once thought to be uncommon, however in Brazil, ocular toxoplasmosis has been reported to comprise approximately 50% of all uveitis, and incidence of acquired subclinical toxoplasmic infection that causes retinochoroiditis is considered much more frequent than previously believed (Silveira *et al.*, 1988; Glasner *et al.*, 1992). Besides, the brazilian are usually fond of eating beef well charcoal-grilled so called "churrasco" and rarely eat raw meat, however, Japanese Brazilians have a preference for eating them raw, like "sashimi" or "shabu shabu", as the Case 1 practiced. This custom of eating may increase their chance to contract ocular toxoplasmosis. In the context of the control of the disease, particular attention has to be paid among the Japanese settlers.

Toxoplasmic retinochoroiditis is usually seen as a late manifestation of quiescent congenital forms of toxoplasmosis. Based on the various epidemiological studies, Stagno (1980) anticipated that approximately 6 of every 1,000 pregnant women will acquire toxoplasmosis during the 9 months of gestation. And the average incidence of congenital toxoplasmosis was reported to be 1:1000 live birth and 10% showed ophthalmic involvement (Kean *et al.*, 1991). The Case 2, unfortunately, suffered from this congenital ocular toxoplasmosis, manifesting central and bilateral lesions as are usually the case with congenital ones. Subsequent siblings of the Case 2 were thought to be protected by their mother's acquired immunologic defense mechanism, as one may theoretically expect them to be, and in fact, no ocular lesion was recognized on them. However, the considerably high antibody titers were detected for the family, so that their long-term and intense exposure to the organism was thought to be actually taking place. Their close contact with cats will be the most probable cause of their repetitive infections, although the survey of the oocysts produced from the cats have not been conducted.

Our previous serological study on the prevalence of toxoplasmosis in the same area (Sato *et al.*, 1989) also suggested the significant health hazard caused by the disease. Dedicated efforts have to be made for the control of toxoplasmosis in Tomé-Açu.

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