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Immune Response of Cholera Patients against the components of *Vibrio cholerae*

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Abstract: There was a sporadic outbreak of cholera in 1994 in Okinawa. Immune response of the cholera patients against *Vibrio cholerae* cell components and extracellular proteins were examined. A potent response to cholera toxin and hemolysin, and generally poor response to purified cellular components were seen. Although the antigen was not defined, marked elevation of vibriocidal antibody and cell agglutination antibody was observed. Only little elevation of the agglutination titer against heat-killed organisms suggests that the antigen (agglutinin) is heat labile protein on the cell surface.
Identification and purification of this protein antigen will elucidate a protective antigen of *V. cholerae*.

INTRODUCTION

Cholera patients acquire potent protective immunity after the infection (Benenson et al., 1968), however, the ideal vaccine for cholera is not developed yet, and even the protective antigen of cholera vibrio is not sufficiently elucidated. Protective antigen should be immunoresponsive, therefore, immune response to *Vibrio cholerae* O1 has been intensively studied in human volunteers, but a few studies in naturally infected cholera patients (Pierce and Sack, 1977; Richardson et al., 1989). In those papers, the antibodies against LPS, cholera toxin (CT), whole bacterial cell, and some outermembrane proteins were studied in relation to vibriocidal activities.

There was a sporadic outbreak of cholera in Okinawa in July 1994, and 6 bacteriologically confirmed patients were officially reported. The organisms isolated from the patients were all *V. cholerae* O1 El Tor, serovar Ogawa. This paper described their immune response to a variety of cellular components and extra-cellular proteins of *V. cholerae*.

MATERIALS AND METHODS

Patients and sera: The convalescent sera from 4 patients with severe diarrhea and a patient with mild self-limited diarrhea were obtained to determine the immune response against the pathogens. In addition, the sera from 6 healthy individuals without the history of cholera and traveling abroad were used as the control. Some informations about the sera were shown in Table 1.

Antigens: Antigens to examine the immune response included *V. cholerae* whole cells, cytosol proteins (complex and purified 40 kDa protein), innermembrane proteins, outermembrane proteins (complex and purified OmpS), flagellar core protein, 5 kinds of *V. cholerae* pili (VCF, V10, TCP, Mini, V14), cholera toxin (CT) and hemolysin. V10 pili, V14 pili and hemolysin were obtained from *V. cholerae* non-O1, Mini-pili from *V. cholerae* O139. The other antigens were obtained from *V. cholerae* O1.

Preparation of the antigens: The organisms cultured in heart infusion broth at 37 C for over night were harvested. The heavy suspension of the organisms in normal saline solution was treated with biomixer (Nihon Seiki CO., Tokyo, Japan) to detach pili and flagella. The cells without pili and flagella were frozen at -80 C for a few days, once made them thaw, refrozen at -20C, and then crushed the cells with X-press. The crushed materials were centrifuged at 85,000×g for 1 hour, and the supernatant was regarded as cytosol. The pellet was washed 2 times with saline and collected with centrifugation. The final pellet was suspended in 10 mM PBS (pH 7.4) supplemented with 1% sodium lauroyl sarcosinate, and homogenized in a glass-homogenizer under mild sonication at times. The homogenized sam-

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Table 1 Informations about the sera

Serum	Age	Sex	sampling*	diarrhea	other symptoms
P1	52	M	17	Severe	muscle cramp, acute renal failure
P2	80	F	9	Severe	muscle cramp
P3	44	F	10	Severe	acute renal failure
P4	39	M	9	Severe	muscle cramp
P5	51	F	11	mild (self-limited)	
H1	21	F	—	healthy	
H2	25	M	—	healthy	
H3	24	M	—	healthy	
H4	21	M	—	healthy	
H5	26	M	—	healthy	
H6	28	M	—	healthy	

*: Days after onset, P: Patients, H: Controls (healthy subjects)

ple was centrifuged at 85,000×g for 1 hour, and the supernatant was regarded as innermembrane. The pellet was repeatedly homogenized and sonicated in 1% SLS, and the final pellet was regarded as outermembrane. Flagellar core was purified from a *V. cholerae* O1 El Tor by detaching with biomixer, sedimentation in polyethylene glycol, and DEAE column chromatography. The other antigens were purified in accordance with the references as VCF pili (Ehara et al., 1987; Iwanaga et al., 1989), V10 pili (Yamashiro et al., 1993), TCP (Osek et al., 1994), Mini-pili (Yamashiro et al., 1994), CT (Nakashima et al., 1995; Spyrides and Feeley, 1970), hemolysin (Iwanaga and Ichinose, 1991), etc.

Detection of antibodies: Presence of antibodies against various antigens were examined by western blotting as described by Towbin et al. (8) using 1:200 dilution of the patient's and control sera as the first antibodies. Horse radish peroxydase (HRP) conjugated anti-human IgG goat serum (CAPPEL) diluted to 1:1000 was used as the second antibody.

Titration of the antibodies: Vibriocidal antibody was titrated by using microplate technique as described by Bennenson (1968). Agglutination titers were examined by serial 2 fold test tube dilution method using live and heat-killed organisms. Vibriocidal- and agglutination activities of the sera were examined for the organisms isolated from the patient corresponding to each serum. Antibody titers were expressed as the reciprocal of the maximum dilution in the positive findings. Anti-CT and anti-hemolysin antibodies were titrated by ELISA using serially 2 fold dilution of the patient's sera and the antigen coated plastic plates. HRP conjugated anti-human IgG goat serum diluted to 1:1000 was used as the second antibody, and 2,2'-azino-bis (3-ethylbenzthiazoline sulfonic acid) was used as the substrate. Antibody titers were expressed as reciprocal of the maximum dilution of the patient's sera giving the OD_{415} more than 0.3.

RESULTS

Agglutination titers: Agglutination titers of the patient's sera against live organisms were markedly elevated when live organisms were used as the antigen. However, serum of the patient with mild diarrhea revealed low elevation of the titer as 256. The highest titer in the control sera was 32. The agglutination against heat-killed organisms was very poor (Table 2).

Vibriocidal titer: Vibriocidal activities of the patient's sera showed the titer more than 20,480 except the serum of the patient with mild diarrhea. The control

Table 2 Titration of the antibodies

Serum	Cell Agglutination		Vibriocidal
	Live	Heat-killed	
P1	>8,192	8	>20,480
P2	4,096	8	>20,480
P3	1,024	8	>20,480
P4	>8,192	8	>20,480
P5	256	<4	10,240
H1	8	<4	80
H2	<4	<4	<10
H3	<4	<4	40
H4	32	<4	2,560
H5	8	<4	320
H6	<4	<4	640

Table 3 Immunoreactivities as examined by Western blot (No. of positive/examined)

CPs				IMPs				OMPs			
MW	HC	Pts	P-value	MW	HC	Pts	P-value	MW	HC	Pts	P-value
115	4/6	4/5	0.575	115	3/6	4/5	0.348				
85	0/6	1/5	0.455	85	1/6	3/5	0.197				
77	1/6	1/5	0.727	77	0/6	1/5	0.455				
71	0/6	1/5	0.455	67	2/6	3/5	0.392				
56	1/6	1/5	0.727	56	3/6	2/5	0.608				
				45	1/6	4/5	0.067	45	4/6	2/5	0.392
43	0/6	1/5	0.455	43	0/6	2/5	0.182	43	4/6	3/5	0.652
				33	0/6	1/5	0.455	33	1/6	3/5	0.197
28	0/6	1/5	0.455	23	1/6	2/5	0.424				
14	2/6	1/5	0.576	14	1/6	2/5	0.424				

CPs: Cytosol proteins, IMPs: Innermembrane proteins, OMPs: Outermembrane proteins,
MW: molecular weight of reacted protein, HC: sera of healthy controls,
Pts: patients' sera, P-value: calculated by applying Fisher's exact probability

Table 4 Immunoreaction frequency of the sera against purified antigens as examined by Western blot (No. of positive/examined)

Antigens	HC	Pts	P-value
Flagellin	0/6	0/5	1.000
40kp	0/6	1/5	0.455
OmpS	1/6	1/5	0.727
Cholera toxin			
A subunit	0/6	2/5	0.182
B subunit	1/6	5/5	0.013
Hemolysin	0/6	4/5	0.015
Pili			
TCP	0/6	0/5	1.000
VCF/classic	0/6	0/5	1.000
VCF/El Tor	0/6	0/5	1.000
Mini	0/6	0/5	1.000
V10	0/6	0/5	1.000
V14	0/6	2/5	0.182

HC: Control sera, Pts: Patient's sera, 40kp: 40kDa cytosol protein
P-value: calculated by applying Fisher's exact probability

serum with its agglutination titer of 32 revealed vibriocidal titer of 2,560 (Table 2).

Antibodies against cellular components: Some of the cytosol proteins, innermembrane proteins, and outermembrane proteins reacted to the patient's sera and to the control sera with the simillar extent (Table 3). Reactions of the sera to the purified components such as pili, flagella, OmpS (maltose induced 43kDa outermembrane protein), 40kDa cytosol protein, etc. were almost negligible, except that 2 sera showed a potent reaction to V14 pili (Table 4).

Table 5 ELISA titer of sera to extracellular proteins

Serum	CT	Hemolysin
P1	400	1,600
P2	200	400
P3	800	<50
P4	800	200
P5	400	800
H1	100	50
H2	<50	<50
H3	<50	<50
H4	50	50
H5	<50	<50
H6	<50	<50

Antibodies against extracellular proteins: Contrary to the cellular components, the patient's sera potently reacted to extra-cellular proteins such as cholera toxin and hemolysin, whereas the control sera did not react (Table 4, 5).

DISCUSSION

Although there have been many studies on immune response to *V. cholerae* in cholera infection, the attention was directed to the response against some limited antigens (Hall et al., 1991; Majumdar et al., 1981; Richardson et al., 1989). The protective antibody for cholera has not been identified so far. In the present study, the response to a variety of antigens such as extracellular proteins, cytosol proteins, innermembrane proteins,

outermembrane proteins, flagellin, and pilins was examined. The organism of *V. cholerae* has uncountable number of antigens. However, the present study revealed that the host immune response was not seen against all the antigens but a limited number of the antigens. A protective antigen of a certain pathogen is supposed to induce a potent immune response and to be a pathogenic factor. Cholera toxin is a main pathogenic factor of *V. cholerae* and induce potent immune response. Animals immunized with CT are protected from the illness to some extent, however in human beings, vaccination with CT does not protect the vaccinees from cholera (Noriki, 1976; Pierce and Sack, 1977). Hemolysin is also a potent immunogen but not likely a protective antigen. The elevation of the antibodies against a variety of the purified cellular components including a group of proteins in cytosol and membranes was not commonly seen. Besides, the antibodies against those proteins were also detected from some of the control sera with the simillar frequency. It suggests that there must be some cross antigens in the intestinal floral organisms. Anti-pili antibodies were not detected at all except anti-V14 pili. TCP has been recognized to be involved in colonization and not to elevate the antibody in cholera patients (Hall et al., 1991). The other pili excluding V14 pili were proved not to be colonization factors (Iwanaga et al., 1989; Nakasone and Iwanaga, 1990), therefore, it is no wonder that anti-pili antibodies were not elevated in these cholera patients. V14 pili have been newly identified in the recent, and this pilus is likely a colonization factor according to the studies so far (unpublished data). In this viewpoint, the immunological reaction against V14 pili, although 2 cases out of 5, is noteworthy.

Outstanding immune response of cholera patients, except the elevation of antibodies against CT and hemolysin, was seen in marked elevation of vibriocidal antibody and agglutination antibody against live organisms. Vibriocidal activity of those sera was elevated in parallel with agglutinating activity against live organisms but not against heat-killed organisms. It suggests that the agglutinin reacting to the patient's sera is heat labile substance located at the surface of the organisms. The antigen for vibriocidal antibody and the heat labile agglutinin could be identical, therefore, the identification of non-LPS heat labile agglutinin may be a key to elucidate protective antigen of *V. cholerae* O1.

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NEUTRALIZATION OF *CRYPTOSPORIDIUM MURIS* SPOROZOITES BY RABBIT ANTI-*C. MURIS* SERUM

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Abstract: The neutralization of *Cryptosporidium muris* sporozoites by rabbit antiserum was examined in vitro and in mice. The sporozoites of *C. muris* could be separated from intact oocysts by repeated centrifugation. The antiserum reacted strongly with three bands of sporozoite antigens at >200, 78, and 47.5 kilodaltons during immunoblotting. Heat-inactivated antiserum caused morphological changes seen ultrastructurally in the sporozoites. The antiserum reduced the viability of the sporozoites as measured with fluorescein diacetate. When antiserum-treated sporozoites (1×10^5) were used to inoculate 7-day-old mice per os, endogenously developed parasites were first detected 10 days later by an indirect fluorescence antibody test. When sporozoites treated with normal rabbit serum were used to inoculate mice, parasites were first detected 6 days later. The results suggest that the antiserum partially neutralizes the infectivity of *C. muris* sporozoites.

INTRODUCTION

Cryptosporidium is a genus of parasitic protozoa that infect respiratory and gastrointestinal epithelial cells of a wide range of vertebrates, causing cryptosporidiosis in humans and domestic animals (Dubey *et al.*, 1990). *C. muris* parasitizes the stomachs of cattle and rodents, causing gastric lesions and decreasing weight gains in cattle (Anderson, 1989).

Chemotherapy is not effective in this infection (Dubey *et al.*, 1990). Mata (1986) suggested that breast-fed children are partly protected against *Cryptosporidium*. Hyperimmune bovine colostrum and anti-parasite antibodies are said to be at least partially efficacious in preventing and treating infection by *C. parvum* in mice (Riggs and Perryman, 1987; Fayer *et al.*, 1989; Perryman *et al.*, 1990; Fayer *et al.*, 1990; Tilley *et al.*, 1991; Riggs *et al.*, 1994) or an immunodeficient child (Tzipori *et al.*, 1986). However, other workers have found that antibodies and lacteal factors are not useful for the prevention or treatment of infection in mice (Moon *et al.*, 1988; Arrowood *et al.*, 1989) or humans (Saxon and Weinstein 1987; Sterling *et al.*, 1991).

Reports on experimental infections with *C. muris* are limited to studies by Uni *et al.* (1987), Iseki *et al.* (1989), and McDonald *et al.* (1992). The purpose of this study is to identify the effects of antiserum to *C. muris* on sporozoite viability and infectivity in an examination of the efficacy of immunotherapy for *C. muris* infection.

MATERIALS AND METHODS

The RN 66 strain of *C. muris* was maintained by subinoculation into specific-pathogen-free (SPF) mice in our laboratory. Purified oocysts were obtained from the feces of infected mice by sucrose flotation and stored in 2.5% $K_2Cr_2O_7$ at 4°C for up to 1 month before use (Iseki *et al.*, 1989).

Sporozoite preparation: Purified oocysts (4×10^7) were suspended in minimum essential medium (MEM, Flow Laboratories, Irvine, Scotland) and centrifuged at $600\times g$ for 5 min at 4°C to remove light oocysts. This process was repeated a total of three times. The sedimented oocysts were suspended in MEM and incubated for 30 min at 37°C. This excystation preparation, in which 75% of the oocysts were excysted, was centrifuged at $600\times g$ for 5 min at 4°C. The supernatant (the upper 3/4 of the 10 ml in the plastic tube) was taken out with care not to

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shake the tube and contained both sporozoites (1×10^8) and oocysts (1×10^7). MEM was added to the supernatant and the mixture was centrifuged at $600\times g$. The supernatant obtained was centrifuged one more time in the same way. The supernatant included sporozoites (4×10^6) and intact oocysts (1×10^3), which were collected by further centrifugation at $1,500\times g$ for 15 min and used as the sporozoite preparation in this experiment.

Preparation of antiserum against oocysts and sporozoites: Excysted oocysts (4×10^7; about 0.5 mg/ml) in phosphate-buffered saline (PBS) were homogenized with an ultrasonic homogenizer (Nihonseiki Co., Ltd., Tokyo). One healthy New Zealand White rabbit without coccidia infection was immunized by a subcutaneous injection of an emulsion of excysted oocysts with Freund's complete adjuvant, and a booster injection with excysted oocysts (1×10^8) in Freund's incomplete adjuvant was made 5 weeks later. Blood was collected 8 days after the final injection of the antigen and the serum was obtained.

The specificity of the antiserum for *C. muris* sporozoites was examined by an indirect fluorescence antibody test (IFAT). Air-dried sporozoites fixed in acetone were incubated with diluted rabbit antiserum for 30 min, washed with PBS, and incubated for 30 min with goat anti-rabbit immunoglobulin G (IgG) labeled with fluorescein isothiocyanate (Organon Teknika, Malver, PA).

Electrophoresis and western blotting: A sample of the sporozoite preparation (5×10^7 sporozoites) was suspended in 200 μl of buffer (150 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 50 mM Tris, and 0.02% sodium azide, pH 7.4) containing 0.5% nonionic detergent Nonidet P-40 (Mead *et al.*, 1988). The preparation was freeze-thawed twice and homogenized by ultrasonication. A 50-μl sample was diluted with an equal volume of sample buffer [100 mM Tris buffer, 4% sodium dodecyl sulfate (SDS), 10% 2-mercaptoethanol, 0.2% bromophenol blue, and 15% glycerin] and the mixture was boiled for 4 min. SDS-polyacrylamide gel electrophoresis (PAGE) of the sporozoite sample was done with 5% stacking gels and 10% separating gels at a constant current of 20 mA in a slab-electrophoresis cell (Rapidas AE-6450, Atto Corp., Tokyo).

After SDS-PAGE, the antigens were transferred from the gels to nitrocellulose membranes ("Clear blot membrane-P", Atto) with a device for semi-dry western blotting (Horize-Blot AE-6675P, Atto). Electrophoretic transfer was done with a constant current of 1.5 mA/cm^2 for 1 hr. The nitrocellulose strips were treated with goat serum, incubated with the rabbit antiserum (1:500), and treated with biotinylated anti-rabbit IgG. The strips were then treated with avidin-biotin complexes (ABC) bound with horseradish peroxidase (Vectastain; Vector Laboratories, Inc., Burlingame, CA). Antigens were made visible with 4-chloro-1-naphthol. In the control experiment, the nitrocellulose strips were treated with normal rabbit serum in the same way. Molecular mass standards for comparison were rabbit skeletal muscle myosin (200 kDa), *Escherichia coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), hen egg white ovalbumin (42.6 kDa), bovine carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa), and hen egg white lysozyme (14.4 kDa) (Bio-Rad Laboratories, Richmond, CA).

Sporozoite viability assay: A portion of the sporozoite preparation (1×10^5 sporozoites in 1 ml of MEM) was mixed with 100 μl of heat-inactivated (56°C, 30 min) antiserum, incubated at 37°C for 30 min, and washed in PBS. A 100-μl sample of this sporozoite preparation in PBS was mixed with 900 μl of fluorescein diacetate (0.1 mg/ml, Lambda Probes & Diagnostics, Graz, Austria) and the mixture was incubated for 15 min at room temperature. The sporozoites were examined under a fluorescence microscope. In the control experiment, a portion of the sporozoite preparation was incubated with MEM or heat-inactivated normal serum and treated with fluorescein diacetate. Sporozoites boiled for 5 min also were treated with fluorescein diacetate in the same way.

Transmission electron microscopy (TEM): A portion of the sporozoite preparation (1×10^5 sporozoites in 1 ml of MEM) was mixed with 100 μl of heat-inactivated antiserum, incubated at 37°C for 30 min, and fixed in 1% paraformaldehyde-3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). For the control specimen, some of the sporozoite preparation was incubated with heat-inactivated normal serum and fixed in the same way. The fixed specimens were dehydrated, embedded, and sectioned as described previously (Uni *et al.*, 1987). The specimens were examined with a JEOL 1200-EX electron microscope at 80 kV.

Sporozoite infectivity assay: A portion of the sporozoite preparation (1×10^6 sporozoites in 1 ml of MEM) was mixed with 100 μl of heat-inactivated antiserum and incubated at 37°C for 30 min. In the control experiment, some of the sporozoite preparation was incubated with MEM or heat-inactivated normal rabbit serum. After the incubation, a sample of the sporozoite preparation (1×10^5 sporozoites in 0.1 ml of the incubation medium per mouse) was given orally to 7-day-old BALB/c

CrSlc mice (Japan SLC Inc., Shizuoka) through a polyethylene catheter (20 gauge, Terumo Corp., Tokyo). For evaluation of the infectivity of the contaminating oocysts in the sporozoite preparation, a portion of the sporozoite preparation was suspended in distilled water and used to inoculate mice; the step of incubation mentioned above was omitted. (Sporozoites are destroyed by osmosis, but intact oocysts remain viable.) In all experiments, one or two mice from each treatment group were killed and examined daily for parasites on days 6 to 10 and on day 13 postinoculation (PI).

Impression smears of the gastric wall (the entire posterior glandular part was used) were prepared from inoculated mice, and IFAT was used to find the parasites. The posterior glandular part of the gastric wall is the parastic location of *C. muris*.

RESULTS

Excystation and sporozoite preparation: A high percentage (70-90%) of excystation was achieved by the incubation of *C. muris* oocysts for 30 min at 37°C in MEM without treatment with sodium hypochlorite, sodium taurocholate, or trypsin when the oocysts were used within 2 weeks after isolation from experimentally infected mice. The oocysts were sticky, and usually adhered to the surface of the plastic tube. After centrifugation ($600 \times g$) for 5 min, sporozoites and a few intact oocysts were found in the medium, and almost all oocysts were found at the bottom of the plastic tube. By repeated centrifugation of the supernatants, the contaminating oocysts in the sporozoite preparation could be reduced to 0.03% of the number of sporozoites.

Characterization of sporozoite antigens recognized by rabbit antiserum: Twelve bands (>200, 200, 78, 47.5, 40, 33.5, 31.5, 30, 29, 27.5, 26.5, and 26 kDa) of antigens from *C. muris* sporozoites were detected with the antiserum (Fig. 1, lane A). There were strong bands at >200, 78, and 47.5 kDa. Three bands (27.5, 26.5, and 26 kDa) were seen faintly in the low-molecular-weight range, where a diffuse mass of antigens had moved. Use of normal rabbit serum resulted in no bands (lane B).

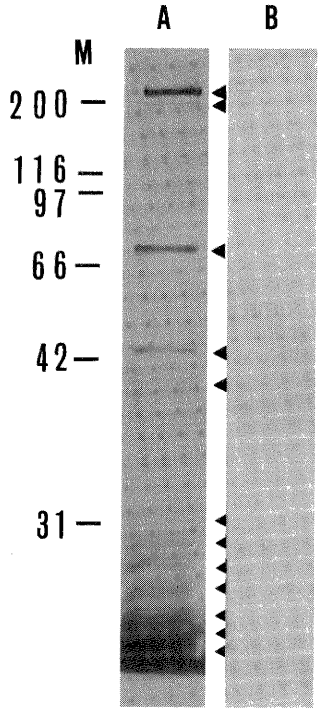


Figure 1. Western blotting of sporozoite antigens reacting with rabbit anti-*C. muris* serum (lane A) and normal rabbit serum (lane B). M, molecular weight marker proteins (10^3).

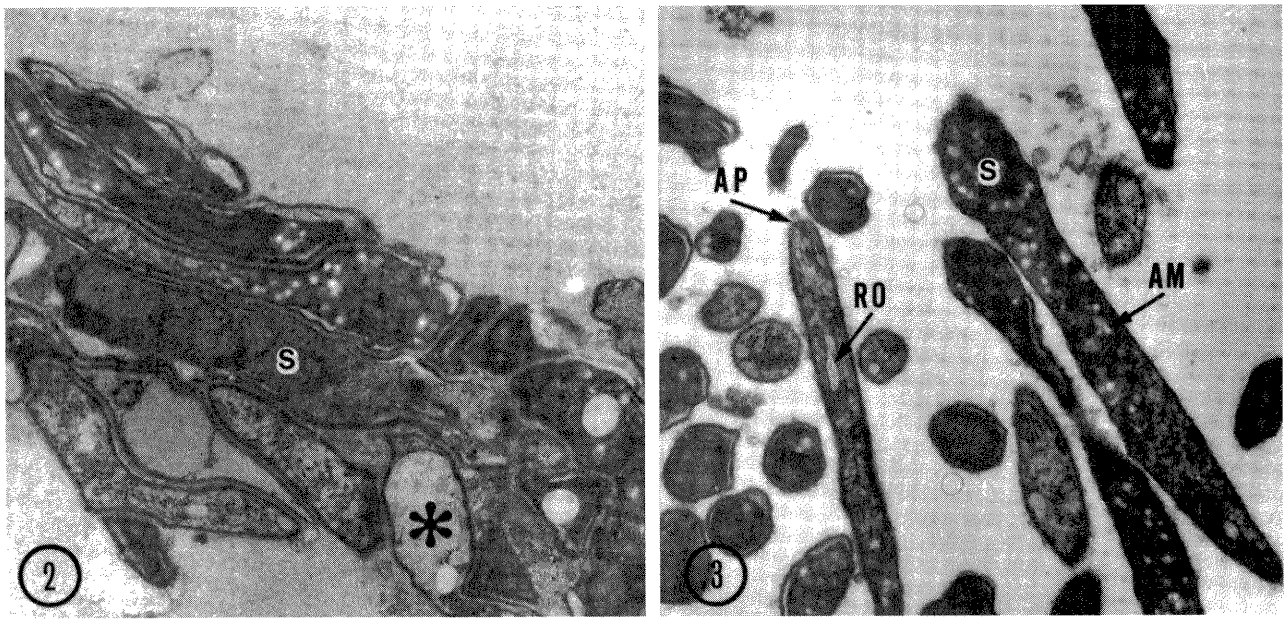
The antiserum had a titer of 1:8,000 for sporozoites by IFAT. The serum reacted also with merozoites, meronts, macrogametes, and oocyst walls in impression smears, but did not react with microgametes.

Viability of sporozoites treated with antiserum: In vitro, 23% of sporozoites treated with heat-inactivated antiserum did not fluoresce (were not viable) after treatment with fluorescein diacetate. In contrast, 1% of sporozoites treated with normal serum or medium alone did not fluoresce (Table I). Boiled sporozoites did not fluoresce.

Table I. Viability of *C. muris* sporozoites assayed with fluorescein diacetate

Treatment	Sporozoites		
	Fluorescent	Not fluorescent	Total
1×10^5 sporozoites (sp) + immune rabbit serum diluted (1:10) in medium	278	84	362
Sp+normal rabbit serum diluted (1:10) in medium	119	1	120
Sp+medium	297	3	300

$p < 0.01$, χ^2 test.



Figures 2-3. Transmission electron micrographs of sporozoites treated with immune and normal sera. Cytoplasmic vacuolization (*) and agglutination are seen in the sporozoites treated with the antiserum (Fig. 2). ×11,000. Sporozoites treated with normal serum (Fig. 3). ×11,000. AP, apical complex; RO, rhoptry; AM, amylopectin body; S, sporozoite.

Morphological changes in sporozoites treated with antiserum: Morphological changes such as swelling, cytoplasmic vacuolization, and agglutination of the sporozoites were seen by TEM in the sporozoites treated with heat-inactivated antiserum (Fig. 2). Normal serum caused virtually no morphological changes (Fig. 3).

Infectivity of sporozoites treated with antiserum: Parasites were first detected on day 10 PI in the mice when heat-inactivated antiserum was used (Table II). In control mice, parasites had already appeared on day 6 PI. In preliminary experiments, the parasite was not detected till day 5 PI. On day 13, a mean of 615 parasites per smear was found in the four control mice, but there were fewer than 10 parasites in the one mouse infected when inoculated with sporozoites treated with the antiserum. The parasite was not detected on day 13 PI in the mice inoculated with the sporozoite preparation treated with distilled water.

DISCUSSION

It was essential for detection of neutralization of the sporozoites to have infectious sporozoites without intact oocysts. Sodium hypochlorite, sodium taurocholate, and trypsin have been used in the excystation of

Table II. Neutralization of *C. muris* sporozoites by antiserum

Inoculum	Number of infected mice/number of mice examined on postinoculation days					
	6	7	8	9	10	13
1×10^5 sporozoites (sp) +immune rabbit serum (1:10)[a]	0/4	0/4	0/8	0/4	5/8	1/3
Sp+normal rabbit serum (1:10)	2/3	3/4	5/6	3/3	5/5	4/4
Sp+medium	0/2	1/2	2/4	2/2	3/3	3/3
Sp+distilled water	0/3	0/1	0/4	0/1	0/4	0/4

[a]Dilution was with the medium.

oocysts of *C. parvum* parasitizing the intestine (Dubey *et al.*, 1990), but in *C. muris* parasitizing the stomach, these treatments were not necessary for excystation. Oocyst contamination (2.2%) of the sporozoites isolated by isopycnic Percoll gradients (Arrowood and Sterling, 1987) and 0.06% oocyst contamination of the sporozoites separated by DEAE-cellulose anion-exchange chromatography (Riggs and Perryman, 1987) were found in experiments with *C. parvum*. We collected sporozoites of *C. muris* with 0.03% oocyst contamination by repeated centrifugation. The omission of the use of sodium hypochlorite probably allowed oocysts to adhere to the tube, accounting for the low contamination rate. The experiment with the sporozoite preparation treated with distilled water showed that the few oocysts remaining had virtually no effect on results of this sporozoite infectivity assay.

Treatment with antiserum did not completely prevent *C. muris* infection of mice. Nevertheless, the prepatent period was longer and parasites were fewer in mice inoculated with sporozoites treated with antiserum. The result in mice seems to be correlated with the data on the viability of sporozoites in the in vitro assay. In infections by *C. parvum*, a high titer or concentration of antibodies in the passively administered bovine colostrum is said to be effective in reducing the infection (Taghi-Kilani *et al.*, 1990, Doyle *et al.*, 1993). Arrowood *et al.* (1989) found that neonatal mice receiving an oral dose of antisporozoite monoclonal antibodies before oocyst inoculation are as susceptible to infection as the control mice, but that daily oral treatment with the same monoclonal antibodies results in fewer parasites in the infected mice.

The antiserum may include antibodies that can agglutinate sporozoites. A monoclonal antibody that agglutinates *Eimeria tenella* sporozoites has been pre pared (Speer *et al.*, 1985). Another monoclonal antibody causes circumsporozoite precipitation (CSP) and abolishes the infectivity of malaria sporozoites (Yoshida *et al.*, 1980). CSP is accompanied with cytoplasmic vacuolization of the sporozoites (Cochrane *et al.*, 1976). Riggs *et al.* (1994) have reported that bovine antibodies against *C. parvum* have an immunother apeutic effect against cryptosporidiosis in severe combined immune-deficient (SCID) mice, and elicit a CSP-like reaction to the sporozoites. Here also, some morphological changes observed in *C. muris* sporozoites treated with heat-inactivated antiserum suggested that there was a CSP-like reaction.

When antigens obtained from *C. muris* oocysts and sporozoites are used in immunoblotting, bands at 110 kDa, 54-51 kDa, and in the region of low molecular weights have been found (Nina *et al.*, 1992), unlike our finding of sporozoite antigens with bands at 78, 47.5, and 27.5 kDa. *C. parvum* sporozoites have surface antigens of 55, 28, 20, and 15 kDa that may be responsible for induction of the neutralization antibodies (Mead *et al.*, 1988; Riggs *et al.*, 1989; Tilley *et al.*, 1991). The *C. muris* sporozoite antigen of 27.5 kDa that was recognized by this antiserum seems to be similar to the *C. parvum* sporozoite antigen of 28 kDa. Antibodies that neutralize sporozoites reduce the infectivity of merozoites also, because *C. parvum* sporozoites and merozoites share epitopes (Bjorneby *et al.*, 1990; Tilley *et al.*, 1991). However, the 15-kDa antigen of *C. parvum* was not detected in *C. muris* from cattle (Tilley *et al.*, 1991).

The results from our experiments show that antibodies in anti-*C. muris* serum may neutralize the viability and infectivity of *C. muris* sporozoites. Secretory immunoglobulin of animals (e.g., cattle) resists digestion in the human gastrointestinal tract; when colostrum was administered to patients, Saxon and Weinstein (1987) neutralized gastric acidity to help increase such resistance. Thus, immunotherapy with oral administration of monoclonal antibodies or of immune serum or immune colostrum from suitable animals may be useful for the control of gastric cryptosporidiosis, which might be caused by *C. muris* in immunocompromised patients.

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THE SIMULIIDAE (DIPTERA) FROM BOUGAINVILLE ISLAND, PAPUA NEW GUINEA

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Abstract: Seven black fly species were identified, chiefly on the basis of reared adults, pupae and mature larvae collected from Bougainville Inland, Papua New Guinea. All were assigned to the genus *Simulium* Latreille s. l. and were further classified into two subgenera, *i.e.*, six species in *Simulium* (*Morops*) and one in *Simulium* (*Gomphostilbia*). *S.* (*M.*) *noroense* and *S.* (*G.*) *hiroshii* were newly recorded from Bougainville Island, and five other species were described as new species. Under the subgenus *Morops*, a new species-group was proposed.

Smart and Clifford (1965) reported 52 species of *Simulium* Latreille s. l. from Papua New Guinea. Crosskey (1967) classified these species, and those of Australia and the Western Pacific, into subgenera and species-groups. Stone and Maffi (1971) recorded two black fly species from Guadalcanal, the Solomon Islands. Seven other species were also collected from the Solomon Islands (Takaoka, 1994; Takaoka and Suzuki, 1994 & 1995). However, no study has previously been made on black flies of Bougainville Island.

In this study seven species of the genus *Simulium* s. l. were recognized. They were reared or light-trapped adults, pupae and mature larvae collected from Bougainville Island in 1965 by Dr. R.W. Crosskey and in 1987-1989 by Miss Cathy Yule.

Descriptions of five new species are given, and a new species-group within the subgenus *Morops* is proposed.

Type specimens will be deposited in the Natural History Museum (BMNH), London, U.K., and in the Australian National Insect Collection (ANIC), Canberra, Australia.

Genus *Simulium* Latreille s. l.

Subgenus *Gomphostilbia* Enderlein

This subgenus was first defined by Crosskey (1967) and recently revised by Takaoka and Davies (1995). This is characterized by the bare pleural membrane and haired katepisternum in adults of both sexes.

1. *Simulium* (*Gomphostilbia*) *hiroshii* Takaoka, 1994

Simulium (*Gomphostilbia*) *hiroshii* Takaoka, 1994: 97-101.

SPECIMEN EXAMINED. 1 ♀ (ANIC), in alcohol, caught inside the house, 10/39 Arawa, Bougainville Island, Papua New Guinea, 1. X. 1987, by C. Yule.

DISTRIBUTION. Bougainville Island (new record) and Solomon Islands (New Georgia Island and Guadalcanal Island).

REMARKS. The female specimen well agreed with the original description of *S.* (*G.*) *hiroshii* reported from the Solomon Islands (Takaoka, 1994). The female of this species is easily distinguished from the other *Morops* species by the large basal tooth of the tarsal claws as well as the bare pleural membrane. It is interesting that the female genitalia of *S.* (*G.*) *hiroshii* including the spermatheca with internal setae are very similar to those of the *sherwoodi*-group of the subgenus *Morops*, as noted later.

Subgenus *Morops* Enderlein

This subgenus was well defined by Crosskey (1967) and was divided into several species-groups (Crosskey, 1967; Colbo, 1976). The subgenus *Morops* is characterized in the adults by the presence of hairs on both pleural membrane and katepisternum. In this study, all

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the seven, but one in the subgenus *Gomphostilbia* Enderlein, were assigned to this subgenus; two species were further classified into the *clathrinum*-group and four were into the newly proposed *sherwoodi*-group.

(A) *clathrinum*-group

This species-group is easily distinguished from other species-groups in having the pit-like cuticular organ at the base of pupal gill filaments (Crosskey, 1967). The identification of species within this species-group is usually difficult in the adult and larval stages but is most reliably made in the pupal stage by comparing the size and feature of pit-like organ, as well as the shape of gill filaments. Takaoka and Suzuki (1995) proposed *plo* ratio (= A/B as shown in Fig. 1) to compare relative sizes of the pit-like organ.

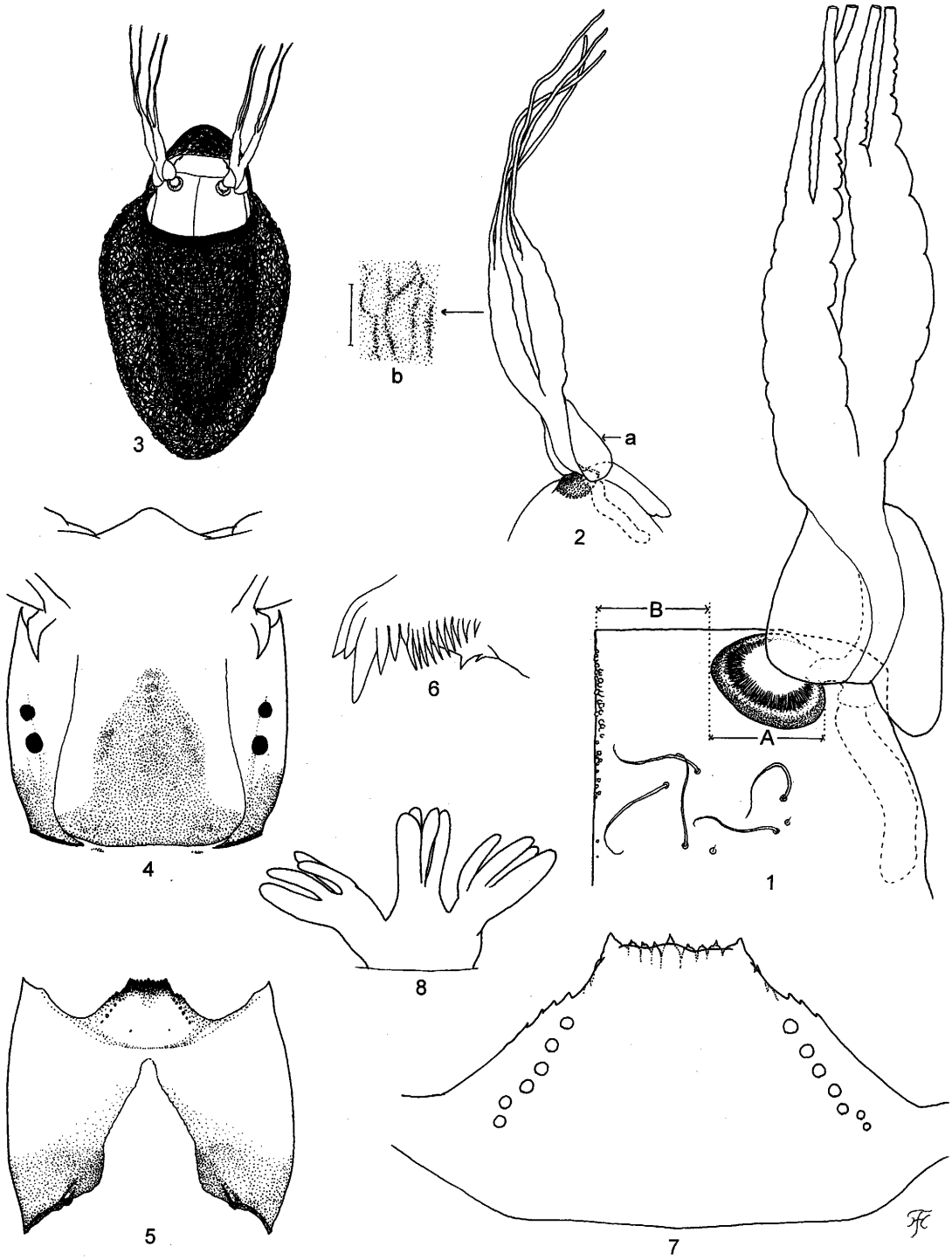
The *clathrinum*-group species were recorded from Australia (one species) (Mackerras and Mackerras, 1948), Papua New Guinea (four species), New Britain (one species) (Smart and Clifford, 1965) and Solomon Islands (four species) (Takaoka and Suzuki, 1995). Our recent investigation in Indonesia indicates the presence of species of this species-group in Halmahera (two species), Ambon (two species), Seram (three species), Biak (one species) and Irian Jaya (two species), extending its distribution up to Halmahera Island in the west (unpublished data). Interestingly, more specialized pit-like organs, such as that of *S.* (*M.*) *aropaense,* are at present recognized only in four species, each on Papua New Guinea, Bougainville Island, the Solomon Islands and Australia.

2. *Simulium* (*Morops*) *aropaense* sp. nov.

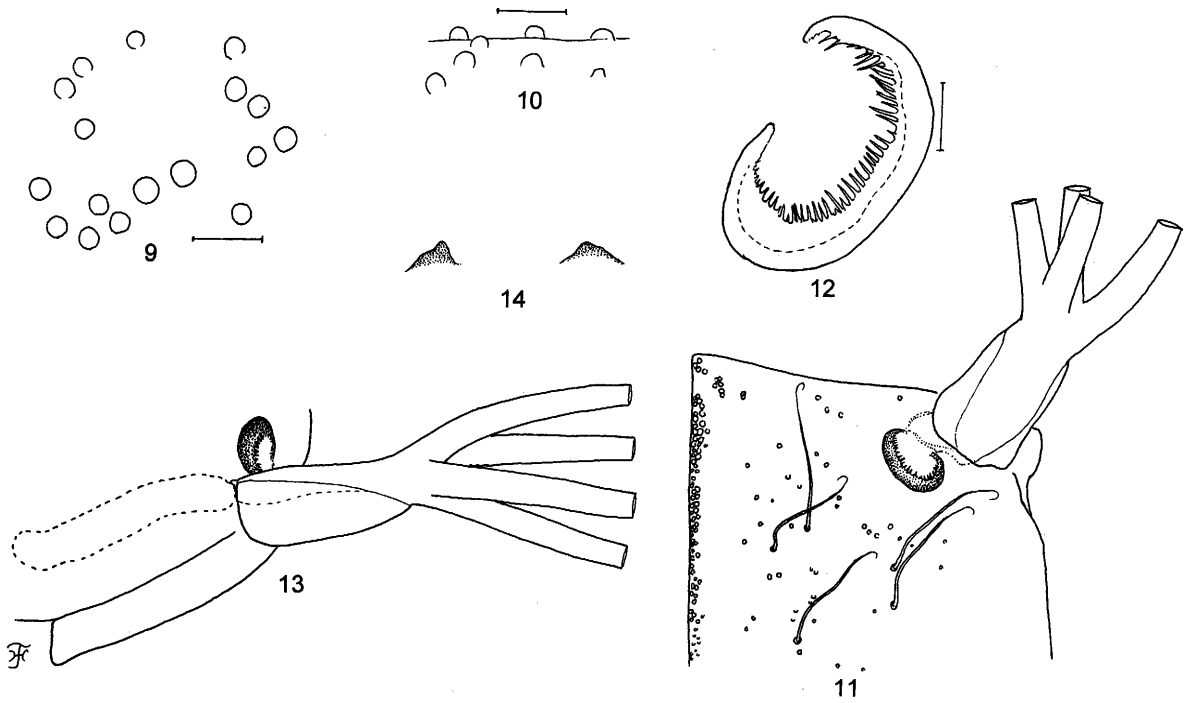
DESCRIPTION. **Female** and **Male**. Unknown.

Pupa. Body length (excluding gill filaments) *ca.* 2.6 mm. *Head.* Integument dark yellow, bare, with 4 pairs of trichomes, all long and simple. Antennal sheath with smooth surface. *Thorax.* Integument dark yellow, bare on anterior 1/2 except area along middle longitudinal suture with round tubercles, and moderately covered with cone-shaped tubercles on posterior 1/2; pit-like organ large, circular, thickly fringed with distinct, comb-like processes, and its diameter (A in Fig. 1) nearly as long as the distance (B in Fig. 1) from its innermost margin to middle longitudinal suture of thorax, *i.e., plo* ratio (= A/B) 1; thoracic trichomes 6 pairs (5 anterodorsally and 1 posterolaterally), all long and simple. Gill (Figs. 1 & 2) composed of 2 inflated, tubular filaments each with 2 slender filaments at its apex (total length *ca.* 1.8 mm); basal stalk of moderate length and with a large, transparent pouch (Fig. 2a), which expands laterally, thus partially covering the pit-like organ; tubular filaments subequal in length and thickness to each other, with several annular constrictions along their length, and, on their surface, with many longitudinal ridges, covered with somewhat larger tubercles on ridges and with smaller ones on interspaces (Fig. 2b); slender filaments subequal in length and thickness to each other, with well-defined annular ridges and furrows on their surface, and covered with minute tubercles; all filaments pale yellow to dark yellow except basal portion of tubular filaments dark brown. *Abdomen.* Terga 1 and 2 almost transparent, without tubercles; tergum 1 with 1 long seta on each side, tergum 2 with 5 simple spinous setae and 1 much longer seta on each side; terga 3 and 4 each with 4 hooked spines directed forward on each side; terga 7 and 8 each with a transverse row of spine-combs and comb-like groups of minute spines directed caudad on each side; tergum 9 with comb-like groups of minute spines and a pair of small, terminal hooks but lacking spine-combs. Sternum 4 with 1 distinct simple hook and a few minute setae on each side; sternum 5 with a pair of bifid hooks on each side; sterna 6 and 7 each with a pair of inner bifid and outer simple hooks on each side; last segment without grapnel-like hooklets ventrolaterally. *Cocoon* (Fig. 3). Shoe-shaped, moderately woven, somewhat extending ventrolaterally; anterior margin thickly woven; individual threads visible; interspaces thinnly walled but without perforations.

Mature larva. Body length 4.0-4.6 mm. Body greyish. Cephalic apotome pale yellow, with faint, positive head spots in 24 larvae, but markedly darkened medially and posteriorly on posterior 1/2 (Fig. 4) in 30 larvae. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.0 : 1.0 : 0.64. Labral fan with *ca.* 35 main rays. Mandible (Fig. 6) with comb-teeth decreasing in size from 1st to 3rd; mandibular serration composed of 2 teeth (1 large and 1 small), with or without supernumerary serrations. Hypostomium (Fig. 7) with a row of 9 apical teeth; median tooth as long as each corner tooth, longer than 3 intermediate teeth on each side; lateral margin serrate; hypostomal bristles 6 or 7 in number per side, lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 5) nearly triangular, wide basally, gradually narrowed anteriorly with a narrow apex, which is approaching posterior border of hypostomium. Thoracic cuticle bare. Abdominal cuticle bare except last segment moderately covered with numerous colorless setae on



Figs. 1–8. Pupal and larval characters of *Simulium* (*Morops*) *aropaense* sp. nov. 1, pupal gill and anterior portion of thoracic integument (dorsal view) showing pit-like organ, trichomes and tubercles along middle longitudinal suture (A, diameter of pit-like organ; B, distance from innermost margin to middle longitudinal suture); 2, pupal gill filaments (side view) showing a transparent pouch (a) and an enlargement of surface pattern of inflated filament (b); 3, pupa and cocoon; 4 & 5, larval head capsule (4, dorsal view; 5 ventral view); 6, apex of larval mandible; 7, larval hypostomium; 8, larval rectal papilla. Scale bar 0.02 mm for Fig. 2b.



Figs. 9-14. Pupal characters of *Simulium* (*Morops*) *kietaense* sp. nov. 9 & 10, tubercles on frontal integument (9, dorsal view; 10, side view); 11, base of gill and anterior part of thoracic integument showing a pit-like organ, trichomes and distribution of tubercles (dorsal view); 12, pit-like organ (anterodorsal view); 13, basal portion of gill filaments (side view); 14, terminal hooks. Scale bar 0.02 mm for Figs. 9, 10 & 12.

each side of anal sclerite. Rectal papilla (Fig. 8) compound, each of 3 lobes with *ca.* 4 finger-like, secondary lobules. Anal sclerite of usual X-form, with anterior arms *ca.* 0.7 × as long as posterior ones, broadly sclerotized at base. Ventral papillae absent, though last abdominal segment expanded ventrolaterally to form double bulges on each side. Posterior circlet with *ca.* 94 rows of up to 16 hooklets per row.

TYPE SPECIMENS. Holotype pupa (BMNH), slide mounted, together with its cocoon, collected from a stream near Aropa, 13 Km S of Kieta, Bougainville Island, 8. VII. 1965, by R.W. Crosskey. Paratypes: 19 pupae and 53 mature larvae (BMNH), same data as holotype.

DISTRIBUTION. Bougainville Island.

REMARKS. The species *aropaense* refers to the locality where this species was collected.

This new species belongs to the *clathrinum*-group of the subgenus *Morops,* defined by Crosskey (1967) by having the pit-like organ (Fig. 1), as well as four gill filaments. Among nine known species, *S.* (*M.*) *lalokiense* from central Papua New Guinea (Smart and Clifford, 1965), *S.* (*M.*) *clathrinum* from Australia (Mackerras and Mackerras, 1948), and *S.* (*M.*) *solomonense* from New Georgia Island, Solomon Islands (Takaoka and Suzuki, 1995) have a pit-like organ fringed with comb-like processes. The pit-like organ of this species is as large as those of *S.* (*M.*) *lalokiense* and *S.* (*M.*) *solomonense* (*plo* ratio 1.1 and 1.0 respectively) but is somewhat larger than that of *S.* (*M.*) *clathrinum* (*plo* ratio *ca.* 0.7). However, *S.* (*M.*) *aropaense* differs from these three known species in the arrangement of the pupal gill filaments.

3. *Simulium* (*Morops*) *kietaense* sp. nov.

DESCRIPTION. **Female** and **Male.** Unknown.

Pupa. Body length (excluding gill filaments) *ca.* 2.4 mm. *Head.* Integument dark yellow, moderately covered with round tubercles (Figs. 9 & 10) on frons, and with 4 pairs of trichomes, all long and simple. Antennal sheath with smooth surface. *Thorax.* Integument dark yellow, sparsely covered with round tubercles on ante-

rior 1/2, densely along middle longitudinal suture, and moderately covered with cone-shaped tubercles on posterior 1/2; pit-like organ (Fig. 11) small, circular, moderately fringed with distinct, comb-like processes (Fig. 12), *plo* ratio 0.33; thorax with 5 pairs of trichomes anterodorsally and 1 pair posterolaterally, all long and simple; 5 anterior pairs are much longer than posterolateral one. Gill (Figs. 11 & 13) composed of 4 slender filaments in 2 pairs (outer and inner), subequal in thickness and length (*ca.* 1.5 mm) to each other, though inner pair slightly shorter than outer pair, and gradually tapered toward apical tip; basal stalk somewhat elongate, with a transparent pouch (Fig. 13); inner and outer pairs of filaments sessile or very short-stalked (Fig. 11); surface of filaments with well-defined ridges (forming reticulate pattern on basal 1/4), covered with somewhat larger tubercles on ridges and with smaller ones on interspaces; filaments dark yellow to brown, paler distally. *Abdomen* and *Cocoon*. As in *S.* (*M.*) *aropaense*. **Mature larva.** Almost indistinguishable from *S.* (*M.*) *aropaense* except following features: Body length 4.3-4.6 mm. Body greyish. Cephalic apotome pale yellow, with faint, positive head spots in 1 larva, but markedly darkened medially and posteriorly on posterior 1/2 in 3 larvae. Mandibular serration composed of 2 teeth (1 large and 1 small), with 1 or 2 supernumerary serrations. Rectal papilla compound, each of 3 lobes with 4 or 5 finger-like, secondary lobules. Anal sclerite of usual X-form, with anterior arms *ca.* 0.8 × as long as posterior ones, broadly sclerotized at base.

TYPE SPECIMENS. Holotype pupa (BMNH), slide mounted, together with its cocoon, collected from a stream near Aropa, 13 km S of Kieta, Bougainville Island, 8. VII. 1965, by R.W. Crosskey. Paratypes: 4 pupae and 4 mature larvae (BMNH), same data as holotype.

DISTRIBUTION. Bougainville Island.

REMARKS. This new species is also assigned to the *clathrinum*-group by having the pit-like, cuticular organ (Fig. 12) in the pupa. As in *S.* (*M.*) *aropaense* this organ is furnished with comb-like processes along the inner margin, but is much smaller than that of the former species, and also those of *S.* (*M.*) *clathrinum*, *S.* (*M.*) *lalokiense* and *S.* (*M.*) *solomonense*.

Crosskey (1989) added Bougainville Island as a range of *S.* (*M.*) *raunsimnae* in his list of Simuliidae in the Australasian and Oceanian Regions. Since this was made on the basis of the present pupal specimens of *S.* (*M.*) *kietaense* (which had been identified at that time as *S.* (*M.*) *raunsimnae* by Dr. Crosskey according to the attached label), Bougainville Island should be omitted at present from the range of *S.* (*M.*) *raunsimnae*.

(B) *sherwoodi*-group

The following three new species are distinct among species of the subgenus *Morops* in having the spermatheca with internal setae and the parameres of the male genitalia with distinct hooks. The three ungrouped species, *S.* (*M.*) *sherwoodi* Stone and Maffi, *S.* (*M.*) *kerei* Takaoka and Suzuki, and *S.* (*M.*) *noroense* Takaoka and Suzuki, all from the Solomon Islands, share these characters (except *S.* (*M.*) *kerei* of which the female is still unknown). The new species-group is here proposed to relate the six species which possess the following combination of characters.

DIAGNOSIS. **Female.** Mandible with teeth on inner margin but no or a few teeth on outer margin; fore basitarsus somewhat dilated, without dorsal hair crest; tarsal claw with a minute basal tooth; abdomen lacking dense covering of pubescence on dorsal and lateral surface, and shiny on terga 2 and 5-8 (or 6-8); spermatheca with internal setae. **Male.** Fore basitarsus somewhat dilated, without dorsal hair crest; hind basitarsus slender, not inflated; abdomen lacking dense covering of pubescence, and with a pair of shiny patches on terga 2 and 5-7; ventral plate setose ventrally, posteriorly and dorsally; paramere with distinct hooks. **Pupa.** Frontal trichomes 2 or 3 pairs, simple or branched; gill filaments 5 or 6 in number per side, all slender or 1 or 2 of them inflated; terminal hooks of usual cone-shape or plate-like; cocoon simple, wall-pocket-shaped. **Larva.** Hypostomium with smooth lateral margins; mandible without supernumerary serrations; ventral papillae well developed.

SPECIES INCLUDED. *S.* (*M.*) *kerei*, *S.* (*M.*) *noroense*, *S.* (*M.*) *pangunaense* sp. nov., *S.* (*M.*) *peggyae* sp. nov., *S.* (*M.*) *sherwoodi*, *S.* (*M.*) *yuleae* sp. nov.

S. (*M.*) *kerei*, though the adult female is still unknown, apparently is assignable to this species-group because its adult male has the paramere with distinct hooks. This species had been tentatively placed in the *farciminis*-group mainly because of the similar arrangement of the pupal gill filaments (Takaoka and Suzuki, 1994). As shown later, *S.* (*M.*) *kerei* is very similar to *S.* (*M.*) *pangunaense*.

This species-group is distributed only in the Solomon Islands and Bougainville Island. This fact, together with the close morphological similarities of all the six species, suggests that speciation within this

species-group might have occurred after an ancestor species had arrived on these islands. It is intriguing that among four species of this species-group found in Bougainville Island, successive changes appear to have taken place in morphology of the pupal gill filaments, in particular, of lower paired filaments, as illustrated in Figs. 30-35. If the decreased number and inflation of filaments are considered to be apomorphic, lower two filaments must have changed as follows: first, lower two filaments which were slender (Fig. 30) became inflated (Fig. 31), then, stalk of lower filaments elongated, as shown in Figs. 32 and 33, resulting in the loss of one of two branches (Fig. 34) and, finally, the remaining filament have lost its apical slender portion (Fig. 35). Taking these successive changes into consideration, it is presumed that *S.* (*M.*) *noroense* and *S.* (*M.*) *sherwoodi* are most primitive, *S.* (*M.*) *pangunaense* and *S.* (*M.*) *kerei* are most derivative, while *S.* (*M.*) *yuleae* and *S.* (*M.*) *peggyae* are intermediate in specialization. However, this is not conclusive since there are other characters which suggest the opposite direction of specialization. *S.* (*M.*) *noroense* has a smaller basal tooth of the female claw and a wider fore basitarsus (both of which are generally regarded as being a more derived character than a large claw tooth and a narrow basitarsus, respectively) than those of the other three Bougainville species (Takaoka and Suzuki, 1995); further, pupal terminal hooks are of cone-shape (derived form) in *S.* (*M.*) *noroense* and *S.* (*M.*) *sherwoodi,* whereas these hooks are plate-like (supposedly primitive form) in the other species, as shown in Figs. 38-40.

This species-group appears closely related to the eight *Morops* species (all so far ungrouped) described from the Philippines (Takaoka, 1983) because its members share many characters, such as the paramere with distinct hooks, a pair of dorsolateral shiny spots each on the male abdominal segments 2, 5, 6 and 7, pupal gill with six filaments (four filaments in one Philippine species), plate-like terminal hooks on the pupal abdomen (of usual cone-shape in one Philippine and two Solomon species), wall-pocket-shaped cocoon, larval hypostomium with smooth lateral margins, larval mandible without supernumerary serrations and the presence of the larval ventral papillae. However, these Philippine species differ from the *sherwoodi*-group in having no internal setae of the spermatheca, a few hairs on the pleural membrane, a large tooth on the female tarsal claw (small tooth in one species) and grapnel-like hooklets on the last segment of the pupal abdomen. It should be noted that most characters shared by members of this species-group and eight Philippine *Morops* species were not found in other species-groups of *Morops,* but found in the subgenus *Gomphostilbia,* as previously pointed out by Takaoka (1983). Future studies of these Philippine, Solomon and Bougainville species, though far apart geographically from each other, may clarify the phylogenetic relationship between the two subgenera.

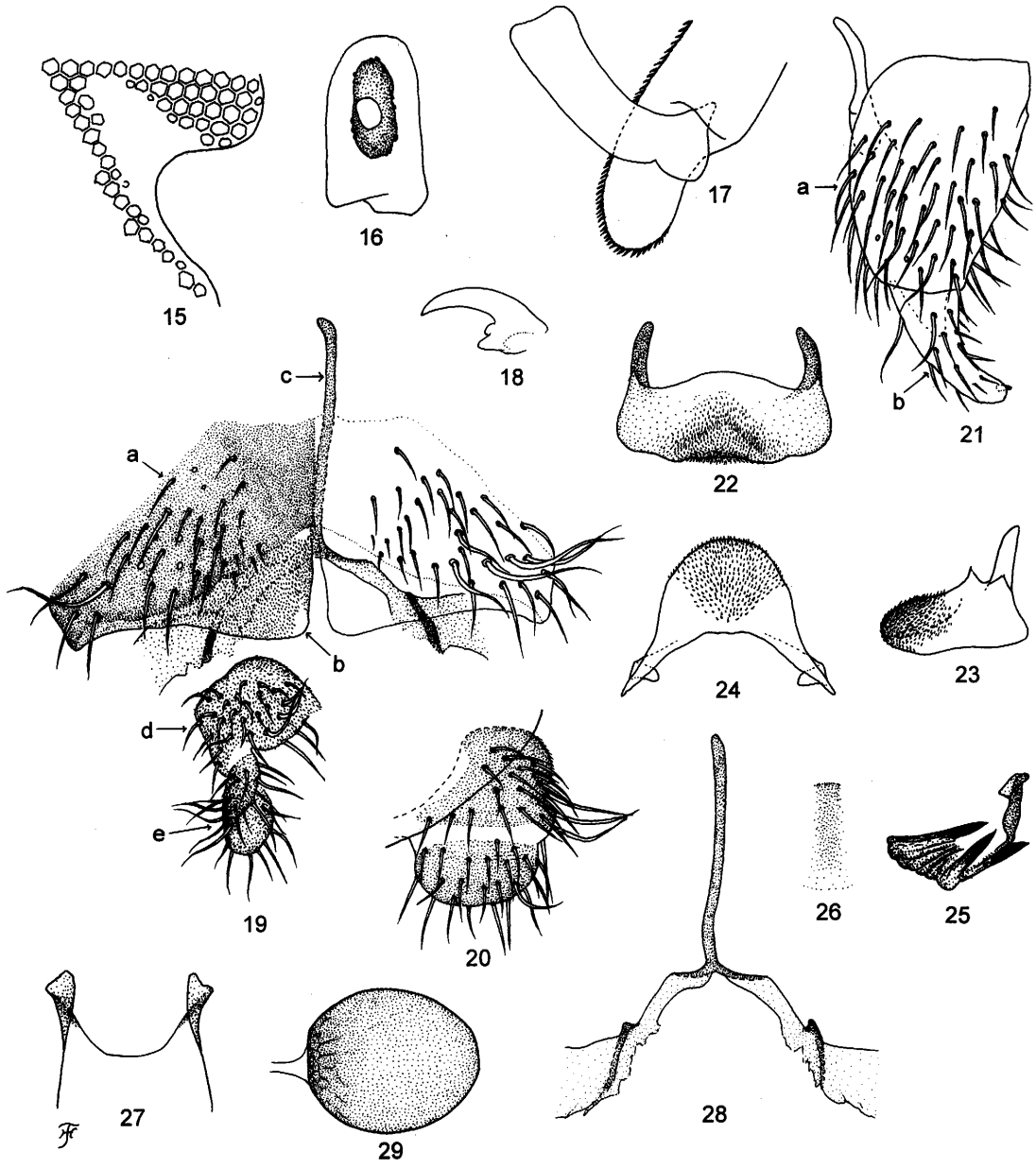
4. *Simulium* (*Morops*) *yuleae* sp. nov.

DESCRIPTION. **Female.** Body length *ca.* 2.2 mm. *Head.* Slightly narrower than thorax. Frons dark brown, whitish grey pruinose, dull, densely covered with yellowish white, recumbent pubescence interspersed with a few dark hairs; frontal ratio 2.1:1.0:3.0. Frons-head ratio 1.0:4.6. Fronto-ocular area (Fig. 15) well developed. Clypeus dark brown, densely covered with yellowish white pubescence, interspersed with several dark hairs. Antenna composed of 2+9 segments, brownish black except scape, pedicel and base of 1st flagellar segment yellowish when viewed from above (scape, pedicel and basal few flagellar segments yellow, apical 5 flagellar segments brownish black when viewed from front). Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.3; 3rd segment somewhat enlarged, sensory vesicle (Fig. 16) much elongate, 2.3 × as long as wide, 0.55 × as long as 3rd segment, with very large opening distally. Maxillary lacinia with 10 inner and 11 outer teeth. Mandible with *ca.* 26 small inner teeth and lacking outer ones, though outer margin weakly erose near apex. Cibarium smooth (nearly as in Fig. 27). *Thorax.* Scutum blackish brown in ground color, whitish grey pruinose, with 3 dark, rather broad, longitudinal vittae, densely covered with whitish yellow, recumbent pubescence, also with dark brown pubescence on longitudinal vittae, and sparsely with dark upright hairs on prescutellar area. Scutellum blackish brown with whitish yellow pubescence and long dark upright hairs along posterior margin. Postscutellum blackish brown, whitish grey pruinose (shining in light), bare. Pleural membrane covered with *ca.* 80 yellowish white pubescence and several dark hairs; several hairs also on sclerotized portion just anterior to pleural membrane. Katepisternum longer than deep, dark brown, whitish grey pruinose (shining in light), with numerous dark hairs and yellowish white pubescence; sulcus distinct. *Legs.* Foreleg: coxa and trochanter yellow; femur dark yellow to pale brown; tibia yellow with distal 1/4 black, outer surface widely white shining when illuminated; tarsus black; basitarsus somewhat dilated, *ca.* 6.2 × as long as its greatest width.

Midleg: coxa dark brown; trochanter yellow; femur yellow with apical cap somewhat dark; tibia yellow on basal 1/3 (posterior surface) or on basal 1/2-2/3 (anterior surface), brownish black on rest; posterior surface of tibia widely white shining when illuminated; tarsus black. Hind leg: coxa dark yellow; trochanter yellow; femur yellow with apical cap brown; tibia yellow on basal 2/3, brownish black on apical 1/3; posterior surface of tibia widely white shining when illuminated; tarsus brown with basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, parallel-sided, *ca.* 7.0 × as long as wide. Calcipala (Fig. 17) well developed, *ca.* 1.5 × as long as wide. Pedisulcus well developed. Hind femur and tibia *ca.* 2.4 × and 1.8 × as wide as hind basitarsus, respectively. All femora and tibiae densely covered with scale-like hairs on outer surface. Claws (Fig. 18) each with a round, minute basal tooth. *Wing.* Length 2.0 mm. Costa with spinules as well as hairs. Subcosta haired except distal 1/4 bare. Hair tuft on stem vein dark brown. Basal portion of radius fully haired. *Abdomen.* Basal scale brown with fringe of yellowish white hairs. Dorsal surface of abdomen black, with dark hairs; tergite of 2nd segment whitish pruinose, tergites of segments 5-8 shiny. *Genitalia* (Figs. 19 & 20). Sternal plate undeveloped on 7th abdominal segment. Sternite 8 bare medially, with *ca.* 35 dark macrosetae on each side. Anterior gonapophysis triangular, thin, membraneous, covered densely with microsetae; inner margin well sclerotized. Genital fork of usual inverted-Y form, with arms folded medially; each arm with a small projection directed forwards. Paraproct not produced ventrally, with ventral margin somewhat depressed in middle. Cercus 0.57 × as long as wide, with posterior border gently rounded when viewed laterally. Spermatheca (depressed) well sclerotized except small area near tubal juncture unsclerotized; internal setae present.

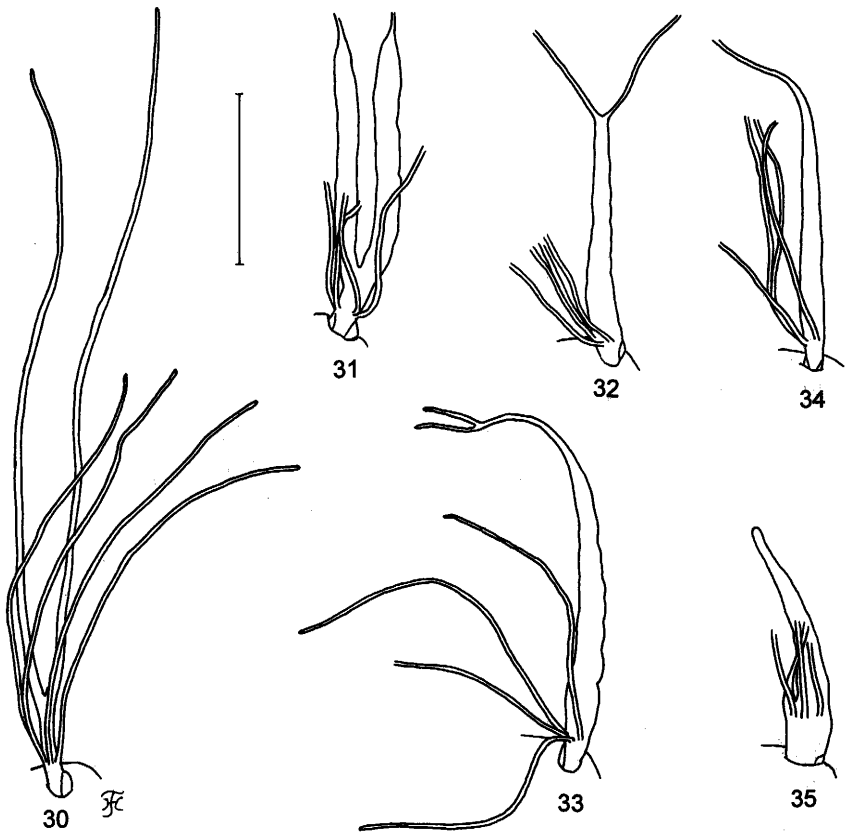
Male. Body length *ca.* 2.2 mm. *Head.* Wider than thorax. Upper eye consisting of 12 vertical columns and 13 horizontal rows of large facets. Clypeus brownish black, whitish pruinose, covered densely with yellow pubescence, interspersed with dark hairs. Antenna composed of 2+9 segments, yellow, but 4 or 5 apical segments brown; 1st flagellomere somewhat elongate, *ca.* 1.6 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.8; sensory vesicle oblong, 0.2 × as long as 3rd segment, with opening near distal end. *Thorax.* Scutum brownish black, densely covered with golden yellow pubescence. Scutellum brownish black with golden yellow pubescence and dark upright hairs. Postscutellum brownish black, white pruinose (white shining in light), bare. Pleural membrane with *ca.* 75 yellow pubescence; 16-20 additional pubescence present just before it. Katepisternum longer than deep, with yellow and dark hairs. *Legs.* Foreleg: coxa pale yellow; trochanter yellow; femur dark yellow to pale brown; tibia yellow with distal 1/3 brownish black, and white shining in light on outer surface; tarsus brownish black; basitarsus somewhat dilated, *ca.* 6.4 × as long as its greatest width. Midleg: coxa dark brown; trochanter yellow; femur yellow with distal cap somewhat darkened; tibia yellow on basal 1/2, brown on distal 1/2 though its border not well defined; tarsus brownish black. Hind leg: coxa dark yellow; trochanter yellow; femur yellow with distal cap brownish black; tibia yellow on basal 2/3, brownish black on rest; tarsus brownish black except basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, parallel-sided, *ca.* 5.8 × as long as wide. Hind femur and tibia *ca.* 2.1 × and 1.8 × as wide as hind basitarsus, respectively. Calcipala well developed (nearly as in ♀ but slightly shorter), *ca.* 1.3 × as long as wide. Pedisulcus well developed. All femora and tibiae densely covered with scale-like hairs on outer surface. *Wing.* Length 1.9 mm. Other features as in ♀ except subcosta with a few hairs. *Abdomen.* Basal scale brownish black, with fringe of yellow hairs. Dorsal surface of abdominal segments brownish black, covered with dark hairs; segments 2, 5-7 each with a dorsolateral pair of shiny, whitish pruinose patches; those on segment 2 widely connected in middle. *Genitalia* (Figs. 21-26). Coxite nearly rectangular in ventral view, much longer than wide. Style shorter than coxite, tapered toward apical tip, gently curved inwards, with an apical spine. Ventral plate transverse, *ca.* 0.4 × as long as wide, with both sides moderately angulated, widely produced ventrally along posterior margin, with microsetae on ventral, posterior and dorsal surface; basal arms converging. Paramere with 3 long hooks and several indistict ones. Median sclerite weakly sclerotized, plate-like, slightly widened toward tip, with round tip.

Pupa. Body length (excluding gill filaments) *ca.* 2.5 mm. *Head.* Integument yellow, moderately covered with tubercles; a pair of facial trichomes long, simple or bifid, and 3 pairs of frontal trichomes all long and simple. Antennal sheath with smooth surface. *Thorax.* Integument yellow, sparsely covered with tubercles; 5 anterodorsal pairs of trichomes all long, simple or bifid, 1 posterolateral pair long and simple. Pit-like organ absent. Gill (Fig. 31) composed of 2 inflated, tubular filaments and 4 slender filaments arising dorsally from



Figs. 15-29. Adult characters of the *sherwoodi*-group of *Simulium* (*Morops*). 15, ♀ fronto-ocular area; 16, ♀ 3rd maxillary palpal segment showing a sensory vesicle; 17, distal tip of ♀ hind basitarsus and 2nd tarsomere showing calcipala and pedisulcus; 18, ♀ claw with a minute tooth; 19, ♀ genitalia in situ (spermatheca omitted; ventral view) showing 8th sternite (a), gonapophysis (b), genital fork (c), paraproct (d) and cercus (e); 20, paraproct and cercus (side view); 21, coxite (a) and style (b) (ventral view); 22-24, ventral plate (22, ventral view; 23, side view; 24, end view); 25, paramere; 26, median sclerite; 27, ♀ cibarium; 28, genital fork; 29, spermatheca. 15-25, *S.* (*M.*) *yuleae* sp. nov.; 27, *S.* (*M.*) *peggyae* sp. nov.; 28 & 29, *S.* (*M.*) *pangunaense* sp. nov.

basal stalk of tubular filaments; tubular filaments subequal in length (*ca.* 1.0 mm) and thickness to each other (although somewhat varied individually), lying horizontally, parallel-sided or somewhat divergent, directing forwards and each apex tapered, bearing a slender apical filament which was cut off, not allowing the measurement of its length; outer tubular filament with several annular constrictions along its length; surface of tubular filaments with many ridges forming irregular patterns, covered with somewhat larger tubercles on



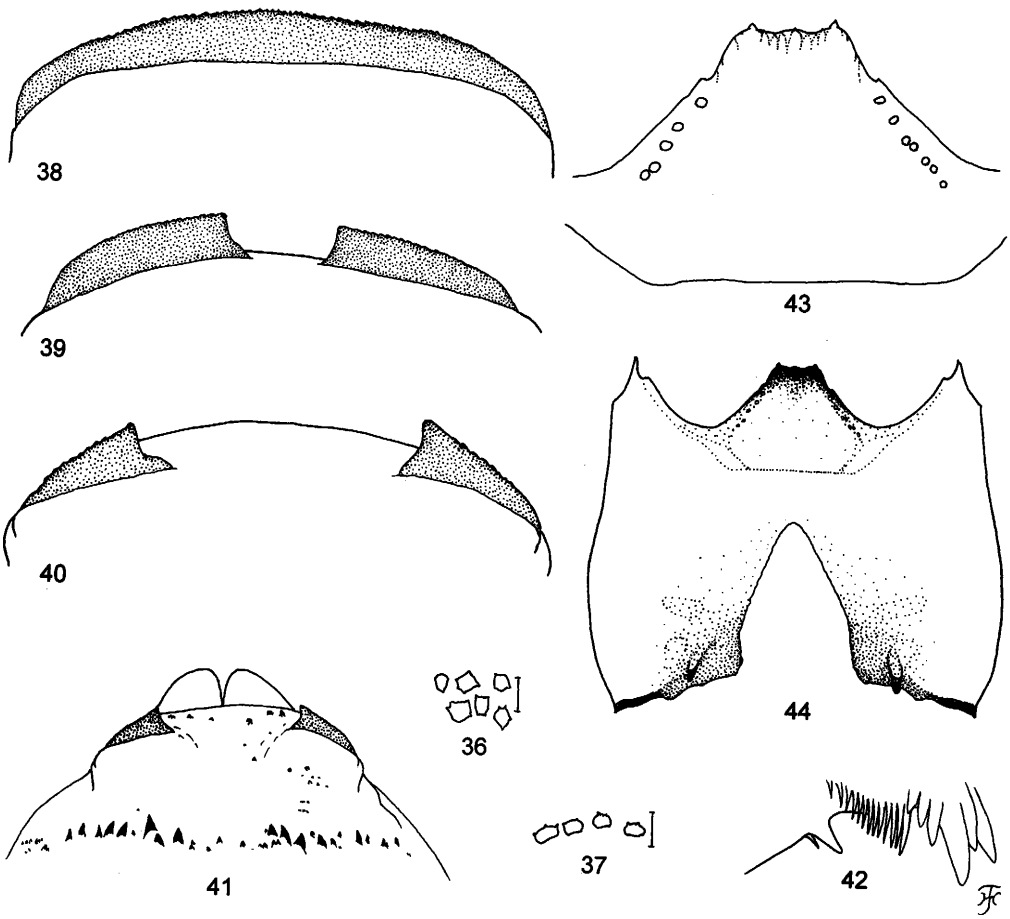
Figs. 30-35. Pupal gill filaments of the *sherwoodi*-group of *Simulium* (*Morops*). 30, *S.* (*M.*) *noroense*; 31, *S.* (*M.*) *yuleae* sp. nov.; 32-34, *S.* (*M.*) *peggyae* sp. nov.; 35, *S.* (*M.*) *pangunaense* sp. nov. Scale bar 0.5 mm for Figs. 30-35.

ridges and with smaller ones on interspaces; slender filaments subequal in length (0.6-0.8 mm) and thickness to each other, with annular furrows on their surface, and covered with minute tubercles; all filaments pale yellow.

Abdomen. Terga 1 and 2 almost transparent, without tubercles; tergum 1 with 1 long seta on each side; tergum 2 with 5 simple spinous setae and 1 much longer seta on each side; terga 3 and 4 each with 4 hooked spines directed forward on each side; terga 6-9 each with a transverse row of spine-combs and comb-like groups of minute spines directed caudad on each side, though spine-combs on tergum 9 are much smaller than those on other terga; tergum 9 with a wide, plate-like, terminal hook, which is moderately serrate along outer margin (Fig. 38). Sternum 4 with 1 distinct bifid hook and 1 spinous seta on each side; sternum 5 with a pair of bifid hooks on each side; sterna 6 and 7 each with a pair of inner bifid and outer simple hooks on each side; last segment without grapnel-like hooklets ventrolaterally.

Cocoon. Wall-pocket-shaped, moderately woven, widely extending ventrolaterally; anterior margin thickly woven; individual threads visible but without perforations.

Mature larva. Body length 5.4 mm. Body greyish. Cephalic apotome pale yellow with distinct, positive head spots. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.0:1.2:1.1. Labral fan with 32 main rays. Mandible (Fig. 42) with comb-teeth decreasing in size from 1st to 3rd; mandibular serration composed of 2 teeth (1 large and 1 small), without supernumerary serrations. Hypostomium (Fig. 43) with 9 apical teeth in row; each corner tooth longer than median tooth, which is longer than 3 intermediate teeth on each side; lateral margin not serrate; hypostomal bristles 5-7 in number per side lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 44) nearly triangular, gradually narrowed anteriorly, *ca.* 2.9 × as long as postgenal bridge. Thoracic cuticle bare. Abdominal cuticle bare except last segment which is moderately covered with numerous colorless setae on each side of anal sclerite. Rectal papilla compound,



Figs. 36-44. Pupal and larval characters of the *sherwoodi*-group of *Simulium* (*Morops*). 36 & 37, tubercles on frontal integument (36, top view; 37, side view); 38-40, terminal hooks; 41, 9th abdominal segment showing a pair of terminal hooks, a transverse row of spine-combs and scattered tubercles (dorsal view); 42, apex of larval mandible; 43, larval hypostomium; 44, larval head capsule (ventral view). 38, 42-44, *S.* (*M.*) *yuleae* sp. nov.; 39, *S.* (*M.*) *peggyae* sp. nov.; 36, 37, 40 & 41, *S.* (*M.*) *pangunaense* sp. nov. Scale bar 0.01 mm for Figs. 36 & 37.

each of 3 lobes with 3-5 finger-like, secondary lobules. Anal sclerite of usual X-form, with anterior arms *ca.* 0.9 × as long as posterior ones, broadly sclerotized at base. Ventral papillae present. Posterior circlet with 98 rows of up to 18 hooklets per row.

TYPE SPECIMENS. Holotype ♂ (ANIC), reared from pupa, pinned together with pupal skin and cocoon, collected from Konaiano Creek (720 m in altitude), Panguna, Bougainville Island, Papua New Guinea, 24. IX. 1988, by C. Yule. Allotype ♀ (ANIC), reared from pupa, same data as holotype except date: 12. III. 1989. Paratypes: 1 ♂, 1 ♀ (BMNH), slide mounted, emerged from pupa, same data as holotype except date: 26. III. 1989, 2 ♀ (ANIC), pinned together with pupal skin and cocoon, same data as holotype except date: 14 and 29. VIII. 1988, 1 ♀ (ANIC), in alcohol together with pupal skin and cocoon, same data as holotype except date: 29. VIII. 1988, 6 pupae and 1 mature larva (ANIC), data same as holotype except date: 18.X.1987; 1 mature larva (ANIC), collected from a stream flowing from Lake Loloru to Mivo River (1515 m in altitude), Mt. Loloru, Bougainville Island, Papua New Guinea, 8.X.1988, by C. Yule.

DISTRIBUTION. Bougainville Island.

REMARKS. The species name *yuleae* acknowledges Miss C. Yule who kindly gave simuliid specimens collected from Bougainville Island.

The adults of this species, as well as the following two new species, differ from those of *S.* (*M.*) *sherwoodi*

and *S.* (*M.*) *noroense* from the Solomon Islands in lacking the brownish pubescence on the median dorsal surface of the scutum, and also from *S.* (*M.*) *sherwoodi* by their frons densely covered with pubescence, and from *S.* (*M.*) *noroense* by their femora which are much paler than that of *S.* (*M.*) *noroense*. The pupa of *S.* (*M.*) *yuleae* is easily separated from the other related species by the fused, plate-like, terminal hook (Fig. 38), as well as the arrangement of the six gill filaments of which two are inflated (Fig. 31).

5. *Simulium* (*Morops*) *peggyae* sp. nov.

DESCRIPTION. **Female.** As in ♀ of *S.* (*M.*) *yuleae* except following features: *Head.* Frontal ratio 1.6:1.0:1.7. Frons-head ratio 1.0:4.4. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:1.9; 3rd segment somewhat enlarged, sensory vesicle much elongate, 2.5 × as long as wide, 0.56 × as long as 3rd segment, with very large opening somewhat distally from middle. Maxillary lacinia with 8 or 9 inner and 12 or 13 outer teeth. Mandible with *ca.* 26 small inner teeth and a few weak outer ones at some distance from apex. Cibarium smooth (Fig. 27). *Thorax.* Pleural membrane covered with *ca.* 62 yellowish white pubescence; a few pubescence also on sclerotized portion just anterior to pleural membrane. *Legs.* Fore basitarsus slender, *ca.* 6.4 × as long as its greatest width. Mid tibia yellow on basal 1/2 and a little more, brownish black on rest. Hind basitarsus slender, parallel-sided, *ca.* 6.7 × as long as wide. Hind femur and tibia *ca.* 2.2 × and 1.7 × as wide as hind basitarsus, respectively. *Wing.* Length 2.0 mm. *Abdomen.* Tergites of segments 6-8 shiny. *Genitalia.* Sternite 8 with *ca.* 20 dark macrosetae on each side. Anterior gonapophysis triangular, thin, membraneous, covered densely with microsetae, interspersed with a few short setae.

Male. As in ♂ of *S.* (*M.*) *yuleae* except following features: *Head.* Wider than thorax. Upper eye consisting of 12 vertical columns and 12 horizontal rows of large facets. Antenna composed of 2+9 segments, yellow, but 3 apical segments brown; 1st flagellomere somewhat elongate, *ca.* 1.5 × as long as 2nd one. Maxillary palp (5th segment lost) with sensory vesicle oblong, 0.28 × as long as 3rd segment, with opening near distal end. *Thorax.* Pleural membrane with *ca.* 60 yellow pubescence; 8-10 additional pubescence present just before it. *Legs.* Fore basitarsus somewhat dilated, *ca.* 6.5 × as long as its greatest width. Hind basitarsus slender, parallel-sided, *ca.* 6.8 × as long as wide. Hind femur and tibia *ca.* 2.2 × and 1.8 × as wide as hind basitarsus, respectively. *Wing.* Length 2.0 mm.

Pupa. Body length (excluding gill filaments) *ca.* 2.4 mm. *Head.* Integument pale yellow to dark yellow, bare; a pair of facial trichomes long, simple or bifid, and 3 pairs of frontal trichomes all long and simple (1 or 2 pairs bifid in some pupae). Antennal sheath with smooth surface. *Thorax.* Integument pale yellow to dark yellow, bare except posterodorsal area moderately covered with tubercles; 5 anterodorsal pairs of trichomes all long, simple or bifid, 1 posterolateral pair long and simple. Pit-like organ absent. Gill (Figs. 32-34) composed of 1 inflated, tubular filament and 4 slender filaments arising dorsally near base of inflated filament; inflated filament directing forwards, gradually tapered toward apex, bearing 1 or 2 slender apical filaments (when 2 filaments are present, bifurcation occurs at different levels in some specimens); total length, *i.e.*, inflated portion (up to 0.8-1.0 mm long from base) plus apical slender filament probably around 1.5 mm; inflated tubular filament with several annular constrictions along its length; surface of tubular filament smooth with no patterns, covered with minute tubercles; dorsal 4 slender filaments subequal in length (0.8-1.0 mm) and thickness to each other, with indistinct, annular furrows on their surface, and covered with minute tubercles; all filaments pale yellow. *Abdomen.* As in *S.* (*M.*) *yuleae* except following features: Terga 7-9 each with a transverse row of spine-combs and comb-like groups of minute spines directed caudad on each side, though spine-combs on tergum 9 much smaller than those on other terga; tergum 6 with no or a few spine-combs; tergum 9 with a pair of wide, plate-like, terminal hooks which are widely separated from each other; each terminal hook with moderate serrations along outer margin (Fig. 39). *Cocoon.* As in *S.* (*M.*) *yuleae.*

Mature larva. Body length 5.3-5.8 mm. Body greyish. Cephalic apotome yellow, with distinct, positive head spots. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.0:0.8:0.7. Labral fan with *ca.* 40 main rays. Mandible and hypostomium as in *S.* (*M.*) *yuleae* except hypostomal bristles 10 in number. Postgenal cleft similar to that of *S.* (*M.*) *yuleae, ca.* 2.8 × as long as postgenal bridge. Thoracic cuticle bare. Abdominal cuticle bare except last segment which is moderately covered with numerous colorless setae on each side. Rectal papilla compound, each of 3 lobes with 3-5 finger-like, secondary lobules. Anal sclerite of usual X-form, with anterior arms *ca.* 0.7 × as long as posterior ones, broadly sclerotized at base. Ventral papillae

present. Posterior circlet with 148 rows of up to 18 hooklets per row.

TYPE SPECIMENS. Holotype ♂ (ANIC), reared from pupa, slide mounted, with its pupal skin and cocoon pinned, collected from Konaiano Creek, Panguna, Bougainville Island, Papua New Guinea, 24. IX. 1988, by C. Yule. Allotype ♀ (ANIC), reared from pupa, pinned together with its pupal skin and cocoon, same data as holotype except date: 3. III. 1989. Paratypes: 2 ♀ (ANIC), reared from pupa, pinned, same data as holotype except date: 16. X. 1988 and 30. III. 1989; 1 ♀ (BMNH), reared from pupa, slide mounted, with its pupal skin and cocoon on pin; 9 pupae, 4 pupal skins and 1 mature larva (ANIC), same data as holotype except date: 31.X.1987; 1 mature larva (ANIC), collected from a stream flowing from Lake Loloru to Mivo River, Mt. Loloru, Bougainville Island, 8. X. 1988, by C. Yule.

DISTRIBUTION. Bougainville Island.

REMARKS. The species *peggyae* is named after the wife of Dr. R.W. Crosskey.

This species is separable from all the other related species of the *sherwoodi*-group by the female abdomen with the tergites 2, 6, 7 and 8 shiny (tergite 5 not shiny), and the pupal gill composed of 4 slender filaments and 1 inflated filament tapering toward apex and ending as the one or two slender filaments (Figs. 32-34). The female and male of this species are also differentiated from those of *S.* (*M.*) *sherwoodi* and *S.* (*M.*) *noroense* by the color pattern of the scutal pubescence, as well as the number of pubescence on the pleural membrane.

6. *Simulium* (*Morops*) *pangunaense* sp. nov.

DESCRIPTION. **Female.** As in ♀ of *S.* (*M.*) *yuleae* except following features: *Head.* Frontal ratio 1.9:1.0:2.0. Frons-head ratio 1.0:4.4. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:1.9; 3rd segment somewhat enlarged, sensory vesicle much elongate, 2.5 × as long as wide, 0.57 × as long as 3rd segment, with very large opening medially or slightly distally. Maxillary lacinia with 9 or 10 inner and 12 or 13 outer teeth. Mandible with *ca.* 27 small inner teeth and a few weak outer teeth at some distance from apex. *Legs.* Fore basitarsus somewhat dilated, *ca.* 5.9 × as long as its greatest width. Mid tibia yellow on basal 1/2, brownish black on rest. Hind basitarsus slender, parallel-sided, *ca.* 7.9 × as long as wide. Hind femur and tibia *ca.* 2.4 × and 2.0 × as wide as hind basitarsus, respectively. *Genitalia.* Sternite 8 with *ca.* 27 dark macrosetae on each side. Anterior gonapophysis triangular, thin, membraneous, covered densely with microsetae, interspersed with a few setae. Genital fork with a more distinct projection directed forward on each arm (Fig. 28). Spermatheca ovoid, well sclerotized except small area near tubal juncture unsclerotized (Fig. 29); internal setae present.

Male. As in ♂ of *S.* (*M.*) *yuleae* except following features: *Head.* Upper eye consisting of 13 vertical columns and 13 horizontal rows of large facets. Antenna composed of 2+9 segments, yellow with 4 apical segments brown; 1st flagellomere somewhat elongate, *ca.* 1.7 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.3; sensory vesicle oblong, 0.29 × as long as 3rd segment, with opening near distal end. *Thorax.* Pleural membrane with *ca.* 70 yellow pubescence, and a few additional pubescence also present just before it. *Legs.* Fore femur brown. Fore basitarsus slightly dilated, *ca.* 7.0 × as long as its greatest width. Hind tibia yellow on basal 1/2 and a little more, brownish black on rest. Hind basitarsus slender, parallel-sided, *ca.* 8.4 × as long as wide. Hind femur and tibia *ca.* 2.7 × and 2.1 × as wide as hind basitarsus, respectively. *Wing.* Length 2.0 mm. Other features as in ♀ except subcosta with a few hairs.

Pupa. Body length (excluding gill filaments) *ca.* 2.5 mm. *Head.* Integument dark yellow, densely covered with tubercles of salient polygonal forms (Figs. 36 & 37); a pair of facial trichomes long, simple or bifid, and 3 pairs of frontal trichomes all long, simple or bifid. Antennal sheath moderately covered with cone-shaped tubercles. *Thorax.* Integument dark yellow, densely covered with cone-shaped tubercles, intermixed with somewhat larger tubercles of salient polygonal forms; 5 anterodorsal pairs of trichomes all long, simple or bifid, 1 posterolateral pair long and simple. Pit-like organ absent. Gill (Fig. 35) composed of 1 inflated, horn-like filament and 4 slender, individual filaments, of which 3 are arising dorsally and 1 is arising inwardly near base of horn-like filament, and arising at some distance anterior to the bases of 3 other slender filaments; horn-like filament (*ca.* 0.9 mm long) directing forward and downward, thick-walled, thickest at base when viewed dorsally, gradually tapered toward apex, with no secondary apical filament; horn-like filament with several annular constrictions along its length; surface of horn-like filament smooth with no patterns, densely covered with minute tubercles; 4 slender filaments subequal in thickness to each other, though 1 innermost filament is

much thicker at base than 3 dorsal filaments, with indefinite, annular furrows on their surface, and covered with minute tubercles; 4 slender filaments probably as long as or a little shorter than the inflated filament; all filaments brown (or pale yellow except basal portion of inflated filament which is dark yellow in alcoholic specimens). *Abdomen.* As in *S.* (*M.*) *yuleae* except following features: Terga 1 and 2 pale yellow, sparsely or moderately covered with minute tubercles, respectively; tergum 9 with a pair of wide, plate-like, terminal hooks which are very widely separated from each other; each terminal hook with weak serrations along outer margin (Fig. 40); several tubercles present around hooks (Fig. 41). Sterna 6 and 7 each with a pair of inner bifid and outer simple spinous seta or hooks (much less developed than inner hook) on each side. *Cocoon.* As in *S.* (*M.*) *yuleae.*

Mature larva. Unknown.

TYPE SPECIMENS. Holotype ♂ (ANIC), reared from pupa, slide mounted together with pupal skin and cocoon, collected from Konaiano Creek, Panguna, Bougainville Island, Papua New Guinea, 28. III. 1989, by C. Yule. Allotype ♀ (ANIC), reared from pupa, pinned, same data as holotype except date: 29. X. 1988. Paratypes: 1 ♂ (BMNH), reared from pupa, pinned, same data as allotype, 1 ♀ (BMNH), reared from pupa, slide mounted, with its pupal skin and cocoon in alcohol, same data as holotype except date: 28. II. 1989, 2 pupae (ANIC), same as holotype except date: 28. II. 1989.

DISTRIBUTION. Bougainville Island.

REMARKS. The species *pangunaense* refers to the locality where this species was collected.

This new species seems to be very closely related to *S.* (*M.*) *kerei,* described from the Solomon Islands (Takaoka and Suzuki, 1994) in having the similar arrangement of the pupal gill filaments and the similar form of the pupal terminal hooks. However, *S.* (*M.*) *pangunaense* is separable in the pupa from the latter by its frontal and thoracic integuments which are densely covered with tubercles. Moreover, in *S.* (*M.*) *kerei,* four slender filaments are subequal in thickness to each other, but, in *S.* (*M.*) *pangunaense,* the innermost filament is basally much thicker than the three dorsal filaments. This new species is readily separated from the other species of the *sherwoodi*-group by the arrangement of the pupal gill filaments (Fig. 35).

7. *Simulium*(*Morops*) *noroense* Takaoka and Suzuki, 1995

Simulium (*Morops*) *noroense* Takaoka and Suzuki, 1995: 243-245.

SPECIMENS EXAMINED. 1 pupa, together with its cocoon, and 2 mature larvae (BMNH), in alcohol, collected from a stream near Aropa, 13 Km S of Kieta, Bougainville Island, 8. VII. 1965, by R.W. Crosskey.

DISTRIBUTION. Bougainville Island (new record) and Solomon Islands (New Georgia Island).

REMARKS. This species was described from New Georgia Island, Western Province, Solomon Islands, based on the adult female, male, pupal and larval specimens (Takaoka and Suzuki, 1995). This species is very similar to *S.* (*M.*) *sherwoodi,* described from Guadalcanal Island (Stone and Maffi, 1971). The pupa of *S.* (*M.*) *noroense* is distinguished from that of the latter species by the thoracic integument moderately covered with tubercles (the thoracic integument is usually bare or sparsely covered with tubercles, especially on the dorsomedial portion in the latter species).

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THE SIMULIIDAE (DIPTERA) FROM THE SOLOMON ISLANDS, SOUTH PACIFIC

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Abstract: Nine black fly species including five new species were taxonomically studied, chiefly on the basis of reared adults, pupae and mature larvae collected from the Solomon Islands, South Pacific. All species, but one in *Simulium* (*Gomphostilbia*), were placed in *Simulium* (*Morops*). Within the subgenus *Morops* four were further classified into the *clathrinum*-group, one into the *papuense*-group and three remained ungrouped. *S.* (*M.*) *papuense* was newly recorded from the Solomon Islands. Descriptions of all new species and redescription of *S.* (*M.*) *sherwoodi* were given; mature larvae of *S.* (*M.*) *sherwoodi* and *S.* (*M.*) *kerei* were described for the first time. The pupal pit-like organs of all five known species of the *clathrinum*-group from Papua New Guinea and Australia were also illustrated.

Black flies (Diptera: Simuliidae) of the Solomon Islands, South Pacific were little studied. Stone and Maffi (1971) described *Simulium* (*Morops*) *sherwoodi*, and recorded the other black fly species (as *S.* (*M.*) sp. nr *avilae*) from Guadalcanal Island. Recently we described two new species: *S.* (*Gomphostilbia*) *hiroshii* from New Georgia and Guadalcanal Islands, and *S.* (*M.*) *kerei* from New Georgia Island (Takaoka, 1994; Takaoka and Suzuki, 1994).

In this study nine species including five new and one newly recorded species were treated on the basis of reared adults, pupae and mature larvae collected from Guadalcanal and New Georgia Islands in 1992 and 1993 by one of us (HS).

Descriptions of five new species and redescription of *S.* (*M.*) *sherwoodi* are given, and mature larva of *S.* (*M.*) *sherwoodi* and *S.* (*M.*) *kerei* is described for the first time.

Type specimens will be deposited in the Natural History Museum (BMNH), London, U.K.

Genus *Simulium* Latreille s. l.

Subgenus *Gomphostilbia* Enderlein

The subgenus *Gomphostilbia* was well defined by Crosskey (1967) and recently revised by Takaoka and Davies (1995). This subgenus is characterized by the bare pleural membrane and haired katepisternum of adults of both sexes. From the Solomon Islands only one species, *S.* (*G.*) *hiroshii*, was known (Takaoka, 1994). It is noteworthy that this species is very unique in having several characters which depart from the diagnosis of *Gomphostilbia* but agree with those of *Morops* or *Simulium* s. str., as mentioned by Takaoka (1994).

1. *Simulium* (*Gomphostilbia*) *hiroshii* Takaoka, 1994

Simulium (*Gomphostilbia*) *hiroshii* Takaoka, 1994: 97-101.

SPECIMENS EXAMINED. 1 pupa, 1 pupal exuvia and 22 mature larvae (all in alcohol), collected from a stream, Noro, New Georgia Island, Western Province, Solomon Islands, 21. II. 1993, by H. Suzuki; 1 pupa and 1 mature larva, collected from a stream, Selwyn College, Guadalcanal Island, Solomon Islands, 12. VIII. 1993, by H. Suzuki.

DISTRIBUTION. Solomon Islands (New Georgia Island and Guadalcanal Island).

REMARKS. This species was originally reported from the Solomon Islands (Takaoka, 1994). The pupa and larva were collected for the first time from the Guadalcanal Island where only the adult female and male had been previously known.

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Subgenus *Morops* Enderlein

This subgenus was well defined by Crosskey (1967) and was divided into several species-groups (Crosskey, 1967; Colbo, 1976). The subgenus *Morops* is characterized by the haired pleural membrane and katepisternum of adults of both sexes. In this study, all the nine, but one in subgenus *Gomphostilbia* Enderlein, are assigned in this subgenus; four species are further classified into the *clathrinum*-group and one is into the *papuense*-group and three remain ungrouped.

(A) *clathrinum*-group

This species-group is easily distinguished by having the pit-like cuticular organ at the base of pupal gill filaments (Crosskey, 1967). To express relative size of the pit-like organ needed for species identification, *pit-like organ* ratio (=A/B), abbreviated as *plo* ratio, is here proposed, where A is a diameter of the pit-like organ and B is a distance from its innermost margin to the middle longitudinal suture of the thorax (see Fig. 12). The pit-like organs of all the five known species of the *clathrinum*-group are illustrated and measured for *plo* ratio.

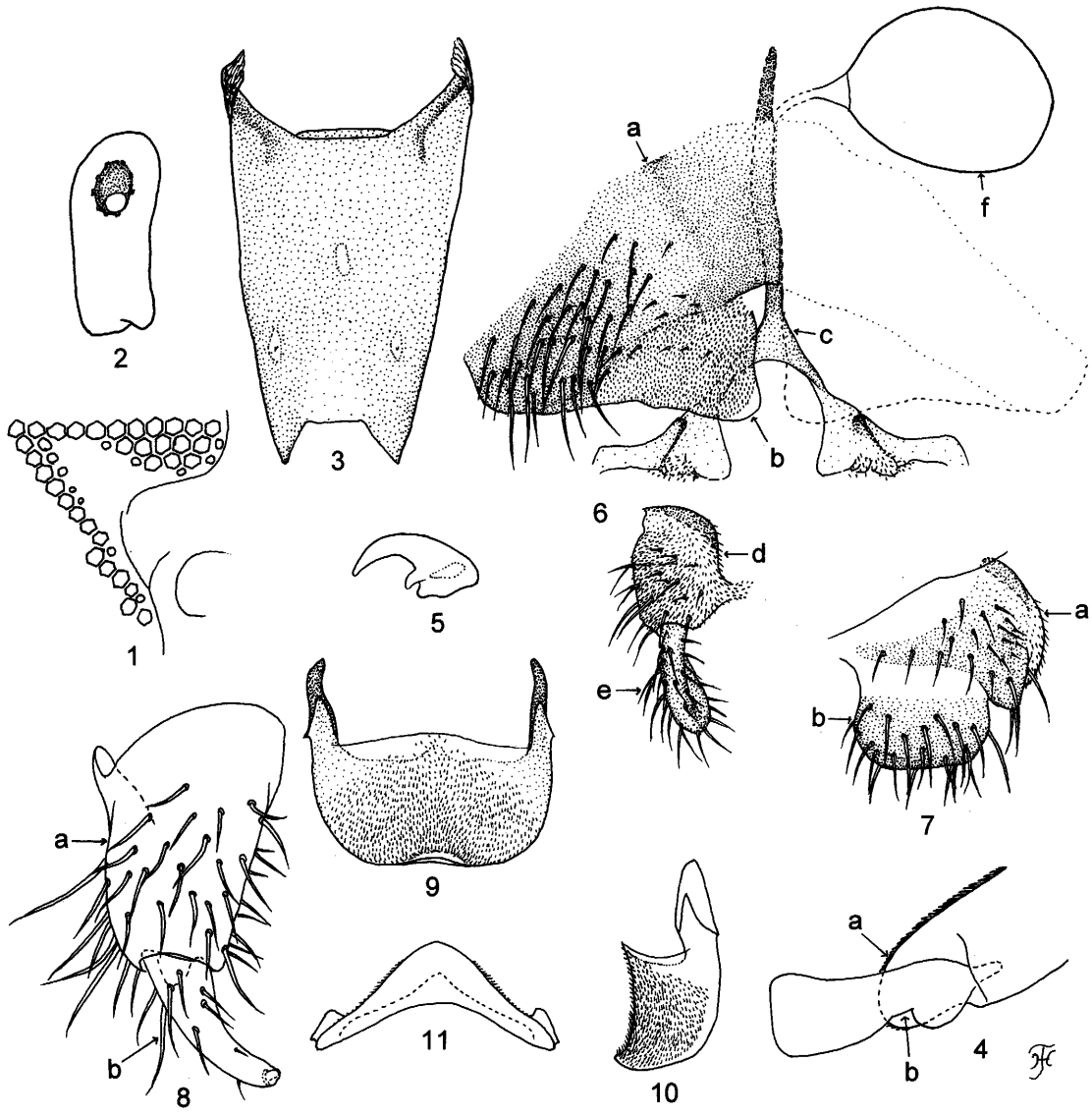
Stone and Maffi (1971) recorded *S.* (*M.*) sp. nr *avilae* Smart & Clifford, on the basis of the pupal and larval specimens from Guadalcanal Island, which was the only species of the *clathrinum*-group from the Solomon Islands. This unidentified species may be *S.* (*M.*) *pohaense* sp. nov. or *S.* (*M.*) *selwynense* sp. nov., both of which are very similar to *S.* (*M.*) *avilae*, as noted later.

2. *Simulium* (*Morops*) *solomonense* sp. nov.

DESCRIPTION. **Female** (coloration incomplete). Body length ca. 2.5 mm. *Head*. Slightly narrower than thorax. Frons dark brown, densely covered with yellowish white, scale-like, recumbent pubescence, interspersed with several dark hairs along both lateral margins; frontal ratio 1.57:1.00:1.87. Frons-head ratio 1.00:4.75. Fronto-ocular area (Fig. 1) well developed. Clypeus dark brown, densely covered with yellowish white, scale-like, recumbent pubescence interspersed with several dark hairs. Antenna composed of 2+9 segments, pale. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:1.7; 3rd segment somewhat enlarged, sensory vesicle (Fig. 2) ellipsoidal, 1.6 × as long as wide, 0.3 × as long as 3rd segment, with a moderate opening near distal end. Maxillary lacinia with 7 or 8 inner and 10 outer teeth. Mandible with ca. 20 small inner teeth and 1 or 2 outer teeth near apex. Cibarium (Fig. 3) with upper margin widely bent forwardly, lacking any processes. *Thorax*. Scutum dark brown in ground color, densely covered with yellowish white, recumbent pubescence except median, large area with dark brown pubescence; 3 longitudinal vittae of yellowish white, recumbent pubescence (1 median vitta narrow and 2 submedian ones rather wide), which are connected anteriorly and posteriorly to areas of the concolored pubescence; long, erect, dark hairs present on prescutellar area. Scutellum blackish brown with yellowish white pubescence and many long, erect, dark hairs. Postscutellum blackish brown, bare. Pleural membrane with ca. 120 yellowish white pubescence and a few dark hairs. Katepisternum longer than deep, dark brown, with numerous, yellowish white pubescence and dark hairs; sulcus distinct. *Legs*. Foreleg: coxa and trochanter pale yellow; femur pale yellow with apical cap brown; tibia pale yellow with basal and distal 1/4 brown; tarsus brown; basitarsus somewhat dilated, ca. 5.8 × as long as its greatest width. Midleg: coxa dark brown; trochanter pale yellow; femur pale yellow with apical cap brown; tibia brownish with median large portion pale; tarsus brown. Hind leg: coxa pale yellowish brown; trochanter pale yellow; femur pale yellow with apical cap brown; tibia brownish with base and median large portion pale yellow; tarsus pale brown with basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, parallel-sided, ca. 6.7 × as long as wide. Calcipala (Fig. 4a) well developed, as long as wide. Pedisulcus (Fig. 4b) well developed. Hind femur and tibia 2.1 × and 1.6 × as wide as hind basitarsus, respectively. All femora and tibiae densely covered with scale-like hairs on outer surface. Claws (Fig. 5) each with a small basal tooth. *Wing*. Length 1.7 mm. Costa with spinules as well as hairs. Subcosta haired. Hair tuft on stem vein dark brown. Basal portion of radius fully haired. *Abdomen*. Basal scale brown with fringe of yellowish white hairs. Dorsal surface of segments 2-5 very densely covered with scale-like, recumbent pubescence, which is yellowish white on segment 2 but brown dorsally and yellowish white laterally on segments 3-5; that of segments 6 and 7 moderately covered with yellowish white, recumbent pubescence and dark hairs; that of segments 8 and 9 moderately covered with yellowish white, short hairs and dark, longer hairs; tergite of 2nd segment whitish pruinose, tergites of segments 6-8 shiny. *Genitalia*. Sternal plate undeveloped on abdominal segment 7. Sternite 8 (Fig. 6a) bare medially, with 32-36 macrosetae on each side. Anterior gonapophysis (Fig. 6b) triangular, thin, membraneous, covered densely with microsetae

interspersed with a few setae; inner margin well sclerotized. Genital fork (Fig. 6c) of usual inverted-Y form, with arms rather broad, folded medially, without projection directed forward; arms furnished with microsetae posteriorly. Paraproct (Figs. 6d & 7a) of usual form. Cercus (Figs. 6e & 7b) 0.5 × as long as wide, with posterior border nearly straight when viewed laterally. Spermatheca ovoid, well sclerotized and with reticulate patterns on surface; tube and small area near tubal juncture unsclerotized; internal setae absent.

Male. Body length ca. 2.8 mm. *Head*. Wider than thorax. Upper eye consisting of 14 vertical columns and 15 horizontal rows of large facets. Clypeus brownish black, covered densely with yellow pubescence, interspersed with several dark hairs. Antenna composed of 2+9 segments, yellowish; 1st flagellomere elongate, 2.0 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:2.0; sensory vesicle globular, 0.2 × as long as 3rd segment, with a small opening. *Thorax*. As in ♀ except



Figs. 1-11. Adult characters of *Simulium* (*Morops*) *solomonense* sp. nov. 1, fronto-ocular area; 2, 3rd maxillary palpal segment showing a sensory vesicle; 3, cibarium; 4, distal tip of basitarsus and 2nd tarsal segment of hind leg showing calcipala (a) and pedisulcus (b); 5, claw with a small tooth; 6, genitalia in situ (ventral view) (a, 8th sternite; b, gonapophysis; c, genital fork; d, paraproct; e, cercus; f, spermatheca); 7, paraproct (a) and cercus (b) (side view); 8, coxite (a) and style (b) (ventral view); 9-11, ventral plate (9, ventral view; 10, side view; 11, end view). 1-7, ♀; 8-11, ♂.

pleural membrane with ca. 130 yellowish white pubescence interspersed with several dark hairs. *Legs*. Coloration as in ♀. Fore basitarsus somewhat dilated, 6.3 × as long as its greatest width. Hind basitarsus slender, almost parallel-sided, 6.5 × as long as wide. Hind femur and tibia ca. 2.3 × and 1.8 × width of hind basitarsus, respectively. Calcipala and pedisulcus well developed, as in ♀. All femora and tibiae densely covered with scale-like hairs on outer surface. *Wing*. Length 1.8 mm. Other features as in ♀ except subcosta bare. *Abdomen*. Basal scale brown, with fringe of dark hairs. Dorsal surface of abdominal segments brownish, covered with dark hairs; segments 2, 5-7 each with a dorsolateral pair of shiny patches. *Genitalia*. Coxite (Fig. 8a) much longer than wide. Style (Fig. 8b) shorter than coxite, tapered toward apex, and with an apical spine. Ventral plate (Figs. 9-11) transverse, much shorter than wide, concave posteriorly (in ventral view), fully setose on ventral surface but bare on posterior and dorsal surfaces; basal arms of moderate length, subparallel to each other. Paramere without hooks. Median sclerite tansparent, probably wide.

Pupa. Body length (excluding gill filaments) ca. 2.8 mm. *Head*. Integument dark yellow, bare with 4 pairs of trichomes, all long and simple. Antennal sheath with smooth surface. *Thorax*. Integument dark yellow, bare except posterodorsal surface very sparsely covered with tubercles; near base of gill there is on each side a large, circular pit-like organ (Fig. 12a) thickly fringed with distinct, comb-like processes (as in Fig. 20); *plo* ratio 1; thoracic trichomes 6 pairs (5 anterodorsally and 1 posterolaterally), all long and simple. Gill (Fig. 13) composed of 4 slender filaments, arising from very long basal stalk, subequal in length and thickness (total gill length ca. 2.0 mm); basal stalk with a large, transparent pouch, which expands laterally and ventrally (Figs. 12b & 13a); surface of distal 1/2 of basal stalk with many longitudinal and transverse ridges, covered with somewhat larger tubercles on ridges and with smaller ones on interspaces (Fig. 13b); slender filaments subequal in length and thickness, with well-defined annular ridges and furrows on their surface, and covered with rather larger tubercles on ridges and smaller ones on interspaces; all filaments dark yellow to yellowish brown. *Abdomen*. Terga 1 and 2 pale brown, without tubercles; tergum 1 with 1 long seta on each side, tergum 2 with 5 simple spinous setae and 1 much longer seta on each side. Terga 3 and 4 each with 4 hooked spines directed forward on each side. Terga 7-9 each with a transverse row of spine-combs and comb-like groups of minute spines directed caudad on each side, though spine-combs on tergum 9 somewhat smaller than those on terga 7 and 8. Tergum 9 with distinct, cone-shaped, terminal hooks (Fig. 14). Sternum 4 with 1 distinct, simple hook and a few minute setae on each side; sternum 5 with a pair of bifid hooks on each side; sterna 6 and 7 each with a pair of inner bifid and outer simple hooks on each side; last segment without grapnel-like hooklets ventrolaterally on each side. *Cocoon*. Shoe-shaped, moderately woven, somewhat extending ventrolaterally; anterior margin not thickly woven; individual threads visible; interspaces thinnly walled, but without perforations.

Mature larva. Body length 4.7-5.2 mm. Body grey to greyish black. Cephalic apotome pale yellow with a large dark brown area medially on posterior 1/2; head spots distinct, often merged in surrounding dark brown area. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.0:1.0:0.7. Labral fan with ca. 44 main rays. Mandible (Fig. 15) with comb-teeth decreasing in size from 1st to 3rd; mandibular serration composed of 2 teeth (1 large and 1 small), with a few supernumerary serrations. Hypostomium (Fig. 16) with a row of 9 apical teeth; median tooth as long as each corner tooth, longer than 3 intermediate teeth on each side; lateral margin serrate; hypostomal bristles 7 in number per side, lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 16) with lateral margins subparallel to each other on basal 1/2, with apex pointed, which often has a narrow extension anteriorly approaching posterior border of hypostomium. Thoracic cuticle bare. Abdominal cuticle bare except each side of anal sclerite of last segment moderately covered with numerous colorless setae. Rectal papilla compound, each of 3 lobes with 4 or 5 finger-like, secondary lobules on posterior surface. Anal sclerite (Fig. 17) of usual X-form, with anterior arms ca. 0.7 × as long as posterior ones, broadly sclerotized at base. Last abdominal segment expanded ventrolaterally forming double bulges on each side, visible as a small ventral papilla when viewed from side. Posterior circlet with ca. 104 rows of up to 16 hooklets per row.

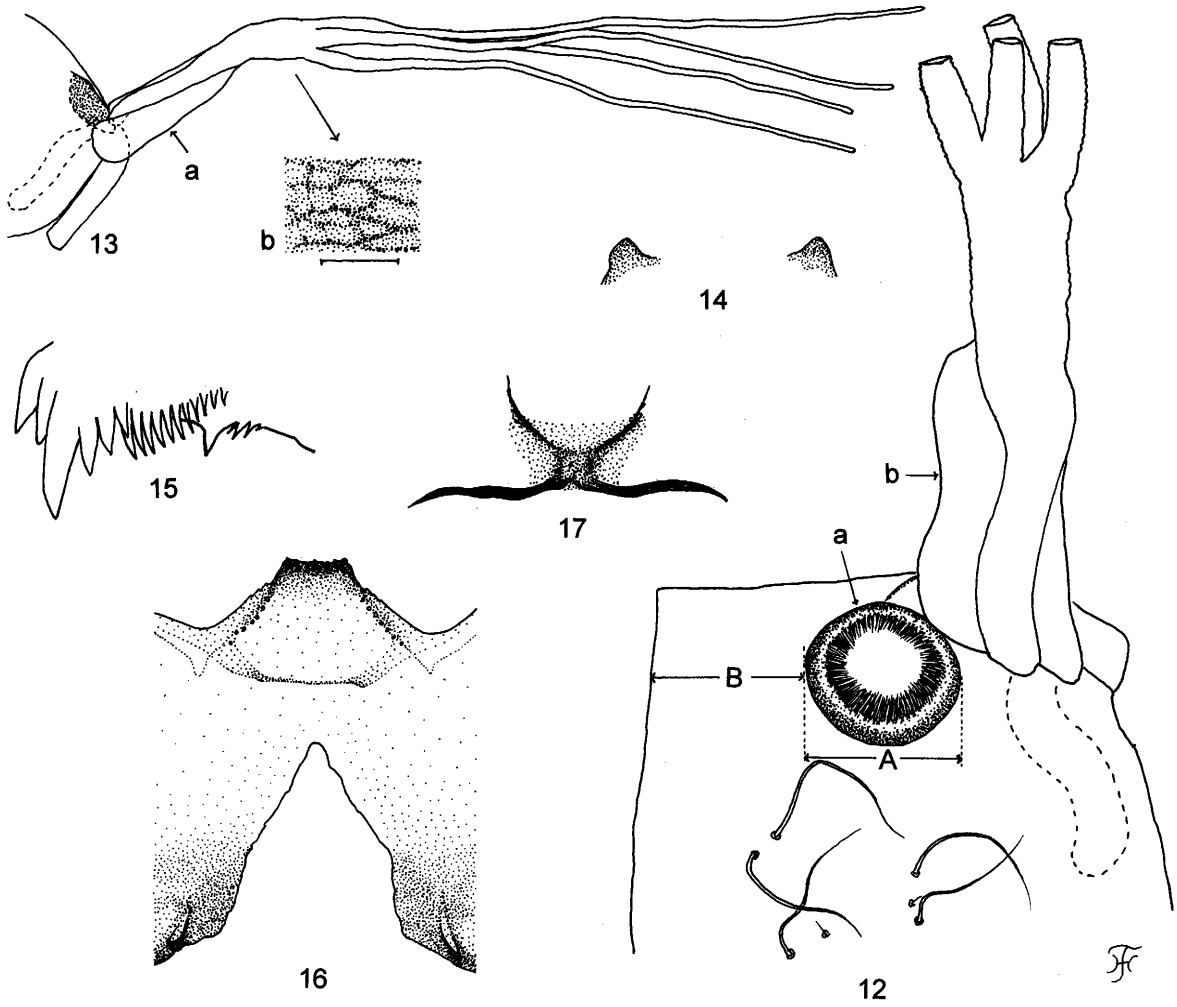
TYPE SPECIMENS. Holotype ♀, reared from pupa, slide-mounted, collected from a stream, Noro, New Georgia Island, Western Province, Solomon Islands, 5. IX. 1992, by H. Suzuki. Allotype ♂, reared from pupa, slide-mounted, same data as holotype. Paratypes: 5 pupae and 4 pupal exuviae, same data as holotype; 1 pupa and 2 pupal exuviae and 11 mature larvae, same data as holotype except date: 21. II. 1993.

DISTRIBUTION. Solomon Islands (New Georgia Island).

REMARKS. This new species belongs to the *clathrinum*-group of the subgenus *Morops*, defined by Crosskey (1967) by having the pit-like organ (Fig. 12a), as well as four gill filaments. Among five known species, *S.* (*M.*) *clathrinum* from Australia (Mackerras and Mackerras, 1948) and *S.* (*M.*) *lalokiense* from central Papua New Guinea (Smart and Clifford, 1965) have a large, pit-like organ with comb-like processes along the inner margin (Figs. 18 & 19). The pit-like organ of this species is as large as that of *S.* (*M.*) *lalokiense* (*plo* ratio ca. 1.1) but somewhat larger than that of *S.* (*M.*) *clathrinum* (*plo* ratio ca. 0.7). However, there are clear differences between this new species and the latter two known species. In the latter two species, the basal stalk is short, and there are tubercles along the middle longitudinal suture of the anterodorsal surface of thorax (Figs. 18 & 19).

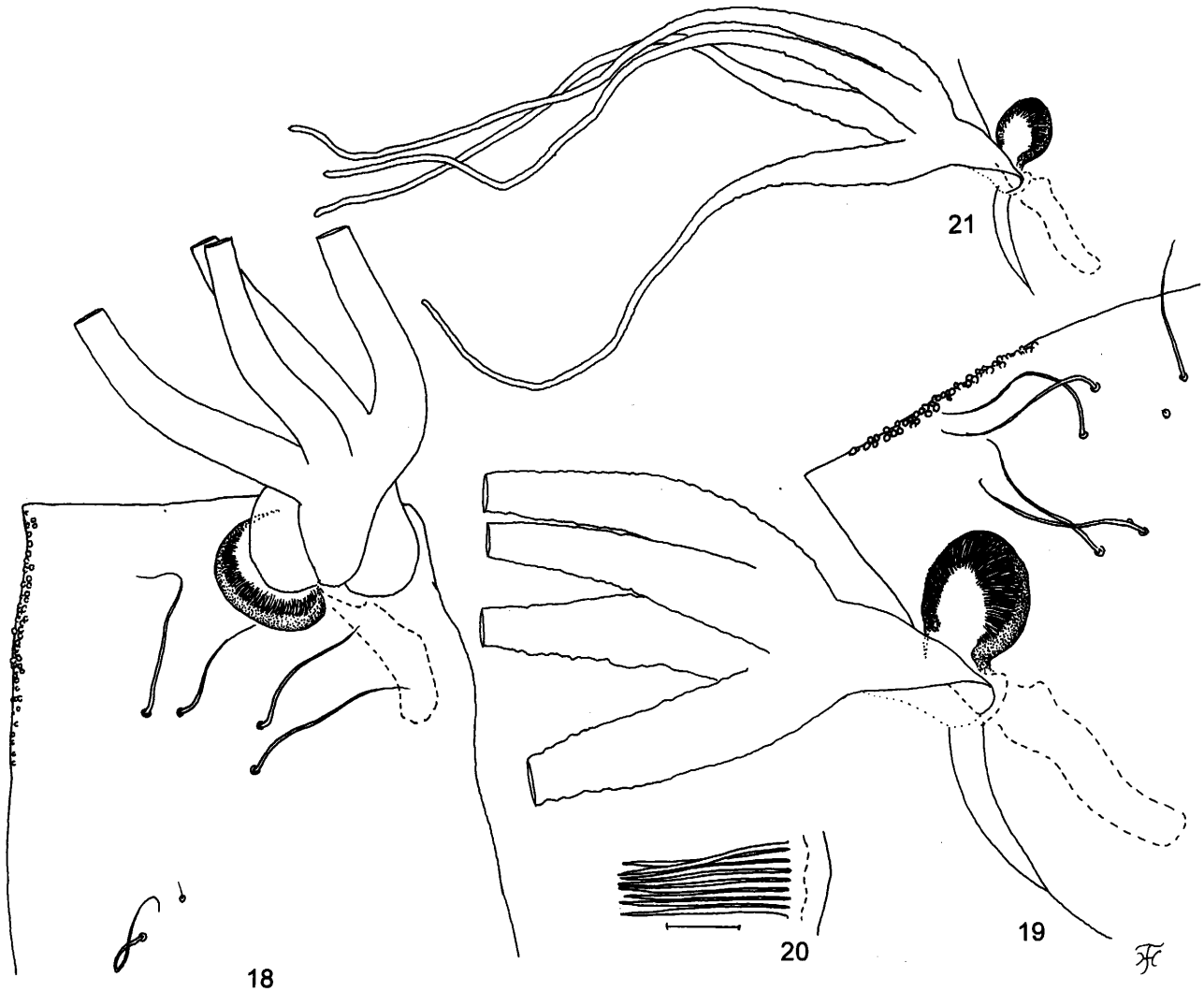
3. *Simulium* (*Morops*) *pohaense* sp. nov.

DESCRIPTION. **Female**. Body length ca. 2.5 mm. *Head*. Slightly narrower than thorax. Frons dark brown, white pruinose, densely covered with white, scale-like, recumbent pubescence, interspersed with several dark hairs along both lateral margins; frontal ratio 1.51:1.00:



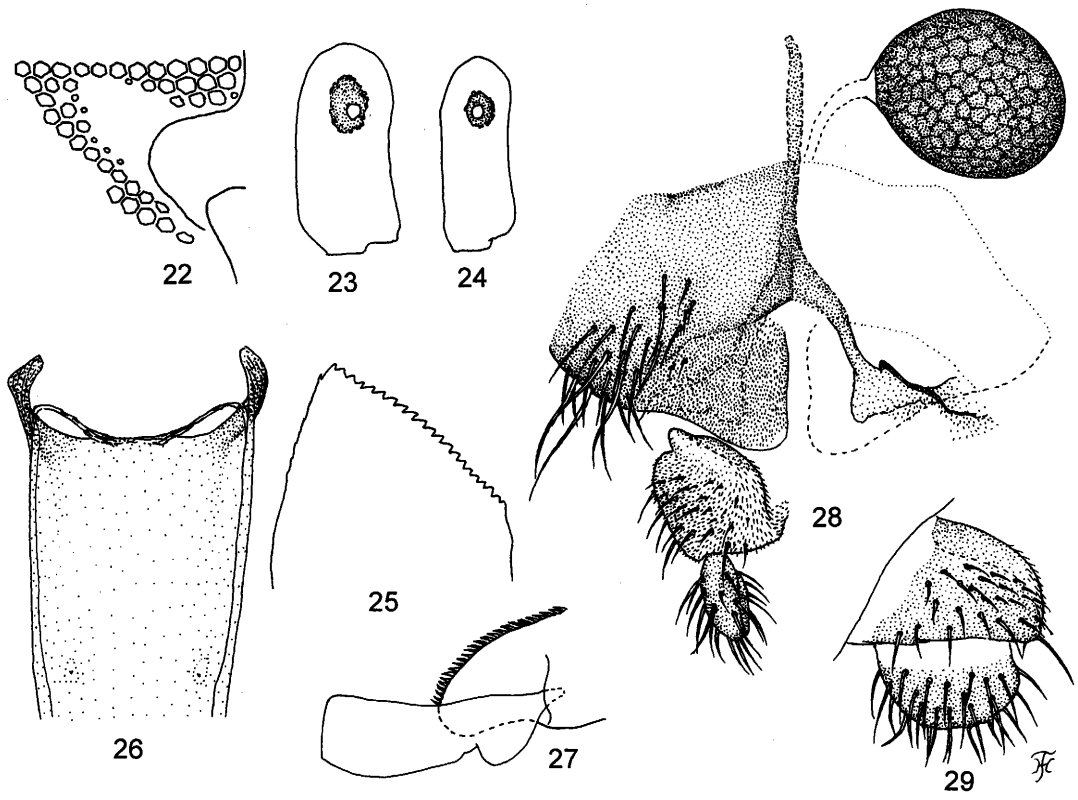
Figs. 12-17. Pupal and larval characters of *Simulium* (*Morops*) *solomonense* sp. nov. 12-14, pupa; 15-17, larva. 12, anterior part of thorax (right half) showing a pit-like organ (a) and a transparent pouch (b) at the base of gill filaments (dorsal view); 13, gill filaments showing a transparent pouch (a) and surface sculpture on the basal stalk (b); 14, terminal hooks; 15, apical tip of mandible; 16, head capsule showing hypostomium and postgenal cleft; 17, anal sclerite. Scale bar 0.02 mm for Fig. 13b.

1.54. Frons-head ratio 1.0: 4.3. Fronto-ocular area (Fig. 22) well developed. Clypeus dark brown, densely covered with white, scale-like, recumbent pubescence interspersed with several dark hairs. Antenna composed of 2+9 segments, dark brown except scape, pedicel and base of 1st flagellomere yellow when viewd from top. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:2.1; 3rd segment somewhat enlarged, sensory vesicle (Fig. 23) ellipsoidal, 1.7× as long as wide, 0.3× as long as 3rd segment, with a medium opening near distal end. Maxillary lacinia with 7 inner and 11 outer teeth. Mandible with 21 small inner teeth and 1 outer tooth near apex; outer margin near apex very slightly erose (Fig. 25). Cibarium (Fig. 26) with smooth posterior margin, which is covered with transparent, elongated projections extending from both arms. *Thorax*. Scutum dark brown in ground color, densely covered with white, recumbent pubescence except median, large area with dark brown pubescence; 3 longitudinal vittae of white recumbent pubescence (1 median vitta narrow and 2 submedian ones rather wide), which are connected anteriorly and posteriorly to areas



Figs. 18-21. Pupal characters of two species of the *clathrinum*-group. 18 & 19, anterior part of thorax showing a pit-like organ (18, dorsal view; 19, side view, slide-mounted); 20, comb-like processes along inner margin of pit-like organ; 21, gill filaments. 18, *S.* (*M.*) *clathrinum* (alcoholic specimen collected from North Queensland, Australia, 15. IX. 1949, by I. M. & M. J. Mackerras, BMNH) ; 19-21, *S.* (*M.*) *lalokiense* (allotype slide-mounted specimen collected from Central District, Papua New Guinea, 17. IX. 1957, by J. Smart, BMNH). Scale bar 0.02 mm for Fig. 20.

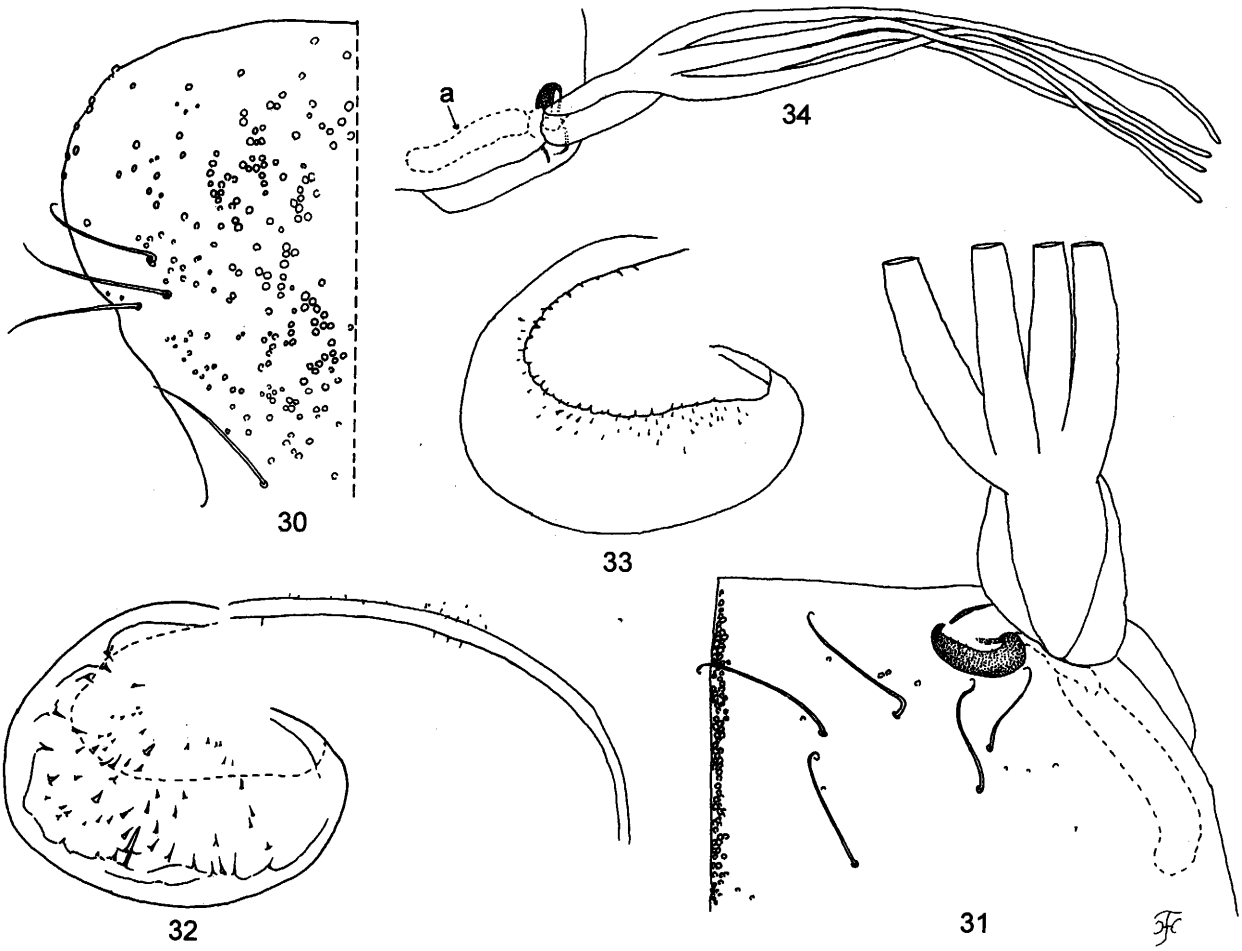
of the concolored pubescence; long, erect, dark hairs present on prescutellar area. Scutellum blackish brown with white pubescence and many, long, erect, dark hairs. Postscutellum blackish brown, bare. Pleural membrane with ca. 120 white pubescence and a few dark hairs. Katepisternum longer than deep, dark brown, with numerous white pubescence and dark hairs; sulcus distinct. *Legs*. Foreleg: coxa and trochanter pale greyish brown; femur pale brown; tibia brown with median, large portion somewhat lighter, white pubescence on outer surface silvery iridescent in certain angles of light; tarsus blackish brown; basitarsus dilated, ca. 5.3× as long as its greatest width; tarsal segments 1-3 moderately furnished with dorsal crest of dark brown, scale-like hairs. Midleg: coxa dark brown; trochanter pale greyish brown; femur greyish brown with apical cap brown; tibia brown with base yellow, white pubescence on posterior surface silvery iridescent in certain angles of light; tarsus brown to blackish brown. Hind leg: coxa and trochanter pale greyish brown; femur greyish brown with apical cap brown; tibia brownish with base yellow and apical 1/4 dark brown, white pubescence on posterior surface silvery iridescent in certain angles of light; tarsus dark brown with basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, parallel-sided, ca. 7.2 × as long as wide. Calcipala (Fig. 27) well developed, ca. 1.2 × as long as wide. Pedisulcus (Fig. 27) well developed. Hind femur and tibia 2.3 × and 1.7 × as wide as hind basitarsus, respectively. All femora and tibiae densely covered with scale-like hairs on outer surface. Claws each with a small basal tooth, similar to that of ♀ *S.* (*M.*) *solomonense*. *Wing*. Length 1.7 mm. Costa with spinules as well as hairs. Subcosta haired on basal 3/5. Hair tuft on stem vein dark brown. Basal portion of radius fully haired. *Abdomen*. Basal scale brown with fringe of white hairs. Dorsal surface of segments 2-5 pale except tergites brown, very densely covered with scale-like, recumbent pubescence which is entirely shiny white on segment 2, and mostly dark brown (intermixed with white ones along posterior margin) on segments 3-5; that of segments 6-8 each with a large, dark brown tergite, moderately covered with white, recumbent pubescence, interspersed with dark hairs; tergites 2, 6-8 shiny; abdomen moderately



Figs. 22-29. Adult characters of *Simulium* (*Morops*) *pohaense* sp. nov. 22, fronto-ocular area; 23 & 24, 3rd maxillary palpal segment showing sensory vesicle; 25, mandible; 26, cibarium; 27, calcipala and pedisulcus; 28, genitalia in situ (ventral view); 29, paraproct and cercus (side view). 24, ♂; others, ♀.

covered with white, scale-like pubescence also laterally. *Genitalia* (Figs. 28 & 29). Sternal plate well developed on abdominal segment 7 and with ca. 50 hairs. Sternite 8 bare medially, with 24-26 macrosetae on each side. Anterior gonapophysis triangular, thin, membraneous, covered densely with microsetae interspersed with a few setae; inner margin moderately sclerotized. Genital fork of usual inverted-Y form, with arms rather broad, folded medially, with a short projection directed forward; arms furnished with microsetae posteriorly. Paraproct of usual form. Cercus 0.5 × as long as wide, with posterior border rounded when viewed laterally. Spermatheca nearly globular, well sclerotized, with reticulate patterns on surface; tube and small area near tubal juncture unsclerotized; internal setae absent.

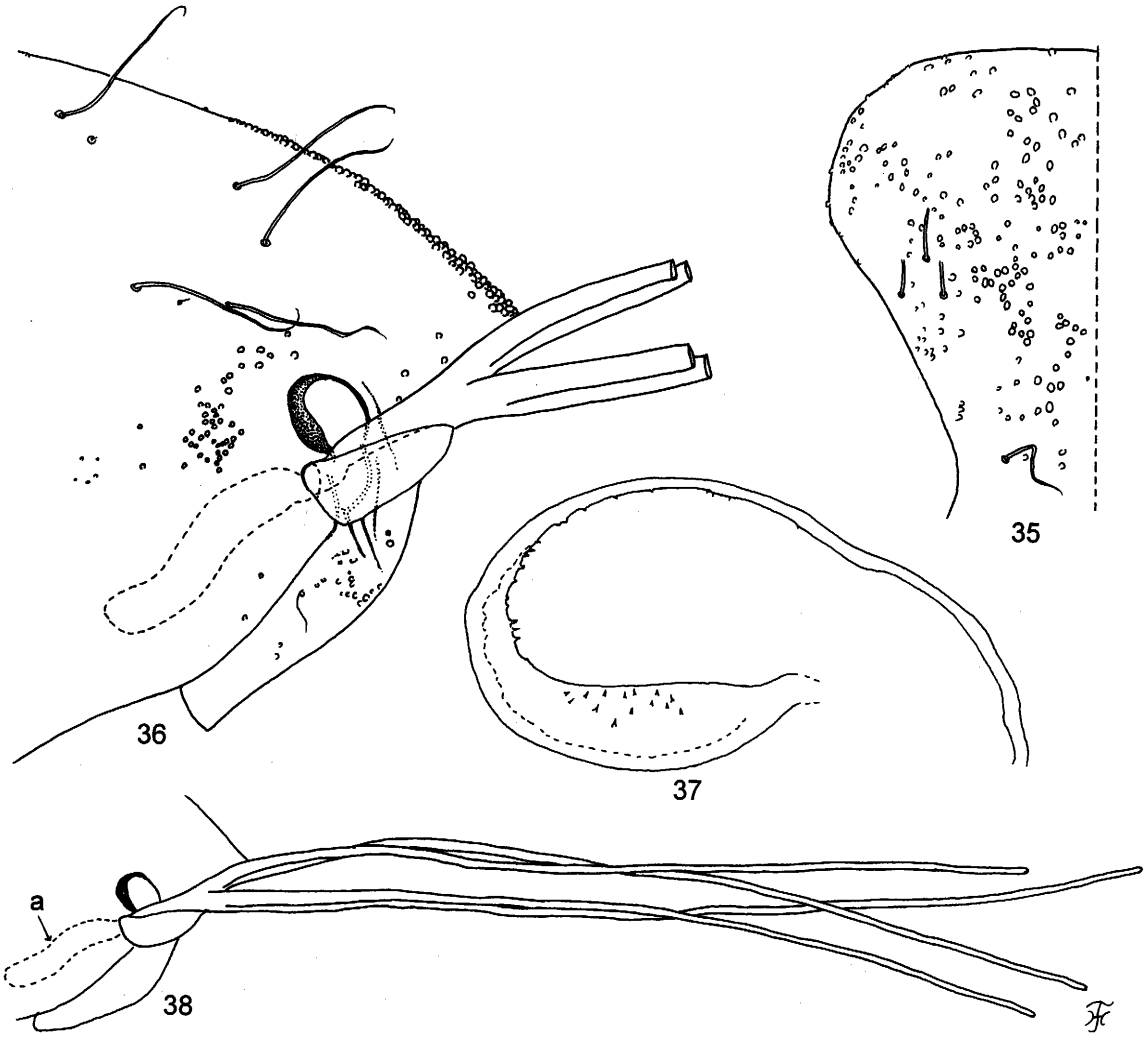
Male. Body length ca. 2.5 mm. *Head*. Wider than thorax. Upper eye consisting of 14 or 15 vertical columns and 13-15 horizontal rows of large facets. Clypeus brownish black, covered densely with whitish yellow pubescence, intermixed with many dark hairs. Antenna composed of 2+9 segments, pale brown, becoming darker apically except scape, pedicel and base of 1st flagellomere yellow; 1st flagellomere elongate, 1.7 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.00:1.03:2.00; sensory vesicle (Fig. 24) globular, 0.2 × as long as 3rd segment, with a medium opening. *Thorax*. As in ♀ except pubescence on scutum and scutellum yellowish; 3 longitudinal vittae of yellow pubescence on scutum not well defined. *Legs*. As in ♀ except following features:



Figs. 30-34. Pupal characters of *Simulium* (*Morops*) *pohaense* sp. nov. 30, frons (right half) showing 4 trichomes and scattered tubercles; 31, anterior part of thorax (right half) showing a pit-like organ, tubercles along middle longitudinal suture, five trichomes and basal portion of gill (dorsal view); 32, pit-like organ showing minute, spine-like processes on floor; 33, dorsal eaves-like wall of pit-like organ showing minute, spine-like processes on under surface and along inner margin; 34, gill filaments (side view) (a, interspiracular trunk).

fore basitarsus somewhat dilated, 6.3 × as long as its greatest width; hind basitarsus slender, almost parallel-sided, 7.0 × as long as wide; hind femur and tibia ca. 2.1 × and 1.8 × width of hind basitarsus, respectively. *Wing*. Length 1.6 mm. Other features as in ♀ except subcosta bare. *Abdomen* and *Genitalia*. As in ♂ of *S.* (*M.*) *solomonense*.

Pupa. Body length (excluding gill filaments) ca. 2.5 mm. *Head*. Integument dark yellow, bare or sparsely (moderately in some pupae, Fig. 30) covered with round tubercles, and with 4 pairs of trichomes, all long and simple. Antennal sheath with smooth surface. *Thorax*. Integument dark yellow, bare or very sparsely covered with round tubercles except posterodorsal surface moderately covered with smaller tubercles and both sides of middle longitudinal suture narrowly covered with round tubercles (Fig. 31); near base of gill there is on each side a medium-sized, pit-like organ sparsely covered with minute, spine-like processes on floor (Fig. 32) and on under surface and along inner margin of dorsal, eaves-like wall (Fig. 33); *plo* ratio variable from 0.31-0.53 (median = 0.43, n = 83); thoracic trichomes 6 pairs (5 anterodorsally and 1 posterolaterally), all long and simple. Gill (Fig. 34) composed of 4 slender filaments,



Figs. 35-38. Pupal characters of *Simulium* (*Morops*) *avilae*. 35, frons (right half) showing four trichomes and scattered tubercles; 36, anterior part of thorax showing a pit-like organ, tubercles along middle longitudinal suture, five trichomes and basal portion of gill (side view, slide-mounted); 37, pit-like organ showing minute, spine-like processes on under surface and along inner margin; 38, gill filaments (side view) (a, interspiracular trunk). (Examined was the paratype slide-mounted specimen collected in Northern District, Papua New Guinea, 19. I. 1958, by J. Smart, BMNH)

arising from long basal stalk, sessile or short-stalked, subequal in length and thickness (total gill length 1.5-2.3 mm, average ca. 1.8 mm); basal stalk with a large, transparent pouch, which expands laterally; each filament rather thick basally, nearly as thick as interspiracular trunk (Fig. 34a), tapered gradually to mid point, then nearly of the same diameter up to apical tip; surface of each filament with well-defined, annular ridges forming polygonal patterns, covered with somewhat larger tubercles on ridges and with smaller ones on interspaces; all filaments dark yellow to yellowish brown. *Abdomen* and *Cocoon*. As in *S.* (*M.*) *solomonense* except tergum 9 lacking spine-combs.

Mature larva. As in *S.* (*M.*) *solomonense* except following features: Body length 4.3-5.0 mm. Cephalic apotome pale yellow with a large, dark brown area medially on posterior 1/2 with head spots distinct, often merged in surrounding dark brown area, or cephalic apotome entirely pale yellow with positive head spots, at least middle longitudinal spot. Proportional lengths of 3 antennal segments from base to tip 1.0:1.0:0.6. Labral fan with 36-42 main rays. Hypostomal bristles 6 in number per side. Rectal papilla compound, each of 3 lobes with 3 or 4 finger-like, secondary lobules on posterior surface. Posterior circlet with 92-100 rows of up to 15 hooklets per row.

TYPE SPECIMENS. Holotype ♀, reared from pupa, slide-mounted, collected from Poha River, Guadalcanal Island, Solomon Islands, 15. VIII. 1993, by H. Suzuki. Allotype ♂, reared from pupa, slide-mounted, same data as holotype. Paratypes: 2 ♀, 5 ♂, all reared from pupa, 23 pupae, 10 pupal exuviae, 5 mature larvae, all in alcohol except 2 pupal exuviae and 3 mature larvae slide-mounted, same data as holotype. Other specimens: 5 ♀, 7 ♂, all reared from pupa, 15 pupae, 33 pupal exuviae and 17 mature larvae, collected from Tenaru River, Guadalcanal Island, 10. VIII. 1992, by H. Suzuki; 8 ♀, 3 ♂, all reared from pupa, 18 pupae and 13 pupal exuviae, collected from Tenaru River, Guadalcanal Island, 16. VIII. 1992, by H. Suzuki; 26 ♀, 13 ♂, all reared from pupa, 49 pupae, 13 pupal exuviae and 3 mature larvae, collected from Tanatita River, Guadalcanal Island, 16. VIII. 1992, by H. Suzuki; 1 ♀, reared from pupa, and 4 pupae, collected at Gold Ridge, Guadalcanal Island, 30. VIII. 1992, by H. Suzuki.

DISTRIBUTION. Solomon Islands (Guadalcanal Island).

REMARKS. This new species also belongs to the *clathrinum*-group by having the pit-like organ (Fig. 31), as well as four gill filaments. The pit-like organ of this species differs in size from one pupa to another, then *plo* ratio also varies from 0.31 to 0.53 with a median of 0.43. Among five known species of the *clathrinum*-group, *S.* (*M.*) *avilae* from Northern District, Papua New Guinea (Smart and Clifford, 1965) has a similar pit-like organ of which *plo* ratio is 0.45 (measured from two slide-mounted specimens loaned from BMNH). However, *S.* (*M.*) *pohaense* is separated from *S.* (*M.*) *avilae* by having the somewhat inflated base of each gill filament (Fig. 34) which is as thick as interspiracular trunk (much thinner in the latter species, see Fig. 38), and by the presence of spinous processes on the floor of the pit-like organ (Fig. 32) (absent in the latter species although there are several processes on the dorsal wall, as seen in Fig. 37).

4. *Simulium* (*Morops*) *selwynense* sp. nov.

DESCRIPTION. **Female**. Unknown.

Male. As in ♂ of *S.* (*M.*) *pohaense* except following features: Body length ca. 2.2 mm. *Head*. Wider than thorax. Upper eye consisting of 12 vertical columns and 13 horizontal rows of large facets. Sensory vesicle nearly globular, 0.21 × as long as 3rd segment, with a small opening medially. *Thorax*. Pleural membrane with ca. 100 yellowish white pubescence interspersed with a few dark hairs. *Legs*. Fore basitarsus somewhat dilated, 5.8 × as long as its greatest width. Hind basitarsus slender, almost parallel-sided, 7.0 × as long as wide. Hind femur and tibia ca. 2.1 × and 1.7 × width of hind basitarsus, respectively. *Wing*. Length 1.5 mm.

Pupa. Body length (excluding gill filaments) 2.5-2.8 mm. *Head*. Integument dark yellow, densely covered with round tubercles, and with 4 pairs of trichomes, all long and simple (Fig. 39). Antennal sheath with smooth surface. *Thorax*. Integument dark yellow, densely covered with round tubercles except anterior surface bare or sparsely covered with tubercles (Fig. 40); near base of gill there is on each side a medium-sized, pit-like organ (Fig. 40), similar to that of *S.* (*M.*) *pohaense*; *plo* ratio 0.45; thoracic trichomes 6 pairs (5 anterodorsally and 1 posterolaterally), all long and simple. Gill (Fig. 41) nearly as in *S.* (*M.*) *pohaense*. *Abdomen* and *Cocoon*. As in *S.* (*M.*) *solomonense* except terga 6-8 each with a transverse row of spine-combs though 1 or 2 in number on tergum 6.

Mature larva. As in *S.* (*M.*) *solomonense* except following features: Body length 4.0-4.2 mm. Proportional lengths of 3 antennal segments from base to tip 1.0:1.1:

0.7. Labral fan with ca. 37 main rays. Hypostomal bristles 6 in number per side. Rectal papilla compound, each of 3 lobes with 3 or 4 finger-like, secondary lobules on posterior surface. Posterior circlet with 85-88 rows of up to 14 hooklets per row.

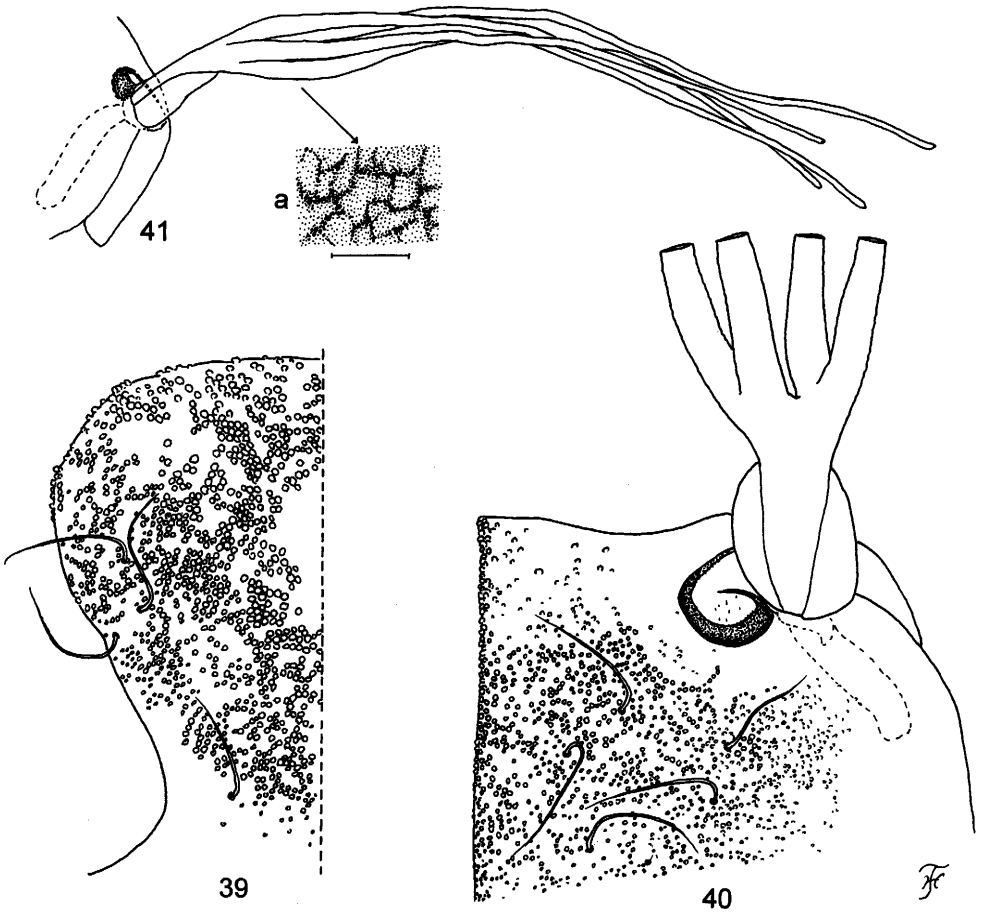
TYPE SPECIMENS. Holotype ♂, reared from pupa, slide-mounted, collected from a stream at Selwyn College, Guadalcanal Island, Solomon Islands, 12. VIII. 1993, by H. Suzuki. Paratypes: 8 pupae and 7 pupal exuviae and 5 mature larvae, all in alcohol, same data as holotype. Other specimens: 3 pupal exuviae, collected from Tenaru River, Guadalcanal Island, 10. VIII. 1992, by H. Suzuki; 3 pupae and 2 pupal exuviae, in alcohol, collected Tanatita River, Guadalcanal Island, 16. VIII. 1992, by H. Suzuki; 1 pupal exuvia, in alcohol, collected at Gold Ridge, Guadalcanal Island, 30. VIII. 1992, by H. Suzuki.

DISTRIBUTION. Solomon Islands (Guadalcanal Island).

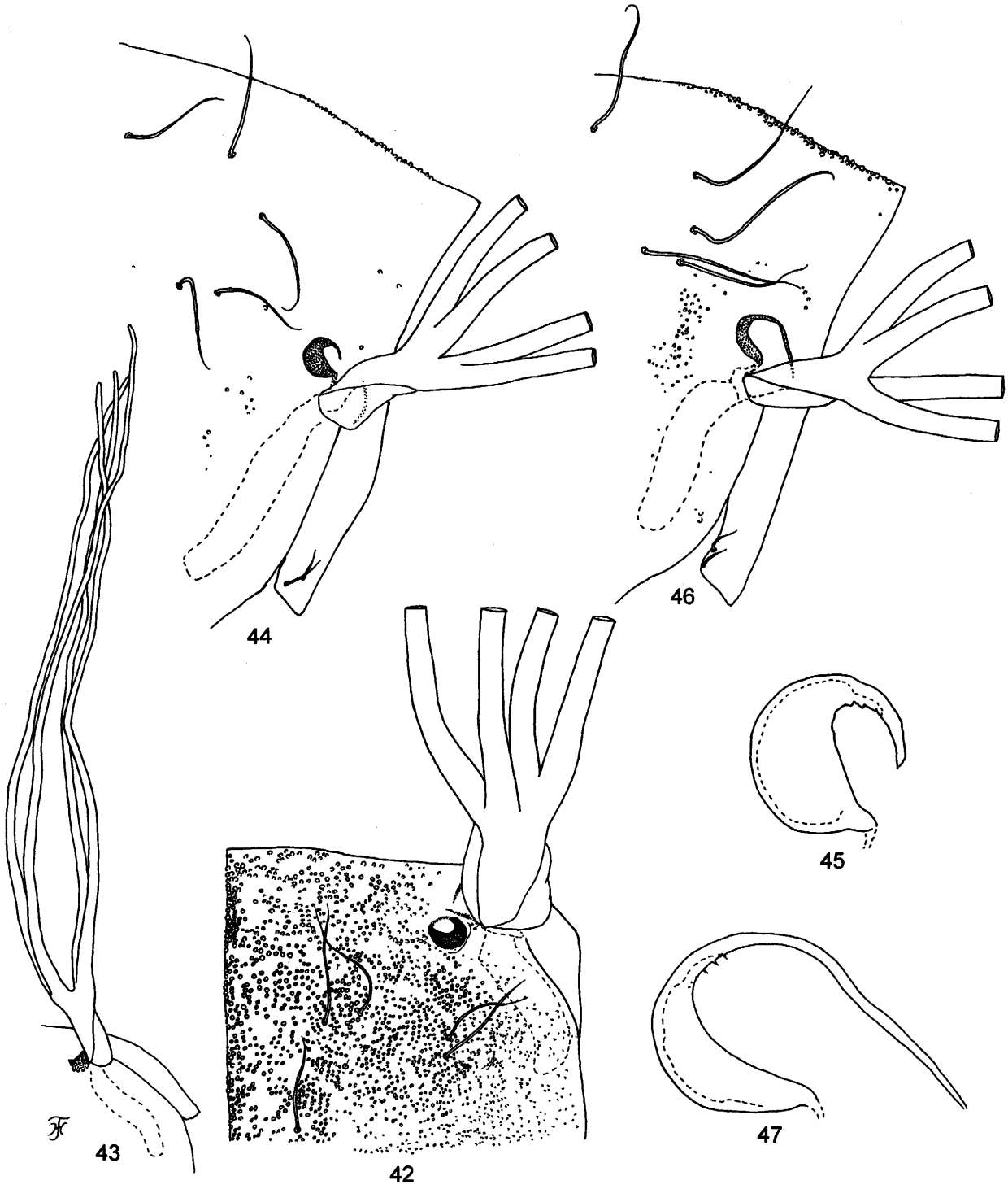
REMARKS. This new species resembles *S.* (*M.*) *pohaense* in many features including the pit-like organ and the gill filaments of the pupa. However this species differs from the latter species by the head and thoracic integument densely covered with tubercles (Fig. 40), which also separates it from *S.* (*M.*) *avilae*.

5. *Simulium* (*Morops*) *kawagishii* sp. nov.

DESCRIPTION. **Female** and **Male**. Unknown.
Pupa. Body length (excluding gill filaments) ca. 2.5 mm.
Head. Integument dark yellow, densely covered with



Figs. 39-41. Pupal characters of *Simulium* (*Morops*) *selwynense* sp. nov. 39, frons (right half) showing four trichomes and dense tubercles; 40, anterior part of thorax (right half) showing a pit-like organ, five trichomes, dense tubercles and basal portion of gill filaments (dorsal view); 41, gill filaments showing surface sculpture near base of each filament (a). Scale bar 0.02 mm for Fig. 41a.



Figs. 42-47. Pupal characters of *Simulium* (*Morops*) *kawagishii* sp. nov. and two related species. 42, 44 & 46, anterior part of thorax showing a pit-like organ, five trichomes, distribution of tubercles and basal portion of gill filaments (42, dorsal view; 44 & 46, side view, slide-mounted); 43, gill filaments (side view); 45 & 47, pit-like organ. 42 & 43, *S.* (*M.*) *kawagishii*; 44 & 45; *S.* (*M.*) *raunsimnae* (paratype slide-mounted specimen collected from New Britain, 14. XI. 1957, by J. Smart, BMNH); 46 & 47, *S.* (*M.*) *gagiduense* (paratype slide-mounted specimen collected from Morobe District, Papua New Guinea, 11-13. I. 1958, by J. Smart, BMNH).

round tubercles, and with 4 pairs of trichomes, all long and simple. Antennal sheath with smooth surface. *Thorax.* Integument dark yellow, densely covered with round tubercles (Fig. 42); near base of gill there is on each side a small, circular, pit-like organ (Fig. 42); *plo* ratio 0.25; thoracic trichomes 6 pairs (5 anterodorsally and 1 posterolaterally), all long and simple. Gill (Fig. 43) composed of 4 slender filaments, arising from long basal stalk, sessile or short-stalked, subequal in length and thickness (total gill length 1.8-2.0 mm); basal stalk with a transparent pouch, which expands laterally to some extent; each filament not so thick basally, much thinner than interspiracular trunk, tapered gradually to mid point, then nearly of the same diameter up to apical tip; surface of each filament with well-defined, annular ridges forming polygonal patterns near base, covered with somewhat larger tubercles on ridges and with smaller ones on interspaces; all filaments yellow. *Abdomen* and *Cocoon.* As in *S.* (*M.*) *pohaense.*

Mature larva. As in *S.* (*M.*) *solomonense* except following features: Body length 4.0-4.5 mm. Body greyish yellow. Cephalic apotome pale yellow with a large dark brown area medially on posterior 1/2 with head spots distinct, often merged in surrounding dark brown area or cephalic apotome entirely pale yellow with indistinct or distinct head spots. Labral fan with ca. 36 main rays. Hypostomal bristles 6 in number per side. Rectal papilla compound, each of 3 lobes with 3-5 finger-like, secondary lobules. Posterior circlet with 82-88 rows of up to 14 hooklets per row.

TYPE SPECIMENS. Holotype pupa, slide-mounted, collected from Danf River, Guadalcanal Island, Solomon Islands, 2. IX. 1992, by H. Suzuki. Paratypes: 2 pupae and 11 mature larvae, same data as holotype.

DISTRIBUTION. Solomon Islands (Guadalcanal Island).

REMARKS. The species name *kawagishii* is given after Mr. N. Kawagishi, ex chargé d'affaires to Solomon, who kindly helped HS during his stays in the Solomon Islands.

This new species also belongs to the *clathrinum*-group by having the pit-like organ (Fig. 42), as well as four gill filaments. The small pit-like organ (*plo* ratio 0.25) of this species separates it from the three preceding new species. Among five known species of this species-group, *S.* (*M.*) *raunsimnae* from New Britain Island and *S.* (*M.*) *gagiduense* from Morobe District of Papua New Guinea (Smart and Clifford, 1965) have a closer value of *plo* ratio, 0.2 and 0.26-0.29, respectively (see Figs. 44 & 46). However, *S.* (*M.*) *kawagishii* differs from both species by the thoracic integument densely covered with tubercles (almost bare or sparsely covered with tubercles in the latter species, as seen in Figs. 44 & 46).

(B) *papuense*-group

6. *Simulium* (*Morops*) *papuense* Wharton, 1948

Simulium papuensis Wharton, 1948: 357-66.
Simulium papuense: Mackerras and Mackerras, 1949: 372-405; Mackerras and Mackerras, 1950: 167-87; Smart and Clifford, 1965: 591.
Simulium (*Morops*) *papuense*: Crosskey, 1967: 44; Crosskey, 1988: 457; Crosskey, 1989: 225.

This species was originally described from Papua New Guinea, based on the female, male, pupal and mature larval specimens (Wharton, 1948). The pupa is distinctive among *Morops* species by the gill of dendroid type (i.e., with ca. 30 slender filaments), and the larva, by the deep postgenal cleft reaching the posterior border of the hypostomium. Crosskey (1967) erected the *papuense*-group for this unique species. In our larval specimens of *S.* (*M.*) *papuense* it is also found that the abdominal segments 1-5 each have a dorsolateral pair of protuberances, a character not mentioned in the original description of *S.* (*M.*) *papuense* (Wharton, 1948). This is the first record of this species from the Guadalcanal Island.

SPECIMENS EXAMINED. 1 ♀ reared from pupa, 1 pupa and 7 mature larvae, collected from a slow stream, Babe, Guadalcanal Island, Solomon Islands, 6, III. 1992, by H. Suzuki.

DISTRIBUTION. Papua New Guinea and Solomon Islands (Guadalcanal Island) (new record).

(C) ungrouped species

7. *Simulium* (*Morops*) *sherwoodi* Stone and Maffi, 1971

Simulium sherwoodi Stone and Maffi, 1971: 299-300.
Simulium (*Morops*) *sherwoodi*: Crosskey, 1988: 458; Crosskey, 1989: 225.

This species was originally described based on the

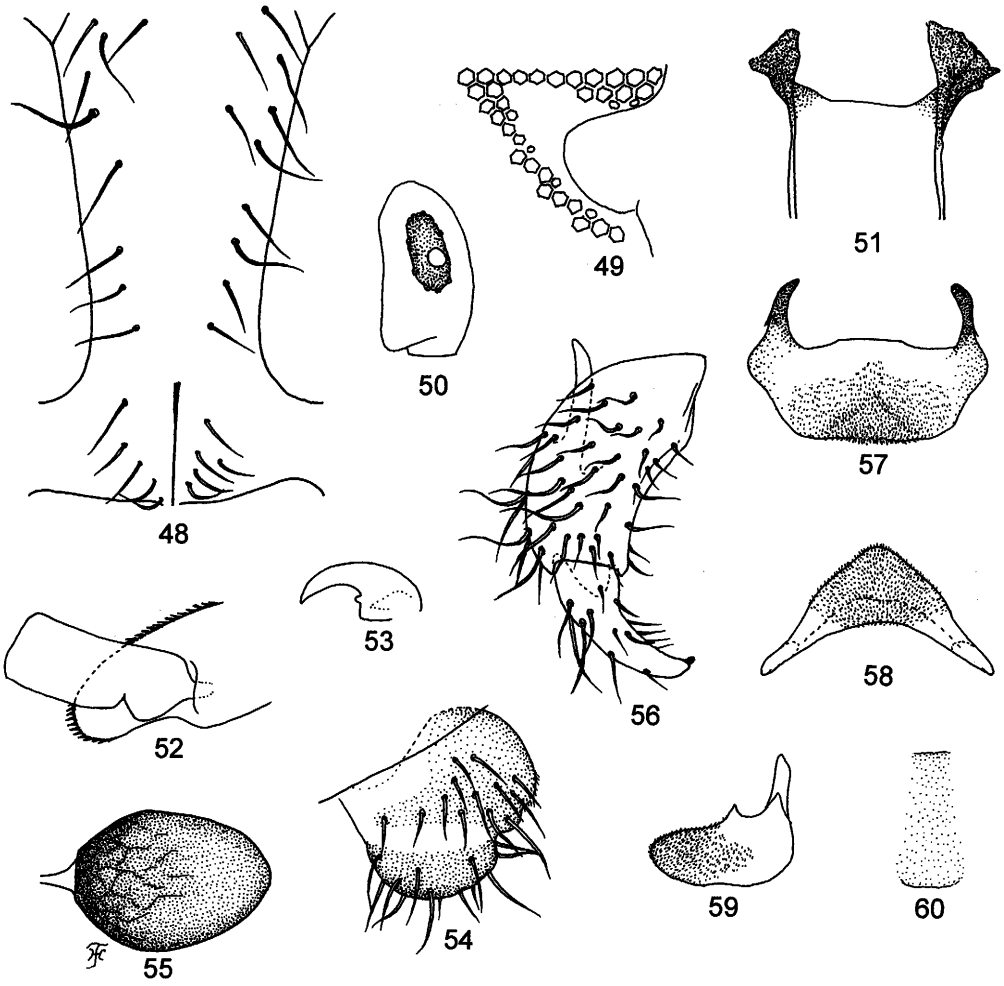
adult female, male and pupal specimens cellected from Guadalcanal Island, Solomon Islands, (Stone and Maffi, 1971). The adult female, male and pupa of *S.* (*M.*) *sherwoodi* are here redescribed, and the mature larva is described for the first time. It is clarified that the female tarsal claws have a minute basal tooth (not simple as mentioned in the original description). Further, the pupae show a transverse row of spine-combs dorsally on the segments 6 to 9 (not segments 5 to 8) and four hooks ventrally on segments 5 to 7 (not segments 6 to 8).

REDESCRIPTION. **Female.** Body length ca. 2.5 mm. *Head.* Slightly narrower than thorax. Frons (Fig. 48) dark brown, shiny, covered with several yellowish white hairs near lower margin and several dark hairs along both lateral margins, without scale-like, recumbent pubescence; frontal ratio 1.6:1.0:2.1. Frons-head ratio 1.0:5.6. Fronto-ocular area (Fig. 49) well developed. Clypeus dark brown, densely covered with yellowish white and dark hairs interspersed with whitish yellow, scale-like, recumbent pubescence on lower 1/2. Antenna composed of 2+9 segments, brownish black except scape, pedicel and base of 1st flagellar segment yellowish. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:2.4; 3rd segment somewhat enlarged, sensory vesicle (Fig. 50) elongate, 2.1 × as long as wide, 0.4 × as long as 3rd segment, with a medium opening slightly distally. Maxillary lacinia with 9 or 10 inner and 12 or 13 outer teeth. Mandible with ca. 25 small inner teeth and lacking outer ones. Cibarium (Fig. 51) smooth. *Thorax.* Scutum blackish brown in ground color, whitish grey pruinose, densely covered with whitish yellow or yellow, scale-like, recumbent pubescence except median large area with brown pubescence. Scutellum blackish brown with dark, scale-like, recumbent pubescence and long, erect, dark hairs along posterior margin. Postscutellum blackish brown, whitish grey pruinose (shining in light), bare. Pleural membrane with ca. 25 yellowish white pubescence. Katepisternum longer than deep, dark brown, whitish grey pruinose (shining in light), with numerous dark hairs and yellowish white pubescence; sulcus distinct. *Legs.* Foreleg: coxa and trochanter whitish yellow; femur dark yellow to pale brown; tibia white with distal 1/4 brownish black, outer surface widely white shining when illuminated; tarsus black; basitarsus somewhat dilated, ca. 4.7 × as long as its greatest width. Midleg: coxa brown; trochanter whitish yellow; femur yellow; tibia whitish yellow on basal 2/5, brown on rest; posterior surface of tibia widely white shining when illuminated; tarsus brown. Hind leg: coxa yellowish brown or pale brown; trochanter yellowish white; femur dark yellow to yellowish brown with apical cap brown; tibia white on basal 3/5, brown to brownish black on distal 2/5; posterior surface of tibia widely white shining when illuminated; tarsus brown with basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, parallel-sided, ca. 6.9 × as long as wide. Calcipala (Fig. 52) well developed, ca. 1.2 × as long as wide. Pedisulcus (Fig. 52) well developed. Hind femur and tibia 2.2 × and 1.7 × as wide as hind basitarsus, respectively. All femora and tibiae densely covered with scale-like hairs on outer surface. Claws (Fig. 53) each with a minute basal tooth. *Wing.* Length 1.5 mm. Costa with spinules as well as hairs. Subcosta haired. Hair tuft on stem vein dark brown. Basal portion of radius fully haired. *Abdomen.* Basal scale brown with fringe of yellowish white hairs. Dorsal surface of abdomen black, with dark hairs; tergite of 2nd segment whitish pruinose, tergites of segments 5-8 shiny. *Genitalia.* Nearly as in *S.* (*M.*) *noroense* sp. nov. (Fig. 71). Sternal plate undeveloped on abdominal segment 7. Sternite 8 bare medially, with 12-13 dark macrosetae on each side. Anterior gonapophysis triangular, thin, membraneous, covered densely with microsetae interspersed with a few setae; inner margin well sclerotized. Genital fork of usual inverted-Y form, with arms rather broad, folded medially, each with a small projection directed forward. Paraproct (Fig. 54) of usual form. Cercus (Fig. 54) ca. 0.5 × as long as wide, with posterior border gently rounded when viewed laterally. Spermatheca (Fig. 55) ovoid, well sclerotized except tube and tubal base unsclerotized; internal setae present.

Male. Body length ca. 2.5 mm. *Head.* Wider than thorax. Upper eye consisting of 9 or 10 vertical columns and 11-13 horizontal rows of large facets. Clypeus brownish black, whitish pruinose, covered densely with yellow pubescence, interspersed with dark hairs. Antenna composed of 2+9 segments, yellow with 6 apical segments brown; 1st flagellomere somewhat elongate, ca. 1.6 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.9; sensory vesicle oblong, 0.33 × as long as 3rd segment, with opening near distal end. *Thorax.* Nearly as in ♀ except pleural membrane with ca. 15 hairs. *Legs.* Foreleg: coxa and trochanter yellow; femur yellow with distal 1/3 brown; tarsus brownish black; basitarsus somewhat dilated ca. 5.0 × as long as its greatest width. Midleg: coxa dark brown; trochanter and femur yellow; tibia brown with basal 1/4 yellow; tarsus brown. Hind leg: coxa dark yellow; trochanter yellow; femur yellow, somewhat darkened toward distal

end, with distal cap brown; tibia yellow on basal 1/2, brown to brownish black on distal 1/2; tarsus brown to brownish black except basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere yellow; basitarsus slender, parallel-sided, ca. 5.6 × as long as wide. Hind femur and tibia subequal in greatest width, ca. 1.8 × as wide as hind basitarsus. Calcipala well developed, ca. 1.2 × as long as wide. Pedisulcus well developed. All femora and tibiae densely covered with scale-like hairs on outer surface. *Wing*. Length 1.5 mm. Other features as in ♀ except subcosta bare. *Abdomen*. Basal scale brown, with fringe of brown hairs. Dorsal surface of abdominal segments brownish black, covered with dark hairs; segments 2, 5-7 each with a dorsolateral pair of shiny, whitish pruinose patches; those on segment 2 widely connected in middle. *Genitalia*. Coxite (Fig. 56) nearly rectangular in ventral view, much longer than wide. Style (Fig. 56) shorter than coxite, tapered toward apical tip, gently curved inward, with apical spine. Ventral plate (Figs. 57-59) transverse, ca. 0.4 × as long as wide, with both sides much angulated, widely produced ventrally along posterior margin, with microsetae on ventral, posterior and dorsal surface; basal arms converging. Paramere with 3 long hooks and several small ones (nearly as in *S.* (*M.*) *noroense* sp. nov., Fig. 76). Median sclerite (Fig. 60) weakly sclerotized, plate-like, slightly widened toward tip, with round tip.

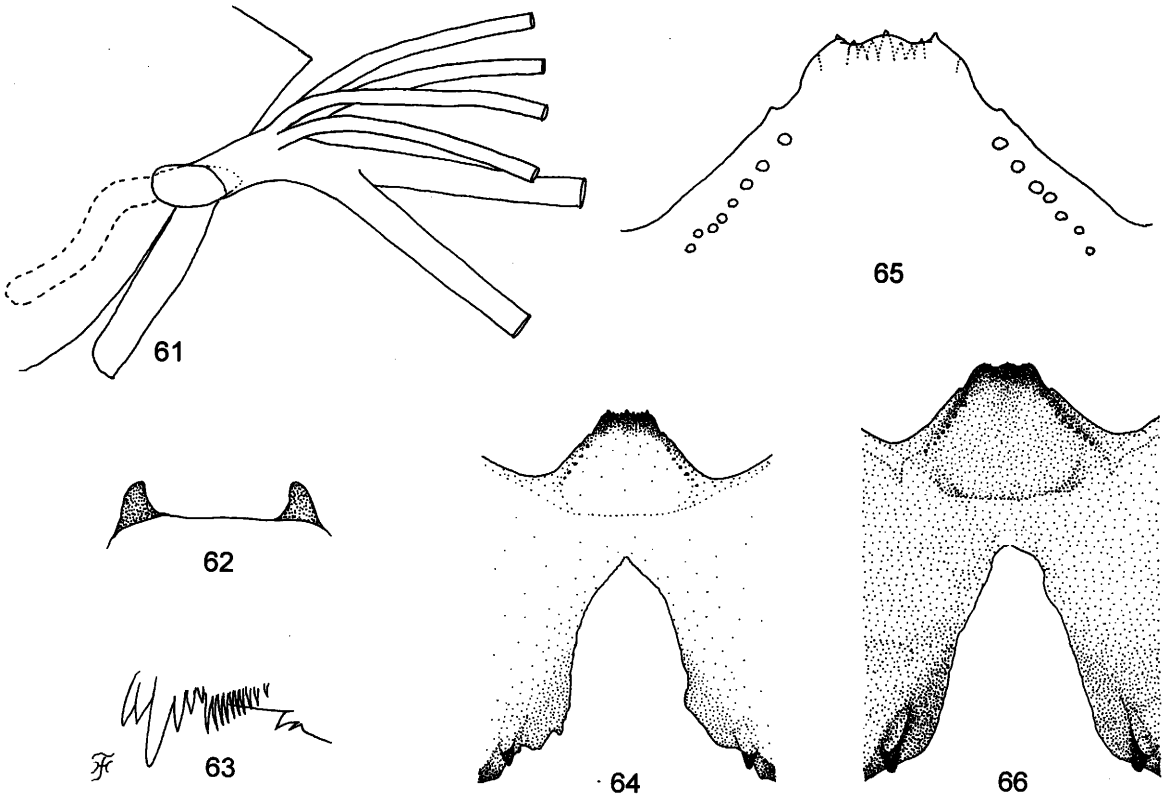
Pupa. Body length (excluding gill filaments) ca. 2.5 mm. *Head*. Integument yellowish brown, moderately covered with round tubercles; a pair of facial trichomes and 2



Figs. 48-60. Adult characters of *Simulium* (*Morops*) *sherwoodi*. 48, frons; 49, fronto-ocular area; 50, 3rd maxillary palpal segment; 51, cibarium; 52, calcipala and pedisulcus; 53, claw with a minute tooth; 54, paraproct and cercus (side view); 55, spermatheca; 56, coxite and style (ventral view); 57-59, ventral plate (57, ventral view; 58, end view; 59, side view); 60, median sclerite. 48-55, ♀; 56-60, ♂.

pairs of frontal trichomes all long and bifid. Antennal sheath with smooth surface. *Thorax*. Integument yellowish brown, bare except anterior surface moderately covered with round tubercles and posterodorsal surface moderately covered with small, cone-shaped tubercles; 5 anterodorsal pairs of trichomes all long and bifid, 1 posterolateral pair long and simple. Pit-like organ absent. Gill (Fig. 61) composed of paired ventral filaments and 4 much slender dorsal filaments arising from basal stalk of paired filaments; paired filaments subequal in length (2.5-3.0 mm) and thickness, lying horizontally, parallel-sided or somewhat diverging, directing forwards and tapered toward apical tip; surface of paired filaments with many transverse ridges (except apical portion) forming reticulate patterns, covered with somewhat larger tubercles on ridges and with smaller ones on interspaces; 4 slender filaments arranged in pairs with variable lengths of stalks (usually sessile or very short-stalked, but in one pupa secondary stalks very long, ca. 1 mm long), subequal in length (1.5-1.8 mm) and thickness, with weak annular ridges and furrows on their surface of basal 1/3, and covered with minute tubercles. *Abdomen*. Terga 1 and 2 pale yellowish brown, without tubercles; tergum 1 with 1 long seta on each side, tergum 2 with 5 simple spinous setae and 1 much longer seta on each side. Terga 3 and 4 each with 4 hooked spines directed forward on each side. Terga 6-9 each with a transverse row of spine-combs and comb-like groups of minute spines directed caudad on each side. Tergum 9 with a pair of spatulate, terminal hooks (Fig. 62). Sternum 4 with 1 bifid hook and 1 simple hook (or spinous seta in some pupae) and 2 slender simple setae on each side; sternum 5 with a pair of bifid or trifid hooks on each side; sterna 6 and 7 each with a pair of inner bifid or trifid and outer simple or bifid hooks on each side; last segment without grapnel-like hooklets ventrolaterally on each side. *Cocoon*. Wall-pocket-shaped, moderately woven, widely extending ventrolaterally; anterior margin thickly woven; individual threads visible; interspaces thinly walled, without perforations.

Mature larva. Body length 3.6-4.2 mm. Body usually greyish, coloring on thorax and abdominal segment 3 weak and much paler than others. Cephalic apotome



Figs. 61-66. Pupal and larval characters of *Simulium* (*Morops*) *sherwoodi* and *S.* (*M.*) *kerei*. 61 & 62, pupa; 63-66, larva. 61, basal portion of gill filaments (side view); 62, terminal hooks (end view); 63, apex of mandible; 64 & 66, head capsule (ventral view) showing hypostomium and postgenal cleft; 65, hypostomium. 61-64, *S.* (*M.*) *sherwoodi*; 65 & 66, *S.* (*M.*) *kerei*.

pale yellow with faint, positive head spots. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.0:0.7:1.0. Labral fan with ca. 36 main rays. Mandible (Fig. 63) with comb-teeth decreasing in size from 1st to 3rd; mandibular serration composed of 2 teeth (1 large and 1 small), without supernumerary serrations. Hypostomium (Fig. 64) with a row of 9 apical teeth, of which median tooth subequal to or slightly longer than each corner tooth; 6 intermediate teeth subequal in size to each other, and much smaller than corner teeth; lateral margin not serrate; hypostomal bristles 6 in number per side lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 64) nearly triangular, gradually narrowed anteriorly, ca. 5.0 × as long as postgenal bridge (in some larvae anterior tip with a narrow extension approaching to posterior margin of hypostomium). Thoracic cuticle bare. Abdominal cuticle bare except each side of anal sclerite of last segment moderately covered with numerous colorless setae. Rectal papilla compound, each of 3 lobes with 1 or 2 finger-like, secondary lobules on posterior surface. Anal sclerite of usual X-form, with anterior arms ca. 0.8 × as long as posterior ones, broadly sclerotized at base. Ventral papillae present. Posterior circlet with 82 rows of up to 14 hooklets per row.

SPECIMENS EXAMINED. 9 ♀, 11 ♂, all reared from pupa, 52 pupae, 4 pupal exuviae and 29 mature larvae, all in alcohol, collected from a stream, Selwyn College, Guadalcanal Island, 12. VIII. 1993, by H. Suzuki; 4 pupae, 3 pupal exuviae and 5 mature larvae, all in alcohol, collected from Poha River, Guadalcanal Island, 15. VIII. 1993, by H. Suzuki; 2 ♂, both reared from pupa, 12 pupae, 1 pupal exuvia and 21 mature larvae, all in alcohol, collected from Tenaru River, Guadalcanal Island, 10. VIII. 1992, by H. Suzuki; 2 ♀, 5 ♂, all reared from pupa, 20 pupae, 2 pupal exuviae and 21 mature larvae, all in alcohol, collected from Tenaru River, Guadalcanal Island, 16. VIII. 1992, by H. Suzuki; 1 pupa and 18 mature larvae, all in alcohol, collected from Gold Ridge River, Guadalcanal Island, 30. VIII. 1992, by H. Suzuki; 5 ♀, 4 ♂, all reared from pupa, 41 pupae, 6 pupal exuviae and 8 mature larvae, all in alcohol, collected from Tanatita River, Guadalcanal Island, 16. VIII. 1992, by H. Suzuki.

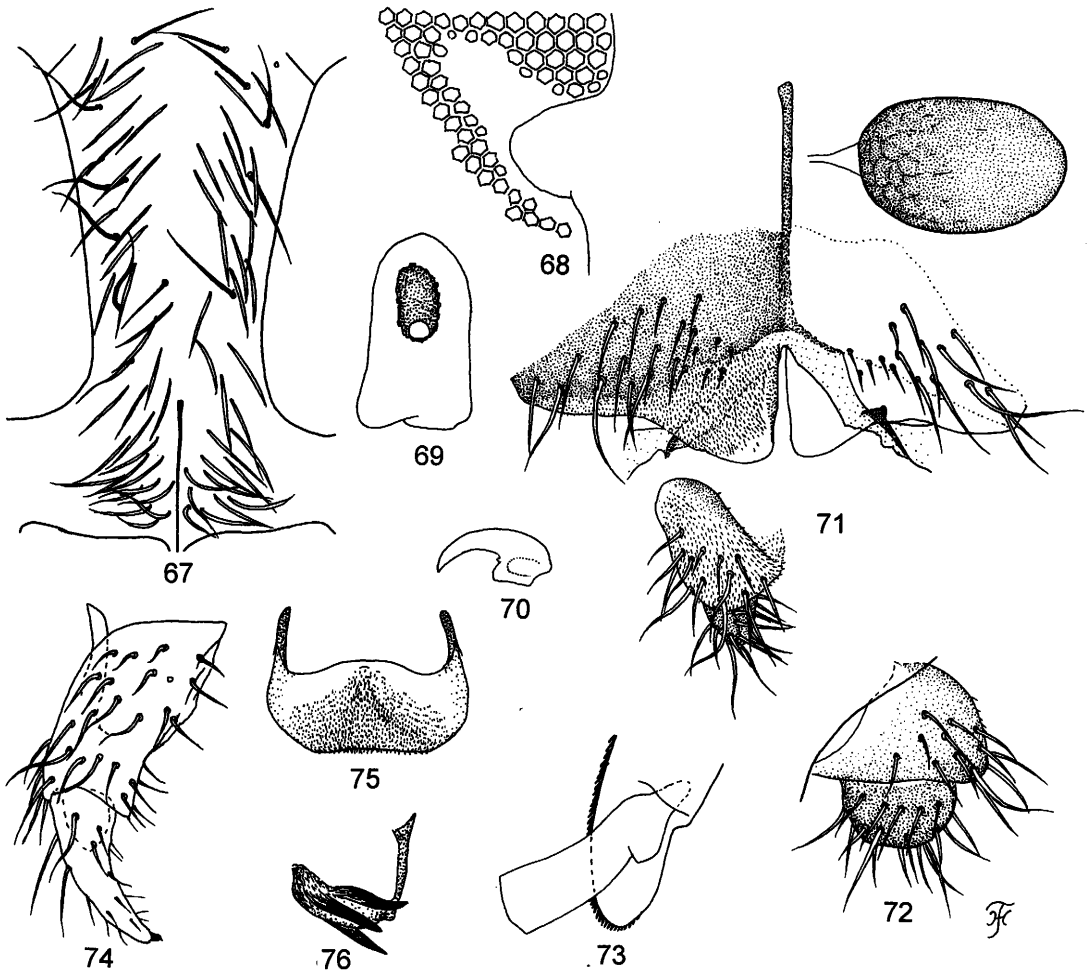
DISTRIBUTION. Solomon Islands (Guadalcanal Island).

REMARKS. This species is characterized in the male by the presence of the parameral hooks and in the pupa by the simple, wall-pocket-shaped cocoon and the six slender gill filaments, of which dorsal four filaments are much slender and shorter than the ventral paired filaments, as described by Stone and Maffi (1971). It is noticeable that the female genitalia are very similar to that of *S.* (*G.*) *hiroshii,* described from the Solomon Islands (Takaoka, 1994), especially in the genital fork with a projection directed anteriorly on each arm, and the spermatheca with internal setae. Takaoka (1983) noted that eight *Morops* species found in the Philippines have more similarities to *S.* (*M.*) *sherwoodi* than all the other *Morops* species reported from Papua New Guinea and Australia in having the parameral hooks in the male genitalia, and 6-filamented gill in the pupa. It is now clear that *S.* (*M.*) *sherwoodi* and all the Philippine *Morops* species are very similar in the male and mature larva, but the former species is readily separated from the latters in the female by the almost bare frons, tarsal claws with a minute tooth, and the spermatheca with internal setae, and in the pupa by the frontal integument with two pairs of trichomes, the absence of grapnel-like hooklets and plate-like, terminal hooks.

8. *Simulium* (*Morops*) *noroense* sp. nov.

DESCRIPTION. **Female**. Body length ca. 2.2 mm. *Head.* Slightly narrower than thorax. Frons (Fig. 67) dark brown, whitish grey pruinose, shiny in certain angles of light, moderately covered with yellowish white, scale-like, recumbent pubescence, interspersed with a few dark hairs along both lateral margins; frontal ratio 1.7:1.0:2.6. Frons-head ratio 1.0:5.3. Fronto-ocular area (Fig. 68) well developed. Clypeus dark brown, densely covered with yellowish white, scale-like, recumbent pubescence, interspersed with several dark hairs. Antenna composed of 2+9 segments, brownish black except scape, pedicel and base of 1st flagellar segment yellowish. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.3:2.7; 3rd segment somewhat enlarged, sensory vesicle (Fig. 69) elongate, 1.9 × as long as wide, 0.4 × as long as 3rd segment, with a large opening near distal end. Maxillary lacinia with 9 inner and 11 or 12 outer teeth. Mandible with ca. 21 small inner teeth and lacking outer ones. Cibarium smooth. *Thorax.* Scutum blackish brown in ground color, whitish grey pruinose, densely covered with whitish yellow, recumbent pubescence except median large area with dark brown pubescence; 3 longitudinal vittae of whitish yellow, recumbent pubes-

cence (1 median vitta very narrow and 2 submedian ones rather wide), which are connected anteriorly and posteriorly to areas of the concolored pubescence. Scutellum blackish brown with brown pubescence and long, erect, dark hairs along posterior margin. Postscutellum blackish brown, whitish grey pruinose (shining in light), bare. Pleural membrane with ca. 25 yellowish white pubescence. Katepisternum longer than deep, dark brown, whitish grey pruinose (shining in light), with numerous dark hairs; sulcus distinct. *Legs*. Foreleg: coxa and trochanter yellow; femur brown; tibia white with distal 1/4 brownish black, outer surface widely white shining when illuminated; tarsus black; basitarsus dilated, ca. 4.6 × as long as its greatest width. Midleg: coxa dark brown; trochanter yellow; femur brown with apical cap dark brown; tibia white on basal 1/3, brownish black on rest, though its border not well defined; posterior surface of tibia widely white shining when illuminated; tarsus black. Hind leg: coxa yellowish brown; trochanter yellowish white; femur brown with apical cap dark brown; tibia white on basal 2/3, brown to brownish black on rest, though its border not well defined; posterior surface of tibia widely white shining when illuminated; tarsus brown with basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, parallel-sided, ca. 6.6 × as long as wide. Calcipala well developed, ca. 1.3 × as long as wide. Pedisulcus well developed. Hind femur and tibia 2.4 × and 1.9 × as wide as hind basitarsus, respectively. All femora and tibiae densely covered with scale-like hairs on outer surface.



Figs. 67-76. Adult characters of *Simulium* (*Morops*) *noroense* sp. nov. 67, frons; 68, fronto-ocular area; 69, 3rd maxillary palpal segment; 70, claw with a minute tooth; 71, genitalia in situ (ventral view); 72, paraproct and cercus (side view); 73, calcipala and pedisulcus; 74, coxite and style (ventral view); 75, ventral plate (ventral view); 76, paramere (ventral view). 67-72, ♀; 73-76, ♂.

Claws (Fig. 70) each with a rudimentary, minute basal tooth. *Wing*. Length 1.5 mm. Costa with spinules as well as hairs. Subcosta haired. Hair tuft on stem vein dark brown. Basal portion of radius fully haired. *Abdomen*. Basal scale brown with fringe of yellowish white hairs. Dorsal surface of abdomen black, with dark hairs; tergite of 2nd segment whitish pruinose, tergites of segments 5-8 shiny. *Genitalia* (Figs. 71 & 72). Sternal plate undeveloped on abdominal segment 7. Sternite 8 bare medially, with 15-18 dark macrosetae on each side. Anterior gonapophysis triangular, thin, membraneous, covered densely with microsetae interspersed with a few setae; inner margin well sclerotized. Genital fork of usual inverted-Y form, with arms rather broad, folded medially, each with a distinct projection directed forward. Paraproct of usual form. Cercus 0.56 × as long as wide, with posterior border gently rounded when viewed laterally. Spermatheca ellipsoidal, well sclerotized except tube and tubal base unsclerotized; internal setae present.

Male. Body length ca. 2.5 mm. *Head*. Wider than thorax. Upper eye consisting of 9 or 10 vertical columns and 11 horizontal rows of large facets. Clypeus brownish black, whitish pruinose, covered densely with yellow pubescence, interspersed with several dark hairs. Antenna composed of 2+9 segments, yellow with 4 or 5 apical segments brown; 1st flagellomere somewhat elongate, ca. 1.5 × as long as 2nd one. Maxillary palp with 5 segments; proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:3.0; sensory vesicle oblong, 0.27 × as long as 3rd segment, with opening somewhat distally (or near distal end). *Thorax*. As in ♀ except pleural membrane with ca. 20 yellow pubescences. *Legs*. Foreleg: coxa and trochanter whitish; femur brown; tibia yellow (though appearing brown due to dense covering of dark pubescence) with distal 1/3 dark brown, silvery shining in light largely on outer surface; tarsus brownish black; basitarsus somewhat dilated, ca. 5.5 × as long as its greatest width. Midleg: dark brown to brownish black except trochanter and base of tibia yellow. Hind leg: coxa brown; trochanter yellow; femur dark brown; tibia white on basal 1/2, brownish black on distal 1/2; tarsus brownish black except basal 3/5 of basitarsus and basal 1/2 of 2nd tarsomere white; basitarsus slender, almost parallel-sided, ca. 5.3 × as long as wide. Hind femur and tibia ca. 1.8 × and 1.6 × width of hind basitarsus, respectively. Calcipala (Fig. 73) well developed, 1.45 × as long as wide. Pedisulcus (Fig. 73) well developed. All femora and tibiae densely covered with scale-like hairs on outer surface. *Wing*. Length 1.5 mm. Other features as in ♀ except subcosta bare. *Abdomen*. Basal scale yellow, with fringe of yellow hairs. Dorsal surface of abdominal segments brownish black, covered with dark hairs; segments 2, 5-7 each with a dorsolateral pair of shiny, whitish pruinose patches; those on segment 2 widely connected in middle. *Genitalia*. Nearly as in *S.* (*M.*) *sherwoodi* except ventral plate. Ventral plate (Fig. 75) transverse, 0.45 × as long as wide, not markedly angulate on both lateral margins.

Pupa. As in *S.* (*M.*) *sherwoodi* except following features: *Head*. Integument yellow. *Thorax*. Integument yellow, moderately covered with round tubercles except posterodorsal surface with small, cone-shaped ones. *Abdomen*. Terga 1 and 2 almost transparent. Tergum 6 with no or a few spine-combs.

Mature larva. As in *S.* (*M.*) *sherwoodi* except following features: Body length 3.8-4.0 mm. Body uniformly greyish. Labral fan with ca. 34 main rays. Rectal papilla compound, each of 3 lobes with 2 or 3 finger-like, secondary lobules on posterior surface.

TYPE SPECIMENS. Holotype ♀, reared from pupa, slide-mounted together with pupal exuvia and cocoon, collected from a stream, Noro, New Georgia Island, Western Province, Solomon Islands, 5. IX. 1992, by H. Suzuki. Allotype ♂, reared from pupa, slide-mounted, same data as holotype. Paratypes: 1 ♀, 3 ♂, all reared from pupa, 2 pupae and 3 mature larvae, all in alcohol, same data as holotype; 2 ♀, 2 ♂, all reared from pupa, 15 pupae, 1 pupal exuvia and 25 mature larvae, in alcohol, same data as holotype except date: 21. II. 1993.

DISTRIBUTION. Solomon Islands (New Georgia Island).

REMARKS. This species is very similar to *S.* (*M.*) *sherwoodi* in many features including the genitalia of both sexes and the pupal gill filaments. However, *S.* (*M.*) *noroense* differs from the latter species by the dark coloration of the femora of both adult sexes, female frons furnished with scale-like pubescence (Fig. 67), male ventral plate not markedly angulate laterally (Fig. 75), and pupal thorax moderately covered with tubercles. The tarsal claw of the female *S.* (*M.*) *noroense* has a minute basal tooth (Fig. 70), which is much smaller than that of *S.* (*M.*) *sherwoodi* (Fig. 53).

9. *Simulium* (*Morops*) *kerei* Takaoka and Suzuki, 1994

Simulium (*Morops*) *kerei* Takaoka and Suzuki, 1994: 207-210.

This species was recently described based on adult male and pupal specimens collected from New Georgia Island, Solomon Islands (Takaoka and Suzuki, 1994). The mature larva is here described.

DESCRIPTION. **Mature larva**. Body length ca. 5.0 mm. Body greyish black. Cephalic apotome pale yellow with distinct, positive head spots. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.00: 0.96: 0.81. Labral fan with ca. 45 main rays. Mandible as in *S.* (*M.*) *sherwoodi*. Hypostomium (Fig. 65) with a row of 9 apical teeth, of which median tooth is subequal in size to each corner tooth; 6 intermediate teeth different in length and much shorter than corner teeth; lateral margin not serrate; hypostomal bristles 7 or 8 in number per side lying subparallel to lateral margin. Postgenal cleft (Fig. 66) nearly triangular, gradually narrowed anteriorly, ca. 4.0 × as long as postgenal bridge. Thoracic cuticle bare. Abdominal cuticle bare except each side of anal sclerite of last segment moderately covered with numerous colorless setae. Rectal papilla compound, each of 3 lobes with 2 or 3 finger-like, secondary lobules on posterior surface. Anal sclerite of usual X-form, with anterior arms ca. 0.9 × as long as posterior ones, broadly sclerotized at base. Ventral papillae present. Posterior circlet with ca. 100 rows of up to 18 hooklets per row.

SPECIMENS EXAMINED. 5 pupal exuviae and 2 mature larvae, Noro, New Georgia Island, Western Province, Solomon Islands, 21. II. 1993, by H. Suzuki.

DISTRIBUTION. Solomon Islands (New Georgia Island).

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We are grateful to Dr. A. Shelley, Ms C. Lowry and Miss T. Howard, Department of Entomology, Natural History Museum, London, U. K. for loaning the type specimens of several Papua New Guinean and Australian species of the *clathrinum*-group. HS thanks Dr. N. Kere, Ministry of Health and Medical Services, Solomon Islands, for his assistance during the collection trips. This work was in part supported by the Grant-In-Aid from the Ministry of Education, Science and Culture of Japan for International Scientific Research programme (No. 03041080; representative, Prof. M. Sasa).

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