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APPLICATION OF SEROEPIDEMIOLOGY IN THE EVALUATION OF A COMMUNITY-BASED MALARIA CONTROL PROGRAM IN PALAWAN, THE PHILIPPINES

PILARITA TONGOL-RIVERA[1], SHIGEYUKI KANO[2,3], ELENA VILLACORTE[1], ALLDRIN DARILAG[1], EDITHA MIGUEL[4] AND MAMORU SUZUKI[2]

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Abstract: Seroepidemiology has several proven applications in malaria endemic areas. In this study, it was used to assess the effectiveness of a community-based malaria control program in the focus of malaria transmission. The first serological survey was done before the implementation of a community-based malaria control program (pre-intervention), and the second one was done after 5 years of intervention in the study area. Comparison of the distribution of the indirect fluorescent antibody (IFA) titers showed a reduction in the high titer-responses after the intervention. Moreover, there was a statistically significant reduction in the geometric mean reciprocal titer (GMRT) after the intervention. These findings were suggestive of a reduction in malaria transmission resulting from the intervention. Results of a parallel parasitologic study revealed the same findings. Therefore, seroepidemiology, when used to complement the parasitologic measurement, is valuable in monitoring the effectiveness of malaria control measures.

Key words: malaria, seroepidemiology, community-based malaria control

INTRODUCTION

In many endemic communities, malaria control has been reoriented to better and more efficient use of resources. Emphasis has been changed from the highly prescriptive, centralized control programs, to those programs adapted to local conditions and responding to local needs of the people (WHO, 1992). The community-based approach to malaria control has been adopted in the Philippines, especially since the provision of health services has been devolved to the local governments (Malaria Control Service, 1996). This type of approach encourages community participation, people empowerment and self-reliance. Sustainability of malaria control measures is thus assured on a long term basis. It is this type of malaria control which was implemented in the focus of malaria transmission in the study area.

In view of the wide applications of seroepidemiology (WHO, 1972; Tharavanij *et al.*, 1986; Ray *et al.*, 1988; Ettling *et al.*, 1989; Kano *et al.*, 1993), this present study attempts to use it to evaluate the effectiveness of a five-years community-based malaria control program in the study community. The findings from this study may be of value in monitoring the present local malaria control program and those of other endemic countries as well.

SUBJECTS, MATERIALS AND METHODS

Study area

This study is limited to the forest fringe part of the same study community (Tongol-Rivera *et al.*, 1993), which was the focus of malaria transmission. An already organized group of volunteer health workers (BHWs) was mobilized for the promotion and implementation of ongoing malaria control interventions in this study community. The target population consisted of 65 families with 344 members. During the five years study period, the BHWs were trained and re-trained. Their activities included the promotion of residual insecticide spraying to cover as many households as possible, active and passive case finding by making blood smears

1 Department of Parasitology, College of Public Health, University of the Philippines, Manila
2 Department of Parasitology, Gunma University School of Medicine, Gunma, Japan
3 Research Institute, International Medical Center of Japan, Tokyo, Japan
4 Palawan Provincial Hospital, Palawan, the Philippines

and bringing them to the microscopist (both government and non-government organizations) for parasitologic diagnosis, administration of Chloroquine and Primaquine tablets (Chloroquine Phosphate at 10 mg base/kg on day 1 and day 2, and 5 mg base/kg on day 3 for *Plasmodium falciparum*; Chloroquine at this dose plus Primaquine at 15 mg base/kg for 14 days for *P. vivax*) to patients with positive blood smears; and distribution and monitoring of the use of Permethrin pre-impregnated mosquito nets (Japan). They were also responsible for dissemination of information about malaria prevention and control and they actively participated in the parasitologic and serologic surveys.

The BHWs did passive and active case detection in the study area during implementation of the project (1992-1996). For suspected malaria cases they made the smears themselves and brought to the microscopists for diagnosis.

Serologic and Parasitologic Surveys

The results of the serologic and parasitologic surveys which were done at the end of the rainy season, in December, 1991 were considered as the pre-intervention data. At that time, a total of 118 blood samples were collected by fingerprick in infants and venipuncture in the older age group (Tongol-Rivera *et al.*, 1993). In February and March, 1997, after five years of community-based malaria control, repeat surveys were done on the same study site. These surveys were scheduled at the end of the rainy season which was unusually delayed. The pre and post-intervention surveys were both done after the rainy season, when malaria transmission was expected to be high. A total of 218 blood specimens were collected by fingerprick (Kano *et al.*, 1989) using blood sampling paper (Nobuto's). The subjects came from the following age groups: 0-9 years of age, 49.5%; 10-20 years of age, 8.3%; and >20 years of age, 42.2%. They were composed of 67% females and 33% males.

Parallel parasitologic surveys were done at the same time as the serologic surveys. Thick and thin blood smears were processed. There were 118 smears during the pre-intervention and 219 (this included 1 subject who did not have plasma specimen) during the post-intervention.

For the pre-intervention serologic survey, the indirect fluorescent antibody test (IFAT) was used with four-fold dilutions of serum ranging from 1:4 to 1:4,096 and reacted to both *P. falciparum* and *P. vivax* antigens (Tongol-Rivera *et al.*, 1993). In the post-intervention survey, the IFAT was done with plasma diluted ten-fold with phosphate-buffered saline (PBS). Fluorescein isothiocyanate-conjugated anti-human IgG (Dako, Japan) was used as antibody and fluorescence was read with an incident light illuminating type fluorescent microscope (model BH-RFC; Olympus, Tokyo, Japan). The highest serum dilution giving a positive reading was the IFAT titer.

Statistical analysis

The Mann-Whitney U test was used to determine the differences in the geometric mean reciprocal titer (GMRT) between the pre and post-intervention serologic surveys because the antibody titers were not normally distributed.

RESULTS

Interviews with the BHWs and the subjects which were done simultaneously with the surveys, revealed that 73.2% have been using the pre-impregnated mosquito nets distributed to them. Those who resided in the study area after the net distribution (21.5%) have been using ordinary mosquito nets. The children, 0 to 10 years old have been the priority group to use the net while sleeping. It was also found out that 64.2% of the subjects' houses have been sprayed with residual insecticides at least once a year. The general observation of the BHWs and the subjects was that the frequency of malaria in their community has been reduced significantly, considering the lesser number of positive blood smears and malaria sick residents, even during the rainy season. Table 1 shows the yearly number of malaria cases which were confirmed by peripheral smear and detected through passive and active case detection by BHWs. Although a trend of increasing number of malaria cases is observed, the actual counts are still lower compared to the 99 cases detected by serology (IFAT) during the pre-intervention survey. Titers of 1:16-1:4,096 are considered positive (Table 2). On closer examination, it is shown that there are 44 malaria cases detected by IFAT in the post-intervention survey, as compared to 39 cases detected by the BHWs through

Table 1 Malaria cases detected by passive and active case detection by BHWs and confirmed by peripheral smear

	1992	1993	1994	1995	1996
P. falciparum	5	3	8	12	26
P. vivax	0	3	1	6	10
Mixed	0	1	1	0	3
Total	5	7	10	18	39

Table 2 Distribution of indirect fluorescent antibody test (IFAT) titers and parasitemia before and after community-based malaria control

Age (in years)	IFAT Titers				Parasitemia		
	Before Community-based Malaria Control		After Community-based Malaria Control			Before Community-based Malaria Control	After Community-based Malaria Control
0-9	1:256-1:4,096	18	1:400-1:1,000	3	*P.f.*	8	0
	1:16-1:64	6	1:40-1:100	7	*P.v.*	0	1
	<1:4	10	1:10	10	Mixed	1	0
			<1:10	88	Negative	25	108
10-20	1:256-1:4,096	24	1:400-1:1,000	0	*P.f.*	7	0
	1:16-1:64	9	1:40-1:100	4	*P.v.*	0	0
	<1:4	4	1:10	2	Mixed	0	0
			<1:10	12	Negative	30	18
>20	1:256-1:4,096	38	1:400-1:1,000	8	*P.f.*	9	0
	1:16-1:64	4	1:40-1:100	22	*P.v.*	0	0
	<1:4	5	1:10	28	Mixed	0	0
			<1:10	34	Negative	38	92
Total		118		218		118	219

*Values are the number of individuals. *P.f.*=*Plasmodium falciparum*; *P.v.*=*Plasmodium vivax*.

active and passive cases detection in 1996, which is the year preceding this survey. These data show that by measuring period prevalence, serology is able to measure the actual estimate of malaria cases. On the other hand, detection of parasitemia in malaria smears, measures point prevalence and misses low parasitemias, past and treated infections.

Figure 1 shows the frequency of antibody titers when graphically plotted. The distribution profiles of both *P. falciparum* and *P. vivax* species followed similar patterns. Figure 1 also shows the comparison of the antibody titer distribution profiles of the pre and post-intervention serologic surveys in the study community. In the pre-intervention survey, a high percentage of subjects showed the highest titer (1:4,096) for both *P. falciparum* and *P. vivax* antigens. A second peak at a lower titer of 1:16 was also noted. In contrast, the post-intervention survey showed a high percentage of negative titers for both *P. falciparum* and *P. vivax* antigens.

Table 2 shows a comparison of the specific titers among the different age groups, and between the pre and post-intervention surveys. In the pre-intervention survey, 68% (n=118) of the subjects had high titers (1:256-1:4,096), 16% had low titers (1:16-1:64) and only 16% had negative titers (1:<4). This is in contrast to the post-intervention results which showed 80% with negative titers (1:<10), 15% with low titers (1:40-1:100) and only 5% with high titers (1:400-1:1,000). Moreover, the difference in the GMRTs of the two surveys was statistically significant ($p<0.01$).

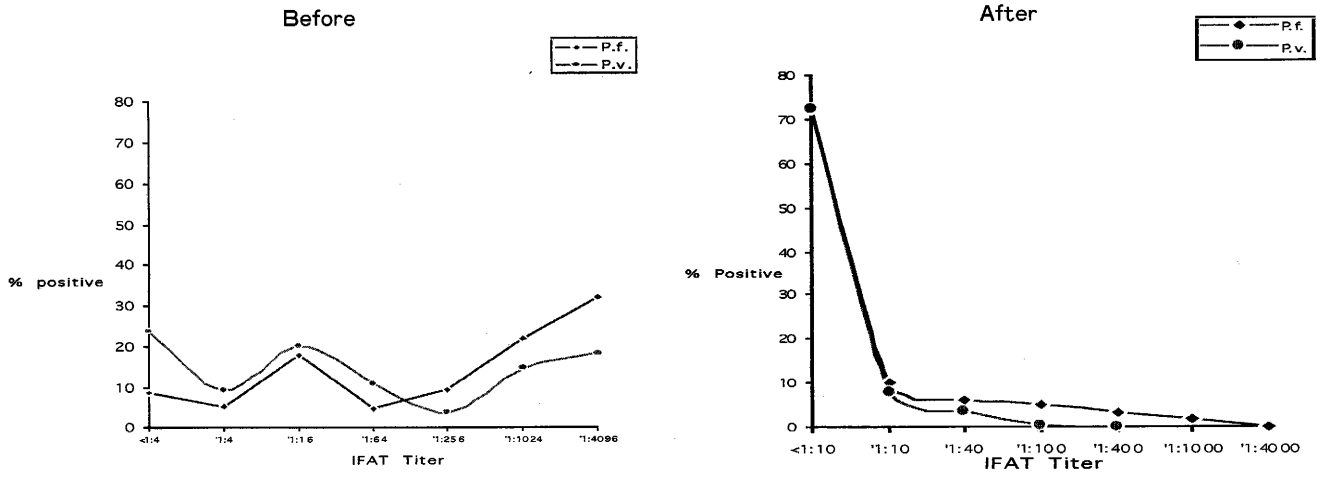

Figure 1 Distribution of the indirect fluorescent antibody test (IFAT) titers before and after community-based malaria control.

The parallel parasitologic study showed that 25 out of 118 subjects had parasitemia during the pre-intervention survey. Of these, 24 had *P. falciparum* while one had mixed infection (Table 2). By the IFAT, there were 99 out of 118 whose titers were 1:16-1:4,096 and were considered to be malaria cases (Table 2). On the other hand, in the post-intervention survey, only one out of 219 was positive and this was *P. vivax*. By the IFAT, more cases were detected, 44 out of 218 whose titers were 1:40-1:1,000. These results show a reduction of malaria cases during post-intervention which may suggest a reduction of the prevalence and transmission of malaria in the study community.

DISCUSSION

The degree of transmission or prevalence of malaria is usually measured by either GMRT or the percentage of the sample population which is serologically positive (Kagan, 1973). In the present study, both of these measurements consistently showed a reduction of malaria transmission and prevalence, which may have been attributed to the implementation of community-based malaria control in this study community. It was shown that there was a statistically significant difference between the GMRTs of the pre-intervention and post-intervention surveys, and which is suggestive of the effectiveness of malaria control. Furthermore, Table 2 shows a high percentage of subjects with high antibody titers to both *P. falciparum* and *P. vivax*, and lower percentages of subjects with low and negative antibody titers before the implementation of community-based malaria control. The opposite was shown after the implementation, when there was a high percentage of negative and low titers, and very low percentage of high titers. Table 1 shows the number of malaria cases per year of the intervention period as determined by passive and active case detection and confirmed by peripheral smear. Comparison with the results of the post-intervention parasitologic survey in Table 2, shows that cases were easily missed by a one-point parasitologic survey. There were more cases detected in the years during the intervention because case detection was done throughout the year. The highest number of 39 cases were detected the previous year in 1996. The post-intervention parasitologic survey in 1997 detected only one case. However, serology even if it was done as a one-point survey detected 44 cases in the post-intervention survey in 1997. Comparison with the results of the pre-intervention serologic survey shows a 44% reduction of cases-44 showed IFAT titers to both *P. falciparum* and *P. vivax* in the post-intervention survey against 99 in the pre-intervention survey. By measuring period prevalence, serology detected cases which occurred in the previous year. The above data may indicate a significant reduction in malaria prevalence and transmission, although complete interruption of transmission was not attained.

Comparison of the antibody distribution profiles (Fig. 1) of the two surveys also shows the same trend, whereas high antibody titers predominate before the implementation of community-based malaria control, low and even negative titers predominate after the implementation of control. Both pre and post-intervention antibody profiles show IFAT titers to both *P. falciparum* and *P. vivax*. However, the post-intervention profile shows a more significant decrease in *P. vivax* and *P. falciparum*. The reason is that *P. falciparum* being dominant during the rainy season causes more recent past infection which are detected by serology in the survey after the rainy season. However, *P. vivax* increases in frequency during the dry season, so that a lesser percentage of *P. vivax* was detected by the same serologic survey done after the rainy season. The paralle parasitologic study (Table 2) shows results which are consistent with the serologic study. More parasitemic subjects, predominantly *P. falciparum* were detected before the intervention, while only one *P. vivax* was detected after the intervention. These data further strengthen the conclusion that malaria prevalence and transmission are significantly reduced in the study community.

Seroepidemiology, as shown in the present study is useful in the assessment of the effectiveness of malaria control programs. Although, the significant reduction in malaria prevalence and transmission in the study community may be attributed to the malaria control implemented, there may be other factors which may have contributed to this improvement in malaria situation. These factors which cannot be controlled in the study include the natural reduction of malaria transmission which may be due to instability of malaria, environmental changes and socio-economic development of the study community.

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INVOLVEMENT OF COMMUNITY HEALTH WORKERS IN TUBERCULOSIS CONTROL IN BANGLADESH

MOHAMMAD AKRAMUL ISLAM[1], YASUHIDE NAKAMURA[1], SOM-ARCH WONGKHOMTHONG[1], SADIA A. CHOWDHURY[2] AND NOBUKATSU ISHIKAWA[3]

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Abstract: Tuberculosis is a major public health problem in Bangladesh. It is estimated that about 52,000 deaths due to tuberculosis and 300,000 new tuberculosis cases occurred in 1997 in Bangladesh. Bangladesh Rural Advancement Committee (BRAC), a Bangladeshi non government organization is implementing a community based program for tuberculosis since 1984 in collaboration with the national tuberculosis program. Community health workers are the nucleus of this initiative. All of them are female and selected from rural community. They identify suspected persons for sputum test and provide treatment to the patients in their own community. In the middle of 1998 this program was reviewed, and the achievements in 1996 and 1997 were analyzed. Treatment outcomes were evaluated through cohort analysis according to WHO/International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines. Outcome indicators defined by WHO/IUATLD were used. A total of 7,946 patients were detected in 34 thanas in 1996 and 1997. Out of them, 6,163 (77.6%) were new sputum positive patients. Their sputum conversion and cure rates were about 90% and 86.7% respectively. This program has achieved the WHO target of 85% cure rate. Community health workers are playing a key role to control tuberculosis in this approach. Thus this model could reduce burden on health facilities, reduce patient's costs and increase case detection and cure rate.

Key Words: Community Health Workers, Tuberculosis Control, Directly Observed Treatment, Bangladesh, Bangladesh Rural Advancement Committee (BRAC)

INTRODUCTION

Tuberculosis is one of leading causes of adult deaths in the world. According to World Health Organization (WHO), more than 3 million people die of tuberculosis in the world every year. It is also estimated that approximately 30 million people will die from tuberculosis in the next ten years if the disease continues to spread at the current rate (WHO, 1996). One thirds of the world's population are already infected with tuberculosis bacillus. Twenty million people are currently suffering from tuberculosis, and 8 million people get tuberculosis disease every year. More than 50 million people may have been infected with drug resistant strains of tuberculosis (WHO, 1995). Tuberculosis is the only disease that the WHO has ever classified as a global emergency declared in 1993 (WHO, 1994).

Tuberculosis has been a major public health threat in Bangladesh. According to the recent review by the government of Bangladesh and WHO, about 52,000 deaths and 300,000 new cases were estimated in 1997 (Government of Bangladesh, 1997). There were only 13 hospitals totally with 1,076 beds and 44 clinics available for tuberculosis services in Bangladesh until 1980s (Chowdhury *et al.*, 1991). The national tuberculosis program had been integrated with general health services basically at thana health complexes as a policy in early 1980s. Thana health complex is a primary health care center of thana (sub-district) covering about 250,000 population. However in 1985, only 124 among 460 thana health complexes provided services for tuberculosis. Only 560 sputum positive patients were identified in those thana health complexes in 1987 (Government of Bangladesh, 1988). Moreover their

1 Department of Community Health, The University of Tokyo, Japan
2 Health and Population Division, BRAC, Dhaka, Bangladesh
3 The Research Institute of Tuberculosis, Tokyo, Japan
Correspondence to: Mohammad Akramul Islam, Department of Community Health, School of International Health, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, Fax: 03-5684-3198; e-mail: akram@m.u-tokyo.ac.jp

treatment completion rate was as below as 25% (Islam, 1987). The World Bank review in 1990 estimated that the overall case detection rate was less than 20% and treatment completion rate was below 50% (Veen and Beex-Bleumink, 1990). In response to these findings, the national tuberculosis program revised its strategy with the guidance from WHO and received the financial assistance from the World Bank and the Government of Netherlands. Since 1993, the revised program has been implemented in order to strengthen the integration of tuberculosis control into the existing primary health care system. It focuses largely on collaboration with non-government organizations (NGOs) as they have already developed primary health care program. Until mid 1997, the national tuberculosis program covered 324 thanas which was about 70% of the whole country. Of these thanas, the government covered 214 thanas and NGOs covered 110 thanas (Ali and Colombani, 1997). The cure rate in thana health complexes under direct government supervision was about 71.1%, while that in the areas supported by NGOs was about 81.5% (Ali and Colombani, 1997). The recent review shows that the overall case detection rate is about 25% and treatment success rate is about 80% (Government of Bangladesh, 1997).

Bangladesh Rural Advancement Committee (BRAC), one of the largest NGOs in Bangladesh has been involved in the activities for poverty alleviation and empowerment of the poor since 1972. Along with the various community development activities i.e. adult and child education, health, income generation, credit and women development, BRAC initiated a pilot community based tuberculosis control project in 1984 in Manikganj thana. It covered a population of 220,000 through more than 200 community health workers in collaboration with national anti tuberculosis association of Bangladesh and government of Bangladesh (Ishikawa, 1985; Chowdhury *et al.*, 1991). The project area was approximately 50 km north west to Dhaka. The aim was to make tuberculosis diagnosis and treatment services available and accessible to the community people through community health workers, who already existed in BRAC initiated community program. In this tuberculosis program, the community health workers were community level service providers in rural villages for educating the community, identifying of symptomatic persons, providing treatment to patients, and following them up to ensure their compliance. Twelve months treatment regimen was then used for treating tuberculosis patients. The treatment completion rate was about 79% (Chowdhury *et al.*, 1991).

With the successful outcome in Manikganj thana, this approach was extended to 10 more thanas in 1992 covering a population of approximately 1.8 million to examine the scope of scaling up. It also showed the treatment completion rate as high as 80% (Chowdhury *et al.*, 1997). Following these encouraging results, BRAC signed a memorandum of understanding with the government of Bangladesh in 1994 to extend tuberculosis control activities to 120 additional thanas. In 1995 BRAC introduced 8 months short course regimen in collaboration with new national program. The program achieved the WHO target of 85% cure rate (Chowdhury *et al.*, 1997). A study showed that the tuberculosis prevalence rate was reduced in BRAC areas nearly by half within four years in compare to non program areas (Chowdhury *et al.*, 1997). This model is currently being applied in 60 thanas covering a population of approximately 13 million. The experience and results of the project after introducing and extending short course treatment are summarized in this paper. The detail strategy and evolution of the program has been published elsewhere (Ishikawa, 1985; Chowdhury *et al.*, 1991; Chowdhury *et al.*, 1997).

Materials and Methods

Manpower development

Community health workers, women of 25-35 years of age, play a key role in tuberculosis control program by BRAC. Each community health worker covers 150 to 200 households. They are mostly illiterate and members of village organizations. Community health workers are selected by the members of village organizations. Village organizations were formed by women of the poorest section in the community. Community health workers were trained by BRAC staff about tuberculosis control for 5 days along with other components of health such as nutrition, reproductive health, safe water supply and sanitation, acute respiratory infection, expanded program on immunization and so on. One day refresher training is also conducted every month to share the information and discuss their performance and problems which they encountered during the last month.

In the beginning of this program a basic training was given to the staff of all levels both in government and BRAC, including medical doctors, field level managers and supervisors following the national tuberculosis program training curriculum. Training materials and logistics were mostly supplied by the national tuberculosis program. WHO training modules for district level managers were used to train medical doctors and

managers. Laboratory technicians were trained centrally by national tuberculosis program.

Identification of patients

The community is informed about the danger of tuberculosis, signs and symptoms, diagnosis and treatment facilities, treatment schedule and prevention of tuberculosis through female forums held by community health workers. In addition, the male seminars, mosque forums, doctors seminars, teachers and elite seminars and Bazaar forums are organized by BRAC staff to disseminate the information. Cured patients also play an important role in disseminating information, identifying and motivating symptomatic persons for sputum examination and motivating the patients to continue treatment until they are cured.

Persons with cough for more than three weeks are mostly identified by community health workers and then referred for sputum examination. Each suspected person is given two sputum containers for collecting sputum samples one at night and the other at early morning, and also given instruction on how to collect sputum. Each suspected person is asked to bring two sputum specimens to the smearing center. Another spot sputum specimen is collected from each suspected person at the center. Sputum collection centers are set up at BRAC field offices and at village levels in remote areas to increase the accessibility of community to the diagnostic facility. Both centers are managed by BRAC staff and community health workers. Sputum smears are prepared and the slides are sent to the thana level laboratory for staining and microscopic examination. The results of the sputum examination are sent back to the symptomatic persons through the community health workers. Fifty percent of positive, 5% negative and 5% follow-up sputum slides are cross checked by another laboratory technician every month. To ensure quality control, randomly selected slides are also re-checked periodically by the national tuberculosis program staff.

Treatment

When two sputum specimens are positive, treatment for tuberculosis is initiated by community health workers under the guidance of BRAC field level staff. If symptoms persist but the sputum is negative, the patients are referred to the government health facility i.e. thana health complex or district tuberculosis clinic. Sputum negative patients as well as extra pulmonary patients are given treatment after consultation with the district tuberculosis clinic consultant.

Patients are asked to deposit Taka 200 (about US $ 4) and to sign a bond with two witnesses as a guarantee of treatment completion. If the patient is too poor to pay, he/she receives waiver of bond money. In some cases, community people also pay the bond money for patients. From the bond money, Taka 25 is given to the community health worker for each patient identification and Taka 100 is given on completion of the treatment. The remaining Taka 75 is refunded to the patient after completion of treatment. If the patient defaulted from the treatment, community health worker is paid proportionally and the remaining is kept by the organization. However if the patient dies, community health worker is paid proportionally and the remaining is returned to the authorized family member mentioned in the bond.

The eight months short course treatment regimen (i.e. isoniazid, pyrazinamide, ethambutol and rifampicin daily for two months followed by isoniazid and thioacetazone daily for six months) is given for new smear positive pulmonary tuberculosis patients as well as for seriously ill smear negative and extra pulmonary patients. Follow up sputum examination is made at 2nd, 5th and 8th months of the treatment to monitor the progress of treatment for sputum positive cases.

Drug taking of the patient is directly observed by community health workers. They collect the drugs from the BRAC field office monthly during refreshers training and store then in their homes. Patients come to the community health worker's home for drug taking during the intensive phase of the new treatment and for the entire period in case of retreatment. If the patient fails to come, the community health worker visits the patient's home and observe the drug taking. In the case of seriously ill patient, the community health worker visits the patient's home and observe him/her swallow the drugs until the patient becomes able to come to the community health worker's house. The streptomycin injections are also administered by community health workers for retreatment (failure and relapse) patients. Patients also collect drugs during ambulatory phase of treatment once a week from the community health workers home. Patients with drug reactions and complications are managed at the community level or sent to the thana health complex or district tuberculosis clinic.

Supervision and monitoring

The program is supervised and monitored by BRAC field and regional level staff. A tuberculosis specialist from central level monitors the activities and provides technical support to the program. Monitoring from the head office is also done by the top managers through management information system. The research and

evaluation division of BRAC also evaluate the program independently. The government staff at thana, district and central level and WHO staff members also assess the quality of the program through periodic field visits and quarterly progress reports.

To monitor the regular treatment in the community, patients are visited by BRAC staff once a week during the intensive phase and twice a month during the continuation phase. Community health workers also visit patients at home once a week during the ambulatory phase of treatment. Community health workers are visited by BRAC staff periodically to monitor their activities, records and provide necessary support.

Record keeping for laboratory register, thana tuberculosis register, along with sputum request forms, treatment cards and referral forms is maintained at the BRAC field office. A copy of the treatment card along with the home visit card is kept at the patient's home. Monthly performance reports, quarterly reports on case finding, sputum conversion and treatment outcome are prepared at thana level by BRAC field level staff to evaluate the outcomes.

Collaboration and Coordination with Government and othes NGOs

Drugs, equipment, reagents and other logistics are mostly supplied by the government to BRAC through the district civil surgeon on a quarterly basis. Coordination meetings with the thana health and family planning officer, district civil surgeon and project director of the national tuberculosis program are held monthly in collaboration with WHO. Meetings with other NGOs involved in tuberculosis control are held quarterly to share ideas and experience and to improve the quality of on going activities in collaboration with WHO. A referral system with the thana health complexes, district tuberculosis clinics and NGOs have been set up to avoid duplication of patient registration. Monthly and quarterly reports are given to the thana health and family planning officer, district civil surgeon and project director of the national tuberculosis program.

Data collection and analysis

Analysis of program performance was done by the authors in the middle of 1998. Quarterly case finding reports, sputum conversion reports, and treatment outcomes from January 1996 to December 1997 were collected from BRAC head office. The national reporting formats recommended by WHO/International Union Against Tuberculosis and Lung Disease (IUATLD) were used for data collection (International Union, 1994). Treatment cohort analysis was done according to WHO/IUATLD guidelines. WHO/IUATLD defined indicators (cured, treatment completed, defaulter, death, failure, transferred/referred) were used for evaluation of clinical outcomes (WHO, 1997b). Patients who complete the treatment full course and become sputum negative at 5th and 8th months are defined as cured. Patients who complete the treatment course but sputum results at 5th and/or 8th months are not available are defined as treatment completed. Patients who stop treatment for 2 or more months at any time during treatment are defined as defaulter. Patients who died during treatment in any cause are defined as death. Patients who become sputum positive at 5th months or later during treatment are designated as failure. Patients who are referred or transferred to another district or institute and treatment outcomes are not known are defined as referred/transferred.

RESULTS

Total number of patients by category in 1996 and 1997 is shown in Table 1. In the two years, a total of 7,946 patients were identified in 34 thanas. Of them, 7,023 (88.4%) were sputum positive, 749 (9.4%) were sputum negative and 174 (2.2%) were extra-pulmonary tuberculosis patients. Among sputum positive patients, a total of 6,163 (87.7%) were new sputum positive patients.

Age and sex distribution of new sputum positive patients is shown in Table 2. Of a total of 6,163 new sputum positive patients, 4,473 (72.6%) were male and 1,690 (27.4%) were female. About two third of new sputum positive patients were between 25-54 years old. Highest number of patients were between 35-44 years old for male and between 25-34 years old for female. Proportionally, there were more patients below 35 years of age in female than in male.

Table 1 Tuberculosis patients identified during 1996 and 1997

Category	1996	1997	Total
Sputum Positive Patients	3,158	3,865	7,023(88.4%)
-New	2,729	3,434	6,163(87.7%)
-Relapse	89	106	195(2.8%)
-Previously Incompletely treated	340	325	665(9.5%)
Sputum Negative	217	532	749(9.4%)
Extra-pulmonary	53	121	174(2.2%)
Total	3,428	4,518	7,946(100%)

Table 2 Age and sex distribution of new sputum positive tuberculosis patients in 1996 and 1997

Age	Male	Female	Total
0-14	32 (0.7%)	45 (2.7%)	77 (1.2%)
15-24	376 (8.4%)	258(15.3%)	634(10.3%)
25-34	950(21.2%)	532(31.5%)	1,482(24.0%)
35-44	1,118(25.0%)	397(23.5%)	1,515(24.6%)
45-54	898(20.1%)	273(16.1%)	1,171(19.0%)
55-64	722(16.2%)	144 (8.5%)	866(14.1%)
65+	377 (8.4%)	41 (2.4%)	418 (6.8%)
Total	4,473 (100%) 〈72.6%〉	1,690 (100%) 〈27.4%〉	6,163 (100%) 〈100%〉

Table 3 Sputum conversion results at 2nd month of new sputum positive tuberculosis patients during 1996 and 1997

	1996	1997	Total
Sputum Negative	2,396(87.8%)	3,152(91.8%)	5,548(90.0%)
Sputum Positive	115 (4.2%)	83 (2.4%)	198 (3.2%)
Death	124 (4.5%)	130 (3.8%)	254 (4.1%)
Defaulted	45 (1.7%)	37 (1.1%)	82 (1.4%)
Transferred/Referred	49 (1.8%)	32 (0.9%)	81 (1.3%)
Total	2,729 (100%)	3,434 (100%)	6,163 (100%)

Table 4 Treatment outcome of new sputum positive tuberculosis patients during 1996 and 1997 (till June)

	1996	till June 1997	Total
Cured	2,312(84.7%)	1,463(89.9%)	3,775(86.7%)
Treatment Completed	6 (0.2%)	—	6 (0.1%)
Died	233 (8.6%)	99 (6.1%)	332 (7.6%)
Failure	52 (1.9%)	22 (1.3%)	74 (1.7%)
Defaulted	55 (2.0%)	28 (1.7%)	83 (1.9%)
Referred/Transferred	71 (2.6%)	16 (1.0%)	87 (2.0%)
Total	2,729 (100%)	1,628 (100%)	4,357 (100%)

Sputum conversion results of new sputum positive patients at 2nd month are shown in Table 3. Of a total of 6,163 new sputum positive patients, 5,548 (90.0%) became sputum negative after initial intensive phase of the treatment. Deaths during the first two months were 254 (4.1%) among new patients.

Treatment outcome after 12-15 months of diagnosis of new sputum positive patients is shown in Table 4. Among 4,357 new sputum positive patients, 3,775 (86.7%) were cured; 332 (7.6%) died; 74 (1.7%) failed in treatment and 83 (1.9%) patients defaulted.

DISCUSSIONS

The concept of using directly observed treatment for tuberculosis emerged more than three decades ago as a result of work in Madras and Hong Kong (Bayer and Wilkinson, 1995). WHO recently claimed that directly observed treatment with short course regimen (DOTS) is the most cost effective strategy for tuberculosis control (WHO, 1997a). However, according to the recent WHO review, only 23% of the worldwide population has an access to the DOTS strategy (Raviglione *et al.*, 1997). And only about 10% of the world's tuberculosis patients are under this strategy (WHO, 1997a).

One of the crucial elements of DOTS strategy is that the health provider watches the patient swallow every single dose of tuberculosis drugs. It can be done in hospitalized condition, but the long period of hospital stay for DOTS increases the burden of hospitals in high epidemic developing countries as seen in Africa (Okot-Nwang *et al.*, 1993). It is also very disruptive and costly for the families of patients (Foster, 1990; Saunderson, 1995). In many developing countries hospital beds are not adequate to admit all infectious tuberculosis patients and therefore it is not feasible such as in Bangladesh and China (Chowdhury *et al.*, 1991; China, 1996).

There are also problems in promoting DOTS at out patient clinics, since health services are not easily accessible to most of the community people particularly in rural areas in developing countries (Maher *et al.*, 1997). Patients either has to come to clinic every day or health worker has to go to patient's house.

To ensure DOTS approach, an alternative model of providing care for tuberculosis patients needs to be explored at community level based on community participation. The BRAC initiative for tuberculosis control through utilization of community based voluntary health workers (i.e. community health workers) has proved to be an example of the alternative approach, achieving WHO target of curing 85% of diagnosed cases consistently over few years (Chowdhury *et al.*, 1997; Kochi, 1997). Utilization of available human resources in the community as trained volunteers and community health workers achieved high cure and treatment completion rates in Africa as well (Wilkinson *et al.*, 1996; Maher *et al.*, 1997). Supervised chemotherapy on out door basis by village doctors in China also contributed to achieve one of the highest cure rate in the world (China, 1996; WHO, 1997a). Involvement of community health workers in the philippines has also increased the cure rate (Mantala, 1997).

The important features of the BRAC TB program are 1) the community based approach; where community health workers are selected from the poorest section of the community; diagnostic and treatment facilities are available at the door step of people and services are organized by them, 2) financial bond for treatment completion and incentive scheme for community health workers to supervise treatment, 3) training and supervision of community health workers by motivated BRAC staff, 4) effective short course drug regimen, and 5) collaboration and support from the government.

Community health workers are more trusted by the community because they are selected by them. They are respected by the villagers in the program and thus they feel prestige to serve the people (Ishikawa, 1985). They could easily find symptomatic patients as they are from same community and know each other. Patients basically get free treatment in their community except the bond money. Therefore, they can continue their economic activities and take care of their families, as majority (77.9%) patients are from economically productive age group between 15-54 years old (Table 2). The system of bond money builds a mutual understanding between patients and community health workers, which makes each accountable to the other for better quality of care and makes treatment less expensive (Ishikawa, 1985). The amount of bond money is also affordable by majority patients, as it was found that 83% patients paid full amount of bond money (Osaki, 1995).

This study shows that the death rate is about 7.6% by the end of treatment (Table 4). More than half (4.1%) of them died within the first two months of treatment initiation (Table 3). On the other hand, there is a big gap between the male-female ratio (73:27), even though BRAC program utilizes female community health workers (Table 2). These indicate that diagnosis is still delayed and strong stigma and fear to tuberculosis exist in the community (Fair *et al.*, 1997). This gap between male and female ratio may be caused by poor women's access to health services due to heavy workload, as well as lack of mobility, independence and access to cash (Hudelson, 1996). However, proportionally more females (49.5%) were diagnosed than males (30.3%) below 35 years of age (Table 2). This may due to females have higher risk to develop disease than males in reproductive age (Dolin, 1998). It was also observed in India that females have 130% higher risk to develop disease than males between the age of 10 and 44 years (Olakowski, 1973).

Furthermore, a cost-effectiveness analysis of this model may determine whether the involvement of community health workers in national tuberculosis program can be more cost-effective approach. In the global expansion of DOTS strategy, each government needs to develop a sustainable program. This model of involving community health workers in tuberculosis control program can be an alternative approach to strengthen the national tuberculosis program by increasing the accessibility of tuberculosis services, particularly in a remote areas, where services are inadequate. The partnership among government, NGOs and community is essential for sustainable implementation of DOTS strategy to control tuberculosis.

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PREVALENCE OF SCHISTOSOMIASIS JAPONICA AMONG SCHOOLCHILDREN AND ANIMAL RESERVOIRS IN ORIENTAL MINDORO, PHILIPPINES

JUN MATSUMOTO[1], MASASHI KIRINOKI[1], SATORU KAWAI[1], YUICHI CHIGUSA[1], EUNICE J. ILAGAN[2], BENEFICO E. DUCUSIN[3], KAZUO YASURAOKA[1] AND HAJIME MATSUDA[1]

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Abstract: A survey was conducted in Oriental Mindoro, Philippines in 1997 and 1998 for the purpose of estimating the current situation of schistosomiasis japonica in the area. The prevalence rate in schoolchil dren determined by enzyme-linked immunosorbent assay detecting the parasite egg-specific immunoglobulin G revealed that the disease was more highly endemic in Malabo (70.7%) than in the other villages studied (31.8% in San Pedro and 36.4% in San Narciso), in spite of the fact that all of these villages were located near to each other. The prevalence rates determined by stool examination or necropsy of animal reservoirs in San Pedro, San Narciso and Malabo were as follows; dogs: 9.7%, 7.4% and 19.2%; rats: 10.4%, 8.7% and 26.1%, respectively. Water buffaloes were all negative in all villages. These results showed that the prevalences of schistosomiasis japonica in animal reservoirs have intimate correlation with that in schoolchildren. In Malabo, the colonies of intermediate-host snails were located very close to the resident area, which might be the major cause of high prevalence of the disease.

Key words: schistosomiasis japonica, Philippines, schoolchildren, animal reservoirs, serological test, stool examination

INTRODUCTION

Schistosomiasis japonica is one of the most important helminthic diseases distributed over Southeast Asia and China. Epidemiological surveys conducted so far in Oriental Mindoro, Philippines (Tubangui and Pasco, 1941; Hunter *et al.*, 1950; Saniel, 1951; Carney *et al.*, 1981; Chigusa *et al.*, 1997) showed that the disease was highly endemic in the area, especially around Lake Naujan.

In contrast to the transmission of other schistosome species, animal reservoirs contribute to the transmission of *Schistosoma japonicum* in endemic areas. Thus, it is indispensable to estimate the prevalences among mammalian hosts to control the disease in the area.

In other islands in the Philippines, investigations were carried out on the prevalences of schistosomiasis japonica in various animals (Pesigan *et al.*, 1958; Oshima *et al.*, 1978; Kamiya *et al.*, 1980; Fernandez *et al.*, 1982; Yasuraoka *et al.*, 1996). These reports showed that several mammals such as dogs, pigs, cows, water buffaloes and field rats were involved in the transmission of the disease. In spite of the fact mentioned above, no survey on the prevalences in animal reservoirs was conducted in Mindoro Island. In this survey, we tried to reveal the prevalences among animal reservoirs living in the given area.

Since 1994, we have continued epidemiological studies on schistosomiasis japonica in Oriental Mindoro, Philippines to know the current situation of the disease and accumulate the baseline data for future control campaigns. The purpose of this report is to show the

1 Department of Medical Zoology, Dokkyo University School of Medicine, 880 Kitakobayashi, Mibu-machi, Shimotsuga-gun, 321-0293 Tochigi, Japan
2 Schistosomiasis Control Team, Victoria, Oriental Mindoro, Philippines
3 Schistosomiasis Control Service, Department of Health, Manila, Philippines
Correspondence: Hajime Matsuda, Department of Medical Zoology, Dokkyo University School of Medicine, Mibu, Tochigi 321-0293, Japan E-mail: hmatsuda@dokkyomed.ac.jp

results of epidemiological studies on the prevalences among schoolchildren and animal reservoirs in three endemic villages (San Pedro, San Narciso and Malabo) in Oriental Mindoro, Philippines, conducted in 1997 and 1998.

Materials and Methods

Study area and period

Mindoro Island is the seventh in size among the Philippine archipelago and is situated just south of Luzon (Fig. 1). The island has a big mountain, which divides the island into two provinces: Oriental Mindoro and Occidental Mindoro. In the lowland of Oriental Mindoro lies Lake Naujan, around which exist many endemic areas of schistosomiasis japonica. As our study area, we selected three villages in the west side of the lake: San Pedro, San Narciso and Malabo. The climate of the area is classified as tropical-wet.

The survey was conducted from 23 July to 20 August in 1997 and from 23 July to 10 August in 1998, during the period of rainy season in the area.

Serological study of schoolchildren

A small amount of blood sample was taken from schoolchildren of the grade one to six (aged six to twelve years) through finger prick and collected in a heparinized capillary tube. The number of specimens collected totaled up to 424: 132 in San Pedro, 118 in San Narciso and 174 in Malabo. And for the purpose of comparing the results, 141 serum samples were collected from schoolchildren in Minas. This village is located about fifteen kilometers west of the villages studied, and proved in our previous study to be a non-endemic area of schistosomiasis (unpublished data). In addition, serum samples were obtained from eight Japanese students without any history of schistosome infection.

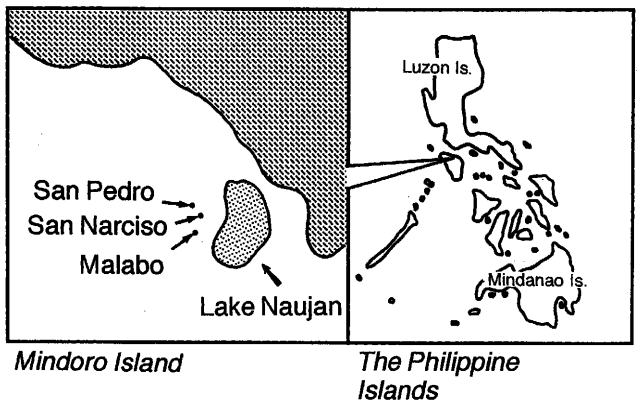

Figure 1 Prevalence of schistosomiasis japonica among schoolchildren and animal reservoirs in Oriental Mindoro, Philippines.
Brief maps of the Philippine Islands (right) and the northeast part of Mindoro Island (left), showing the location of three villages (San Pedro, San Narciso and Malabo) where this survey was conducted.

The parasite specific antibody titer was measured by enzyme-linked immunosorbent assay (ELISA) following the previous report (Matsuda *et al.*, 1984). Briefly, the assay was performed in microtiter plates and plasma samples were used at a dilution of 1 : 200. To detect the parasite specific immunoglobulin, horseradish peroxidase (HRP)-conjugated goat anti-human IgG polyclonal antibody (ICN Pharmaceuticals Inc., U. S.A., cat. No. 674161) was used at a dilution of 1 : 60,000. The substrate was 2-2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) (ABTS, Sigma, St. Louis, U.S.A., cat. No. A-1888). *S. japonicum*-egg (SjE) antigen was used to measure the parasite specific antibodies. The preparation of SjE antigen was previously described (Matsuda *et al.*, 1981). Samples of more than 0.25 of optical density at 415 nm were determined positive, following the previous report (Matsuda *et al.*, 1984).

Stool examination and necropsy

Fecal samples of schoolchildren were brought together to their own schools. The donors of the fecal samples were not necessarily identical to those of the blood specimens. We walked along the path collecting stools of dogs and water buffaloes, and covered almost all resident area. The numbers of stool samples collected from humans and animals in each village are shown in Table 2. All of the fecal specimens were treated by formalin-detergent technique (Moody, 1986) and examined for schistosome infection.

In addition, 202 (in San Pedro), 150 (in San Narciso) and 283 (in Malabo) of field rats (identified as *Rattus rattus mindanensis*) were caught by village volunteers (Table 2). These animals were necropsied, and

Table 1 Prevalence of schistosomiasis japonica among schoolchildren and animal reservoirs in Oriental Mindoro, Philippines.

Area	No. examined	No. positive	positive rate (%)
San Pedro	132	42	31.8
San Narciso	118	43	36.4
Malabo	174	123	70.7
Total	424	208	49.1

The prevalence of schistosomiasis japonica among schoolchildren in Oriental Mindoro, Philippines, estimated by enzyme-linked immunosorbent assay detecting the parasite-specific Immunoglobulin G.

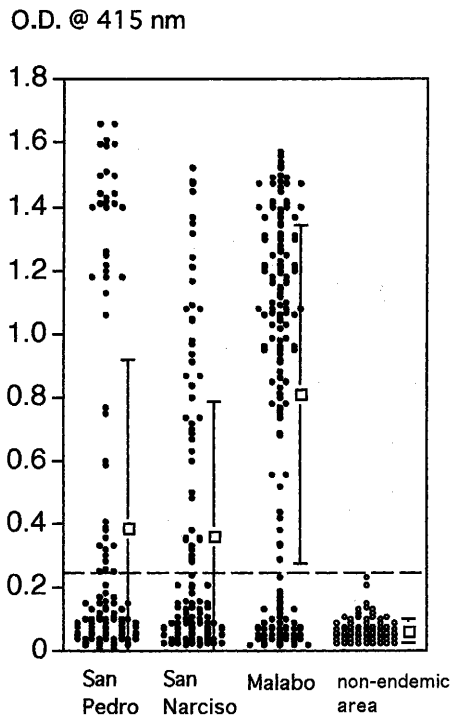

Figure 2 Prevalence of schistosomiasis japonica among schoolchildren and animal reservoirs in Oriental Mindoro, Philippines.
The result of serological examination for *Schistosoma japonicum*-infection among schoolchildren in San Pedro (n=132), San Narciso (n=118), Malabo (n=174) and Minas (a non-endemic area of schistosomiasis, n=141) in Oriental Mindoro, Philippines. The parasite-specific immunoglobulin G was detected by enzyme-linked immunosorbent assay. Open squares and vertical bars represent mean O.D. and S.D., respectively. And dashed line indicates cut off limit. The mean O.D. and S.D. of normal controls of Japanese students were 0.033 and 0.020, respectively.

their mesenteric and portal veins were thoroughly examined for flukes and foci due to schistosome infection microscopically by the press preparation technique of the tissues.

RESULTS

Serological examination

Seroepidemiological survey of schoolchildren (the result is shown in Fig. 2 and Table 1) detecting the parasite specific antibody revealed that schistosomiasis japonica was still endemic in the given area, especially in Malabo in which the prevalence of the disease was relatively higher (positive rate was 70.7%) than the other villages studied.

Table 2 Prevalence of schistosomiasis japonica among schoolchildren and animal reservoirs in Oriental Mindro, Philippines.

Host	Area	No. collected & examined	No. positive	Positive rate (%)
schoolchildren	San Pedro	130	5	3.8
	San Narciso	67	3	4.5
	Malabo	132	22	16.7
	Total	329	30	9.1
dogs	San Pedro	216	21	9.7
	San Narciso	148	11	7.4
	Malabo	255	49	19.2
	Total	619	81	13.1
water buffaloes	San Pedro	86	0	0
	San Narciso	59	0	0
	Malabo	126	0	0
	Total	271	0	0
field rats	San Pedro	202	21	10.4
	San Narciso	150	13	8.7
	Malabo	283	74	26.1
	Total	635	108	17.0

The results of stool examination (schoolchildren, dogs and water buffaloes) or necropsy (field rats) for the detection of *Schistosoma japonicum*-infection in various kinds of final hosts of the parasite in Oriental Mindoro, Philippines.

All of the specimens in Minas, non-endemic area of the disease, were determined to be negative. Normal controls of Japanese students (n=8) were all negative, and their mean O.D. and S.D. were 0.033 and 0.020, respectively.

Stool examination and necropsy

The results of stool examination or necropsy for schistosome infection are summarized in Table 2. In the stool examination also, schoolchildren in Malabo showed higher prevalence of the disease than those in the other two villages studied, although the positive rates estimated were much lower than in the serological test.

The parasitological examinations showed that the prevalence among animal reservoirs were relatively higher in Malabo than those in San Pedro and San Narciso. The positive rates of schistosome-infection in dogs and field rats were more than twice as high in Malabo as in the other two villages, but we could not find any positive case of water buffalo in all the area studied.

DISCUSSION

This survey undergone in three villages (San Pedro,

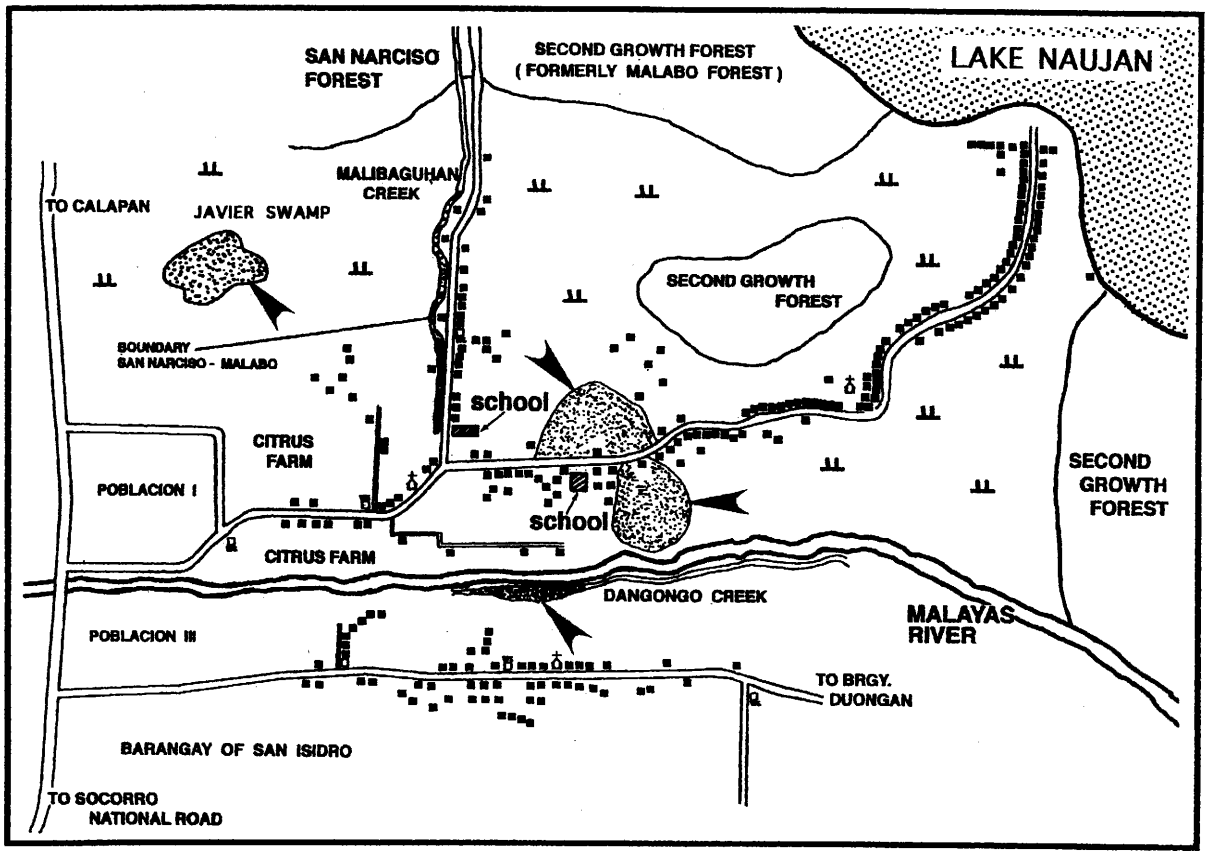

Figure 3 Prevalence of schistosomiasis japonica among schoolchildren and animal reservoirs in Oriental Mindoro, Philippins.
A sketch map of Malabo village, Oriental Mindoro, Philippines. Closed squares indicate houses and arrow heads colonies of intermediate host snails of *Schistosoma japonicum*. As shown in the map, the colonies were located along the central paths in the village.

San Narciso and Malabo) in Oriental Mindoro, Philippines, showed that schistosomiasis japonica is currently endemic in the area.

Seroepidemiological study for schistosome-infection among schoolchildren revealed that the prevalence of the disease was relatively higher in Malabo than in the other villages studied. As shown in the sketch map (Fig. 3), we found snail colonies with highly infected intermediate-host snails (*Oncomelania quadrasi*) beside the paths running through the center of the resident area. Schoolchildren in this village, we suppose, were repeatedly exposed to cercarial infection on the way to and from school every time when the paths are flooded. As indicated by this example, the location of the snail colonies in relation to the resident areas is one of very important factors in the transmission of the disease.

Prevalence rate of the disease among schoolchildren estimated by stool examination was much lower than that determined by serological test. In *S. japonicum* infection, the daily egg count in feces is relatively low and irregular compared with those found in other helminthic infections. Although twice or more of fecal examinations would have improved sensitivity of the test, we could perform the test only once under limited condition, with the result that the prevalence rate may have been underestimated.

On the other hand, serological examinations are highly sensitive and useful in large-scale screening, despite the fact that these kinds of methods cannot be free from following disadvantages, such as possibility of false positive cases mainly due to cross reaction to other parasitic infections, inability to discriminate active infections from very old infections without any living worms in the subjects. In this study, of 147 donors who offered both serum and stool samples, egg-positive cases was 10.2% (15/147), among which as high as 93.3% (14/15) was determied positive in serodiagnosis also (data not shown in results). On the other hand, of all the donors with the both specimens, 47.6% (70/147) was positive in serological test, among which only 20.0%

(14/70) was egg positive in stool examination. These results strongly confirmed that serological diagnoses are highly sensitive for the detection of *S. japonicum* infection compared with stool examinations. In order to make up for the defects in each diagnostic method, both of serological and stool examinations should be employed to estimate the current situation of the disease.

Stool examination of various final hosts indicated intimate correlation between the prevalence rates of the disease among schoolchildren and those of various animal hosts. Among three villages studied, Malabo showed the highest prevalence rates in animal hosts as well as in schoolchildren. As humans are suitable final hosts of *S. japonicum* and play the major role in transmission of the disease due to their behavior, principally water use practices and indiscriminated defecation in the field, it is supposed that the high prevalence in humans has great influence on the prevalences in animal reservoirs in an endemic area. The results of stool examinations in humans and animal reservoirs supported this expectation.

Pesigan *et al.* (1958) determined the transmission index, which express relative transmission potential for different hosts in an endemic area of the disease. In determining the index, we have to take four factors into consideration: population, prevalence and mean daily egg output of the hosts as well as the hatchability of eggs excreted. According to their report, in Leyte Island, dogs showed the highest index rate (14.4%) among animal reservoirs, following humans (75.7%). Although the transmission index in Mindoro Island has not yet determined, we guess that the situation is almost the same. It is our intention to clarify the transmission dynamics of schistosomiasis japonica in Mindoro Island by estimating the index rates of final hosts living in the area.

Due to zoonotic nature of *S. japonicum,* we cannot eliminate the disease without controlling the infection among animal reservoirs. Further surveys are contemplated to monitor the prevalences of the disease among various animal reservoirs under control operation, such as treatment of human cases with praziquantel and vegetation removal followed by chemical molluscicid-ing.

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CLINICAL MALARIA AND TREATMENT OF MULTIDRUG RESISTANCE FALCIPARUM IN THAILAND

SRIVICHA KRUDSOOD, WATCHAREE CHOKEJINDACHAI, UDOMSAK SILACHAMROON, WEERAPONG PHUMRATANAPRAPIN, PARNPEN VIRIYAVEJAKUL, VALAI BUSSARATID AND SORNCHAI LOOAREESUWAN

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Abstract: Clinical manifestations of malaria are nonspecific and range from asymptomatic to severe. The clinical presentations reflect complex interactions between the host, the environment, and the parasites. Signs and symptoms include fever, headache, muscle pain, abdominal pain, anorexia, nausea, vomiting, hepatosplenomegaly, jaundice and dark urine. In mild malaria, these signs and symptoms cannot differentiate malaria from common cold, influenza or other systemic diseases. Fever and malaise in malaria are believed to result from the release of endogenous cytokines [e. g. interleukin 1, 6 and 8 (IL-1, IL-6, IL-8) and tumor necrotic factor-α (TNF-α)] in response to parasite antigens. Other signs and symptoms of malaria are also associated with the rupture of parasitized red cells. In severe malaria, the clinical manifestations included cerebral malaria, pulmonary edema, renal failure, anaemia, and jaundice. Signs and symptoms of cerebral malaria are as follow alteration of consciousness, coma, dysconjugated eyeballs and convulsions. Among fatal cases, 80% died within the first 48 hrs of admission while the rest, death resulted from complications such as acute renal failure, pulmonary edema, bacterial infection, and lactic acidosis. 92% of the survivors had completed recovery. Treatment of multidrug resistant falciparum malaria in Thailand is complicated. New antimalarial drugs have been investigated at the Hospital for Tropical Diseases in the recent years. Artemisinine derivatives such as artesunate, artemether, arteether, dihydroartemisinin are also tested at the Bangkok Hospital for Tropical Diseases. Artesunate and artemether alone with a total dose of 600 to 750 mg produced cure rates of 80 to 95%. Artesunate suppositories have been proved successfully for the treatment of severe malaria. The artemisinin derivatives when used in combination with mefloquine cure rates improved to 95-100%. Dihydroartemisinin alone with a total dose of 480 mg given over 5 days gave a cure rate of 90%. At present, studies with the combination of artemisinin derivatives plus mefloquine in various doses and duration of treatment are being investigated. Until proven otherwise, the drug combinations are still recommended for all adult patients suffering from acute uncomplicated falciparum malaria contracted in multidrug resistant areas.

In severe malaria, the choice of antimalarial chemotherapy depends on the clinical severity, the drug sensitivity of the parasites, and the availability and preparation of the drug. Quinine is widely available drug. Qinghaosu and its derivatives have been used successfully in treating both uncomplicated and severe falciparum malaria. Their effectiveness in eliminating the parasites have been extensively documented, however, the recrudescent rate is rather high (10-30%). In treating severe malaria, early diagnosis and early treatment are vital and the aim is to save patient's life. Prompt administration of an adequate and effective antimalarial drug is needed once the diagnosis is made. Other symptomatic and supportive treatment includes careful monitoring of fluid intake and urine output, frequent observations for complications with appropriate treatment and good nursing care.

Key words: Malaria, drug resistance, treatment, Thailand

INTRODUCTION

Multi-drug resistant falciparum malaria is a serious problem in Thailand. Therapeutic failures with all available antimalarial drugs are well documented. With this deteriorating situation, new drugs are urgently

Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University
Address for correspondence: Professor S. Looareesuwan, Dean, Faculty of Tropical Medicine, 420/6 Rajavithee Road, Bangkok 10400, Thailand, Fax (662)247-1688 E-mail: tmslr@mucc.mahidol.ac. th

needed. The new drugs and drugs in combinations have been studied in Bangkok Hospital for Tropical Diseases since 1988. This paper is an overview of clinical studies on clinical malaria and treatment of multidrug resistance falciparum malaria in Thailand.

Clinical Manifestations

Rupture of intraerythrocytic schizonts coincides with paroxysms of fever and this has led to the traditional categorization of the different types of human malaria. Nowadays, these terms are not recommended, because patterns of fever are variable and some patients die from *Plasmodium falciparum* even though they never develop periodic fever, particularly in nonimmune patients and during the early stage of illness. Symptoms and signs of malaria are vague including fever, headache, malaise, lassitude, fatigue, muscle pain and loss of appetite. These are not specific for malaria and are similar to the symptoms of influenza or other viral infections. It is essential to take blood samples for examination for malaria parasites.

In severe malaria (Table 1), alteration of consciousness is the most prominent manifestation. The patient usually presents with a history of fever for several days and loss of consciousness, which may be sudden following one or more grandmal seizures. In approximately 50% of patients over 6 years of age, coma follows a convulsion. In young children with cerebral malaria, the history may be very short. Vomiting may also occur; this is important to know because aspiration pneumonia could develop before admission to hospital. In many countries in the tropics, empirical antimalarial or antibacterial treatment and antipyretics have often been given before admission to the hospital. This may explain some reports of patients with cerebral malaria in whom no parasites were demonstrated on a peripheral blood smear. On examination most patients are usually febrile and in unarousable coma. A few patients who have very severe infection have a subnormal temperature. Admission temperature usually exceeds 38.0–39.0°C in most cases and rises above 39.0°C during the first two days of hospitalization. There is no classical pattern of fever in cerebral malaria. Rigors are rare in comatose patients but a sustained high fever, which is resistant to antipyretic treatment, is commonly found. The skin temperature is cool relative to the rectal temperature in hypoglycemic or shocked patients.

Jaundice is common in adult patients and anemia develops rapidly in severe malaria. The chest is usually normal on clinical examination although aspiration pneumonia or pulmonary edema may develop. Changes in respiration can be a warning sign of hypoglycemia, metabolic acidosis, pneumonia, and pulmonary edema, but may occur as a result of high fever alone. Therefore careful examination and proper investigations should be performed in all patients.

Hepatosplenomegaly is common. In Thailand, half of the adults and children over 6 years with cerebral malaria have a palpable liver or spleen. Massive splenomegaly is most umusual in severe malaria. Abdominal pain and tenderness are prominent in some patients. In children, the physical signs are similar to adults except that convulsions are more common, hepatomegaly may be prominent, and anemia is common; jaundice is relatively less frequent.

Retinal hemorrhages occur in 15% of cerebral malaria patients in Thailand. Hemorrhages may occur anywhere in the retina. Some appear similar to Roth's

Table 1 Definition of severe malaria and complicated falciparum malaria

1. Cerebral malaria
2. Severe anemia (Hct<15%)
3. Renal failure (no urine or urine output <400 m*l* in 24 hr, or 12 m*l*/kg/24 hr after rehydration, or serum creatinine >3 mg/d*l*)
4. Pulmonary edema or adult respiratory distress syndrome
5. Hypoglycemia (blood sugar<40 mg/d*l*)
6. Shock (systolic BP<70 mmHg in adult or <50 mmHg in children age 1-5 years)
7. Spontaneous bleeding and disseminated intravascular coagulation
8. Repeated convulsions
9. Acidosis (arterial pH<7.25 or plasma HCO_3<15)
10. Macroscopic hemoglobinuria
11. Hyperparasitemia (>5% parasitemia in non-immunes)
12. Hepatic dysfunction
13. Hyperpyrexia (persistence of rectal T>40°C)

spots, which are associated with endocarditis. Exudates are unusual and papilledema is rare (less than 1%). The hemorrhages resolve rapidly in survivors and do not interfere with vision after recovery of consciousness. Systemic bleeding due to disseminated intravascular coagulopathy (DIC) is present in a few patients (less than 5%). Abnormal eye movements such as divergence of the eyes or nystagmus are seen occasionally. Pupillary responses to light, and the oculocephalic and oculovestibular reflexes are always normal. Corneal reflexes are usually present. There may be passive resistance to head flexion but other signs of meningeal

Table 2 Clinical trials of oral antimalarial drug either alone or in combination with other anti-malarial drugs in Bangkok Hospital for Tropical Diseases

Reference and drug	Total dose (mg)	Duration (d)	No. of patients	Cure rate (%)
Looareesuwan *et al.*, 1992				
Artesunate	600	5	40	88
Artesunate followed by mefloquine 25 mg/kg divided into 2 doses	600	5	39	100
Looareesuwan *et al.*, 1993				
Artesunate follow by mefloquine 15 mg/kg divided into 2 doses	300	2.5	50	90
Looareesuwan *et al.*, 1994				
Artesunate plus doxycycline 200 mg/d×7 d	300	2.5	55	80
Looareesuwan *et al.*, 1997				
Artemether	500	5	40	74
Artemether	750	7	58	98
Artemether follow by mefloquine 25 mg/kg divided into 2 doses	600	1	53	98
Looareesuwan *et al.*, 1996a				
Dihydroartemisinin	480	7	53	90
Looareesuwan *et al.*, 1999				
CGP 56697	Artemether 320 and benflumetol 1,920	2	126	69
Looareesuwan *et al.*, 1996c				
Atovaquone	9,000	1	25	72
Atovaquone plus proguanil 4,800 mg	12,000	3	24	100
Atovaquone plus proguanil 2,000 mg	5,000	5	24	100
Looareesuwan *et al.*, 1996b				
Pyronaridine	1,200	3	69	63
Pyronaridine	1,800	5	32	88
Wilairatana *et al.*, 1997				
Biguanide-dapsone	Dapsone 200 plus proguanil 400	3	10	10
Biguanide-dapsone	Dapsone 200 plus Chlorproguanil 70	3	14	29

irritation are absent. Ankle clonus is present in one third of patients. The abdominal reflexes are almost absent. There is no other clinical evidence of autonomic dysfunction in cerebral malaria. The duration of coma is 48-72 hrs in most adult patients in Thailand. After treatment most (92%) of the survivors have complete recovery.

Treatment

1) **Uncomplicated falciparum malaria**

With the emergence of multidrug resistant falciparum malaria in Thailand, new drugs and drugs in combination are urgently needed. The artemisinin derivatives when used in combinations with mefloquine improved cure rates to 95-100% (Looareesuwan *et al.*, 1993). Dihydroartemisinin alone with a total dose of 480 mg given over 5 days gave a cure rate of 90% (Looareesuwan *et al.*, 1996; Wilairatana *et al.*, 1998). Combination of artemether with benflumetol proved safe and effective (cure rate over 90%) in the treatment of acute uncomplicated falciparum malaria (Looareesuwan *et al.*, 1999). Other combinations (artemisinin derivatives combined with doxycycline, mefloquine combined with tetracycline or doxycycline) have also been evaluated with the improvement in cure rates (Table 2) (Looareesuwan *et al.*, 1994).

2) **Severe malaria**

Management of severe malaria bases on the early diagnosis and early treatment with a potent antimalarial drug. Early detection and treatment of complications (convulsions, acute renal failure, pulmonary edema, acidosis, hypoglycaemia, hyperthermia) are essential. The aim of management is to save the patient's life. The choice of antimalarial chemotherapy depends on the clinical severity, the drug sensitivity of the parasites, and the availability and preparation of the drug.

Quinine

Quinine is the most commonly used and available drug in treating falciparum malaria. Recently, its effectiveness has declined when used alone. Quinine resistance is appearing in several parts of the world including Tanzania, Vietnam, and Thailand; however, most of the resistance is at the RI level. Minimal inhibitory concentrations (MIC) of quinine for *P. falciparum* parasites have risen above 10 mg/*l* in some areas. In this situation, and in severe malaria, a loading dose of 20 mg of quinine dihydrochloride per kg body weight, and maintenance doses of 10 mg per kg given every 8 hrs, are required. Persistent parasitemia after a loading dose of quinine is not uncommon. It is believed that the MIC of quinine must be maintained for seven days of effect complete cure of *P. falciparum* infections. Therefore, quinine must be given for at least seven days or longer. In quinine-resistant areas, a second drug such as tetracycline or doxycycline has been added to increase cure rates, which approached 100% in 1986 but declined to 90% in 1991. For children, in whom tetracycline is contraindicated, increasing the dose of quinine to 15 mg/kg in the second half of the treatment period improves the cure rate.

Table 3 Causes of shock in severe falciparum malaria

1. Dehydration (hypovolemia)
2. Septicemia
3. Endotoxemia (TNF)
4. Hypoglycemia
5. Lactic acidosis
6. Hemorrhage (DIC, (jI bleeding, drugs, ruptured spleen)
7. Myocardial failure or cardiac arrhythmias

In severe malaria, if the patient has not received quinine or mefloquine in the previous 24 hrs, then an initial loading dose of 20 mg/kg of quinine dihydrochloride salt should be infused over 4 hrs followed by maintenance doses of 10 mg/kg at 8 hrs intervals until the patient can take oral medication, for a total treatment course of 7 days. The initial doses should never be reduced, the maintenance doses should be reduced by one-third if by the third day of treatment there has been no clinical improvement, or if there is established acute renal failure. Hypoglycemia is the most serious and frequent adverse effect of cinchona alkaloids.

Quinidine

Quinidine, the diastereomer of quinine, has a lower MIC for *P. falciparum* in Thailand. It is as effective as or perhaps more effective than quinine for the treatment of falciparum malaria, as shown in open trials of oral quinidine sulfate and parenteral quinidine gluconate in both acute uncomplicated and severe falciparum malaria in Thailand. Quinidine bisulfate and quinidine slow-release formula was tested in uncomplicated falciparum malaria in Thailand in 1983-1984. They proved effective (with the cure rate of 100%), were well tolerated and had few side effects. They should be considered as alternative antimalarial drugs in the treatment of uncomplicated falciparum malaria.

In places where quinine is not available, such as Japan, America or Europe, quinidine may be used in

place of quinine. It is 4 times more effective but also 4 times more cardiotoxic than quinine. The electrocardiogram shows consistent dose-related prolongation of the QTc interval. In severe malaria, an initial loading dose of 15 mg base/kg is given over 4 hrs, followed by 7.5 mg base/kg given over 4 hrs at 8 hrs intervals. Quinine should be substituted when it becomes available to complete a 7 days treatment course. Hypotension is a serious side effect particularly when it is given over a period of less than 4 hrs.

Combination of quinine, quinidine and cinchonidine

In vitro testing against fresh isolates of *P. falciparum* suggested that a combination-containing equal part (1 : 1 : 1) of the three drugs was more potent than the individual drugs. In clinical trials, this combination was studied in uncomplicated falciparum malaria in Thailand. The combination drug was given every 8 hrs in a dose of 11.4 mg base/kg for 7 days. The results revealed that neither the oral nor the intravenous preparations of the three drugs are toxic and that it is effective in the treatment of chloroquine-resistant falciparum malaria. However, the drug is not generally available.

Qinghaosu (artemisinin) derivatives

Qinghaosu is sesquiterpene lactone peroxide extracted from the qinghao plant (*Artemisia annua L.*). It has been used in Chinese traditional medicine for over 2000 years, to treat the chills and fever associated with malaria. The active constituent, artemisinin, was isolated in 1972. Qinghaosu derivatives are highly effective in killing parasites; however, recrudescence rates are high. Attempts have been made to reduce the high recrudescence rates by increasing the dosage, or lengthening the period of drug administration, or using drug combinations. Two derivatives, (artemether and artesunate) are now widely used and have been registered in Thailand. Other qinghaosu derivatives including arteether and artelinic acid, are under development.

Artesunate

Artesunate is formulated either as tablets (50 mg tablet) or as a dry powder of artesunic acid for injection (supplied in 60 mg vial with an ampoule of 1 m*l* 5% sodium bicarbonate). The powder is dissolved in the 1 m*l* sodium bicarbonate and then further diluted in 1 m*l* of normal saline and use immediately as intravenous or intramuscular injection. Artesunate is manufactured by Guilin No. 2 Pharmaceutical Factory, Guangxi, China. It has rapid antimalarial activity with the clearance of over 90% of parasitemias within 24 hrs (Wilairatana *et al.*, 1997). However, recrudescence rates are high, ranging from 10 to 30% depending upon the dose and duration of initial treatment. A recent study in Thailand has shown that oral artesunate at a total dose of 600 mg given over 5 days had a cure rate of 88% (Looareesuwan *et al.*, 1992). However the efficacy increased to 100%, in both acute uncomplicated and recrudescent falciparum malaria infections, when mefloquine 1,250 mg (divided in 2 doses 6 hrs apart) was given following artesunate treatment. If a half dose of artesunate (300 mg) was given over 2.5 days, followed by 750 mg mefloquine, the cure rate reduced to 90% (Looareesuwan *et al.*, 1993, Looareesuwan *et al.*, 1997). Both oral and parenteral preparations of artesunate have been licensed for use in Thailand. In order to reduce the high recrudescence rate, the optimum dose of artesunate in combination with other drugs such as mefloquine or tetracycline or doxycycline is currently being investigated in Thailand.

Artemether

Artemether (60 mg ampoule), manufactured by Kunming Pharmaceutical Factory, Kunming, China, is formulated in peanut oil for intramuscular injection and licensed for use in Thailand. Oral artemether (50 mg tablet or capsule) is undergoing clinical trials. As with artesunate, the parasiticidal effect is rapid, with clearance of over 90% of parasitemias within 24 hrs. Unfortunately, recrudescence rates are also high. Optimum dose of artemether combined with other drugs such as mefloquine, tetracycline or doxycycline is being under studied in Myanmar and Thailand.

Symptomatic treatment

Supportive and symptomatic treatment of malaria are equally important to antimalarial drugs since most patients have headache, nausea, vomiting, diarrhea, high fever and inability to take any food while they are ill. Therefore, rehydration and the use of antipyretic and antiemetic drugs may be necessary. Antipyretics and antiemetics drugs should be administered a few hours before antimalarials are given, in order to reduce the chance of vomiting and to comfort the patients. After antimalarial drug administration, it is advisable to keep patients under observation for an hour or let the patients lie down, to make sure that they have completed the dose and do not vomit. If they vomit within 1 hr, the dose should be repeated.

In severe malaria, especially in cerebral malaria, intensive care of the unconscious patient and treatment

of life-threatening complications such as pulmonary edema, metabolic acidosis, renal failure, hypoglycemia, bacterial septicemia, and aspiration pneumonia are essential (WHO, 1990). Cerebral malaria is an emergency condition, which requires intensive care during the first 48 hrs of admission (Warell, 1997). It has been calculated that nearly 80% of patients die during this period. Other complications or manifestations should be treated as follows:

Hyperthermia

Cerebral malaria patients usually deteriorate while they have high fever. Temperatures above 38.5°C are associated with an increased incidence of convulsions, especially in children. Temperatures between 39.5°C and 40°C is associated with delirium, and above 42.0°C with coma. High body temperatures may cause permanent severe neurological damage. In pregnant women with malaria, high fever is associated with fetal distress. In the severely ill patient, cooling blankets, fanning, and tepid sponging are necessary. In the tropics, clinicians often accept the risk of agranulocytosis and use parenteral antipyretics such as dipyrone.

Acute pulmonary edema

Although pulmonary edema may develop at any stage of the acute illness, it tends to occur later than the other acute manifestations of malaria. Central venous pressure is a useful measure of hydration. A permeability edema associated with normal pulmonary wedge pressure measurements may develop at any time in the first few days of treatment and is difficult to treat. There is no specific therapy and management should be the same as that for adult respiratory distress syndrome occurring in other conditions. Malaria in pregnancy usually has a high risk of pulmonary edema, particularly after delivery.

Acute renal failure

This results from acute tubular necrosis. Some impairment of renal function is common in adults with cerebral malaria and is also associated with classical blackwater fever but only a minority of patients go on to develop established renal failure. Hypercatabolic acute renal failure should be managed by dialysis or conservative treatment if dialysis is not available. Hemodialysis treatment in acute renal failure from malaria does not shorten the course of acute tubular necrosis, which is usually last for 1-3 weeks. Hemodialysis requires 5-10 times during the 1-3 weeks of anuria. Fluid balance should be strictly monitored.

Hepatic dysfunction

This is manifested by jaundice, raised serum enzymes, prolonged prothrombin time, decreased serum albumin and lactic acidosis. Bilirubinemia is predominantly of the unconjugated type but some patients show an increase in conjugated bilirubin, indicating hepatocellular damage. Saline enema in these patients is recommended (Wilairatana *et al.*, 1994).

Hypoglycemia

Hypoglycemia should be reversed by intravenous dextrose and plasma glucose maintained between 80-120 mg/d*l* Frequent monitoring is essential. This condition should be suspected in any patient whose consciousness is deteriorating, who develops convulsions or who has changed respiratory patterns.

Shock (“algid malaria”)

This is most commonly the result of hypovolemia or complicating septicemia.

Severe anemia

Anemia develops very rapidly in cerebral malaria and blood transfusion is often required. Anemia results from a combination of bone marrow suppression and accelerated red cell destruction of both parasitized and unparasitized red cells. Blood should be crossmatched on admission and transfused to maintain the hematocrit over 21%. Fresh blood is preferable as thrombocytopenia and clotting factor depletion may coexist with anemia. Transfusion rates should be slow to avoid volume overload, and it may be necessary to give a potent diuretic such as furosemide intravenously at the same time.

Metabolic acidosis

This condition is serious and rapidly fatal. Lactic acidosis and renal impairment both contribute to hydrogen ion retention. Lactic acidosis results from the parasite, increased anaerobic glycolysis and a failure of hepatic gluconeogenesis. Sodium bicarbonate provides temporary correction of acidosis. Dichloroacetate has proved to be safe and to decrease the degree of acidosis. However, it did not reduce the mortality rate in a randomised controlled study in adult patients suffering from other diseases. The drug should be tested in cerebral malaria patients.

Hyperparasitemia

In nonimmune patients with severe falciparum malaria, mortality increases with the degree of para-

sitemia. Exchange transfusion may reduce the burden of parasitemia more rapidly than chemotherapy alone and might also remove harmful toxic products e.g. toxins and cytokines (Looareesuwan *et al.*, 1998; Udomsangpetch *et al.*, 1997; Wenisch *et al.*, 1998). This technique replaces blood, plasma, platelets, clotting factors and correct other abnormalities e.g. fluid, electrolytes and acid-base imbalance. Exchange transfusion should be considered in nonimmune patients who have more than 10% parasitemia and who have deteriorated on the conventional chemotherapy and have at least two complications of severe falciparum malaria e.g. cerebral malaria, renal failure, jaundice or acute pulmonary edema. In areas where total exchange transfusion is not possible, partial exchange transfusion with 4-6 units of fresh blood performed manually, alternately venesecting and transfusing the patient is still useful. Monitoring of central venous pressure, vital signs and blood pressure must be done frequently during exchange transfusion.

Bacterial infections

Gram-negative septicemia, aspiration pneumonia, and urinary tract infections may complicate cerebral malaria and should be treated with appropriate antibiotics. Any patient who develops shock at any stage should be investigated because ups to 40% of such cases have been found to have positive blood cultures.

Coagulopathy

Using sensitive measures, activation of the coagulation cascade be detected in all patients with acute symptomatic malaria, but significant disseminated intravascular coagulopathy occurs in less than 5% of patients with severe disease. Replacement therapy (not heparin) is essential, and in the tropics fresh blood is preferable.

Prognosis

Poor prognostic indicators in severe falciparum malaria include deep coma, repeated convulsions with signs of decerebration, high parasitemia (>5% in nonimmunes), peripheral schizontemia, clinical jaundice or more than three-fold elevated serum enzyme concentrations, uremia (creatinine more than 3.0 mg/d*l* and blood urea nitrogen more than 60 mg/d*l* after rehydration), metabolic acidosis (elevated plasma and /or cerebrospinal fluid lactate, or arterial blood gas <7.2), leucocytosis (>15,000/μl), retinal hemorrhages and hypoglycemia (clinical hypoglycemia or blood glucose <40 mg/d*l*).

Chemoprophylaxis

1 *General Principles*

A practical approach to any prophylaxis regimen includes the following concepts:

- Drugs should begin at least 1 week before entering a malarious area. The drugs must be taken regularly and, for most regimens, continued for at least 4 weeks after leaving the endemic area.
- No antimalarial regimen is completely effective, even with absolute compliance. Antimalarial chemoprophylactic regimens do not prevent infection: they suppress the infection sufficiently in most cases so that clinical signs and symptoms do not appear. In areas of increasing drug resistance, 'breakthrough' infections will occur more frequently.
- Chemoprophylaxis should always be combined with personal protective measures such as the use of insecticide impregnated bed nets for sleeping, avoiding peak mosquito biting times, using insect repellents on exposed surfaces, and wearing clothes that minimize exposure.

Antimalarial prophylaxis by people living in malaria endemic areas remains controversial. Pregnant women are at increased risk for severe malaria and should take antimalarial prophylaxis. Antimalarial prophylaxis in children living in endemic areas has been shown to reduce mortality, but some issues remain unsettled.

Recommendations for malaria chemoprophylaxis in travelers are complicated by compliance issues and adverse effects. In areas where the risk of infection is low or there are brief exposures in intermediate or high transmission areas, travelers may carry a treatment course of antimalarial drugs ('standby drugs') instead of taking prophylaxis. If they become ill, especially in areas without medical facilities, the treatment is self-administered.

2 *Current Prophylactic Drugs*

Increasing drug resistance in many parts of the world, especially Southeast Asia and South America, is slowly diminishing the effectiveness of many prophylactic regimens. Knowing the drug resistance patterns of *P. falciparum* in different geographic locations is essential for choosing an appropriate regimen.

The major chemoprophylactic drugs for falciparum malaria include doxycyline, mefloquine.

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SIMULIUM (*NEVERMANNIA*) *BONNINENSE* FROM THE OGASAWARA (BONIN) ISLANDS, JAPAN (DIPTERA: SIMULIIDAE): TAXONOMIC ASSIGNMENT TO THE *VERNUM*-GROUP AND DESCRIPTIONS OF MALE, PUPA AND MATURE LARVA

HIROYUKI TAKAOKA[1], KATSUMI SAITO[2] AND HIROSHI SUZUKI[3]

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Abstract: Descriptions and illustrations of male, pupa and mature larva of a black-fly species, *Simulium* (*Nevermannia*) *bonninense* (Shiraki, 1935), from the Ogasawara (Bonin) Islands in Japan are given for the first time; the female adult is also redescribed. Within the subgenus *Nevermannia*, this species is assigned to the *vernum*-group by the combination of the following characters: male genitalia with a lamellate ventral plate without median keel, an elongate style with a large, broad, inwardly-twisted apex, a single parameral hook per side, and an inverted Y-shaped median sclerite; pupal gill with four slender filaments per side; and larval mandible with supernumerary serrations. Interestingly, this species has the katepisternum haired in both sexes of adults, female genital fork with a prominent projection directed forwards on each arm, and pupal frons with two trichomes on each side, all of which are rare in this species-group. Brief notes on adult blood-feeding, and larval habitats of *S.* (*N.*) *bonninense* are given.

Key words: Simuliidae, *Simulium*, black fly, Ogasawara Islands, *bonninense*

Shiraki (1935) described *Simulium* (*Nevermannia*) *bonninense* (then, under the genus *Eusimulium*) from a single female adult specimen collected in the Ogasawara (Bonin) Islands, ca. 1,000 km south-southeast from Tokyo, in the Pacific Ocean. Stone (1964) gave excellent illustrations as well as a brief description of the female of this species based on 13 females collected in 1958 from Hahajima Is., while reporting three other species of Simuliidae from Micronesia. The immature stages of this species were found for the first time by Saito *et al.* (1974). However, no descriptions have ever been made.

The present paper gives the redescription of the female, and descriptions of the male, pupa and mature larva, of *S.* (*N.*) *bonninense*, based on reared or light-trapped adults, and immature stages recently collected by one of us (HS), as well as those examined by Saito *et al.* (1974), and one female loaned from the Natural History Museum, London, UK (BMNH). This species is assigned to the *vernum*-group within the subgenus *Nevermannia*, as provisionally treated by Takaoka and Davies (1995).

***Simulium* (*Nevermannia*) *bonninense* (Shiraki, 1935)**

Eusimulium bonninense Shiraki, 1935: 21-23.

Simulium (*Eusimulium*) *bonninense*: Tokunaga, 1943: 943; Stone, 1964: 634-635; Crosskey, 1989: 223.

Simulium (*Nevermannia*) *bonninense*: Takaoka and Okazawa, 1988: 98; Takaoka and Davies, 1995: 163; Crosskey and Howard, 1997: 49.

DESCRIPTION. **Female**. Body length 2.7-3.0 mm. ***Head.*** Narrower than thorax. Frons and clypeus brownish black, not shiny, white-pruinose, moderately covered with whitish yellow fine simple hairs, interspersed with brown longer and stouter hairs. Frontal ratio

1 Department of Infectious Disease Control, Oita Medical University, Hasama, Oita 879-5593 Japan
2 Department of Parasitology, School of Medicine, Yokohama City University, Kanazawa-ku, Yokohama City, Kanagawa 236-0004, Japan
3 Information and Reference Center of Tropical Medicine, Institute of Tropical Medicine, Nagasaki University, Sakamoto, Nagasaki City, Nagasaki 852-8523, Japan

1.6:1.0:1.6. Frons-head ratio 1.0:4.9. Fronto-ocular area (Fig. 1) well developed. Antenna composed of 2+9 segments, dark brown to brownish black. Maxillary palp consisting of 5 segments, brownish black, proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:1.7; 3rd segment (Fig. 2) somewhat enlarged, with sensory vesicle elongate, ca. 2.0× as long as wide, and ca. 0.3× as long as 3rd segment. Maxillary lacinia with 14 inner teeth and 15 or 16 outer ones. Mandible with 26-28 inner teeth and 11-15 outer ones. Cibarium smooth. ***Thorax.*** Scutum brownish black, white-pruinose, densely covered with whitish yellow recumbent fine hairs, and with several brown upright hairs on prescutellar area. Scutellum brownish black, with many brown upright hairs as well as whitish yellow recumbent fine hairs. Postscutellum brownish black, bare. Pleural membrane bare. Katepisternum longer than deep, with several fine hairs near upper margin on each side. ***Legs.*** Dark brown to brownish black, except hind trochanter dark yellow to light brown, base of hind tibia yellow, basal 3/5 of hind basitarsus and basal 1/2 of 2nd hind tarsal segment whitish yellow. Fore basitarsus slender, ca. 7.2× as long as wide. Hind basitarsus (Fig. 3) nearly parallel-sided, 5.6× as long as wide, and much narrower than hind tibia. Calcipala and pedisulcus (Fig. 3) well developed. Claws (Fig. 5) each with large basal tooth. ***Wing.*** Length 2.0-2.3 mm. Costa with 2 parallel rows of short spines as well as hairs. Subcosta haired except apical 1/4 bara (in 1 female, apical 1/2 bare). Hair tuft on stem vein brown. Basal portion of radius fully haired. Basal cell absent. ***Abdomen.*** Basal scale brownish black, with fringe of pale long hairs. Dorsal surface of abdominal segments dark brown to brownish black, not shiny, with short dark hairs; terga 3-6 medium, nearly quadrate, subequal in size to one another, tergum 7 nearly 2.0× as wide as terga 3-6, but still confined to dorsal surface, while terga 8 and 9 very wide, extending laterally. ***Genitalia*** (Figs. 6-8). Sternal plate of 7th abdominal segment well developed, nearly triangular. Sternum 8 wide, bare medially but furnished with ca. 12 stout hairs on each side. Anterior gonapophysis thin, membraneous, triangular, densely covered with microsetae and several short setae; inner margin narrowly sclerotized; posteromedian corner rounded. Genital fork with well sclerotized stem and wide arms; each arm with a wide round lobe directed medioposteriorly and a prominent projection directed forward. Paraproct somewhat protruding ventrally. Cercus rounded posteriorly in lateral view. Spermatheca ovoid or pear-shaped, strongly sclerotized except tube and small area of tubal base bare or weakly sclerotized; internal setae absent.

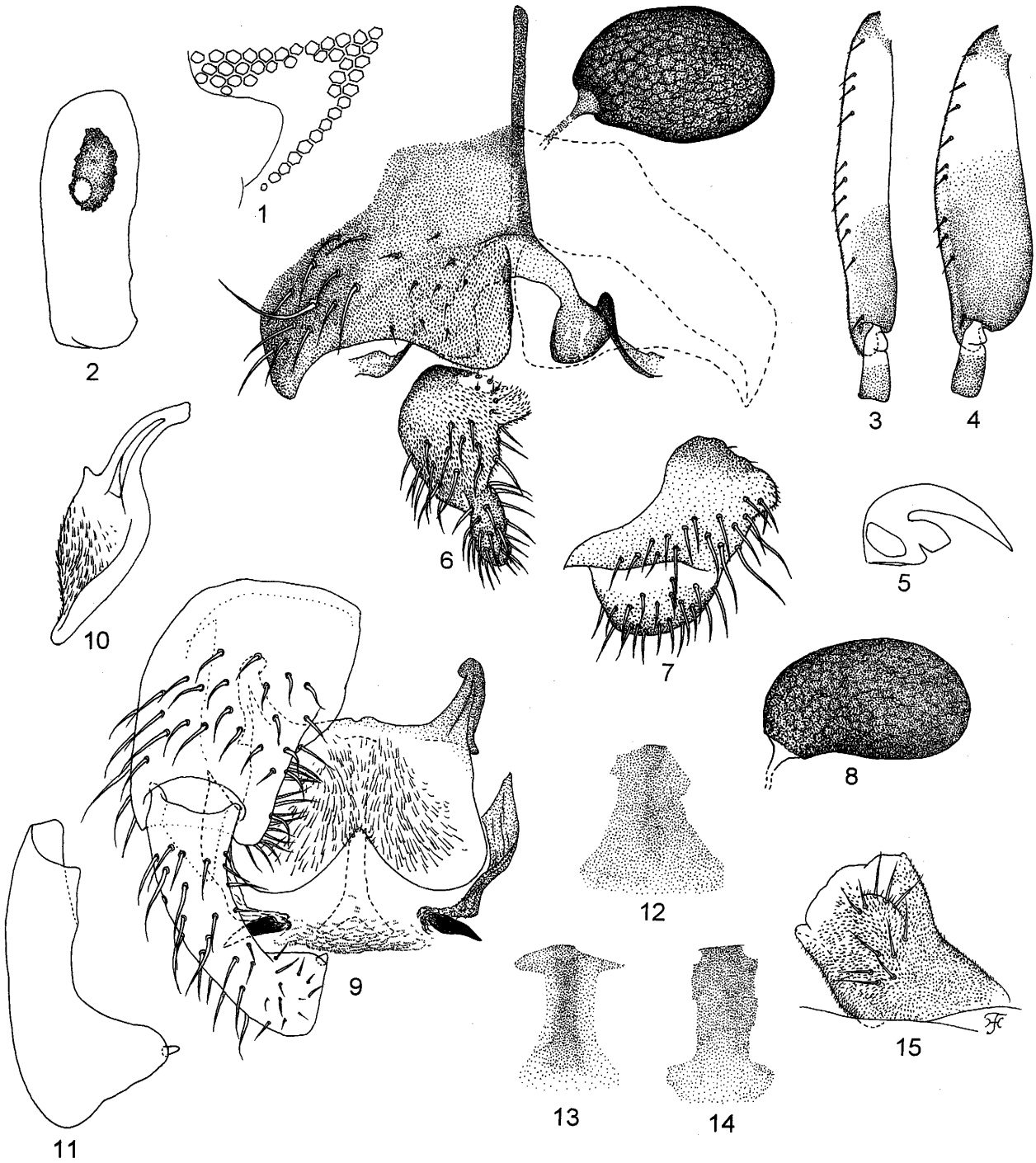
Male. Body length 2.5 mm. ***Head.*** As wide as, or slightly wider than, thorax. Holoptic; upper eye consisting of large facets in 19 vertical columns and 20 horizontal rows. Clypeus dark brown, gray-pruinose, moderately covered with dark simple hairs. Antenna composed of 2+9 segments, dark brown; 1st flagellar segment somewhat elongate, ca. 1.9× as long as 2nd flagellar segment. Maxillary palp brown, composed of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:2.3; sensory vesicle small, ellipsoidal. ***Thorax.*** Scutum brownish black, not shiny, densely covered with yellow recumbent fine hairs, and with several brown upright hairs on prescutellar area. Scutellum brownish black, with many brown upright hairs as well as pale fine hairs. Postscutellum brownish black, bare. Pleural membrane bare. Katepisternum longer than deep, with several fine hairs near upper margin on each side. ***Legs.*** Dark brown to brownish black except basal 2/5 of hind basitarsus and basal 1/2 of 2nd hind tarsal segment whitish yellow. Fore basitarsus slender, ca. 7.9× as long as wide. Hind basitarsus (Fig. 4) enlarged, 3.2× as long as its greatest width, slightly wider than hind tibia but slightly narrower than hind femur. Calcipala and pedisulcus (Fig. 4) well developed. ***Wing.*** As in female except subcosta bare or with a few hairs; length 2.0 mm. ***Abdomen.*** Basal scale brownish black, with fringe of pale long hairs. Dorsal surface of abdominal segments dark brown to brownish black, not shiny, with short dark hairs. ***Genitalia*** (Figs. 9-15). Coxite subquadrate much longer than wide. Style slightly shorter than coxite, broadly truncate apically, twisted, then apical part bent inwards, and with a subapical spine directed inward and forward. Ventral plate lamellate, shorter than wide, with a small medial notch on anterior border, and a deep medial concavity on posterior border, and moderately covered with fine short setae on ventral surface; arms short, stout, and bent inwardly and dorsally. Parameres of normal form, each with a distinct hook. Median sclerite slender, forked apically. Dorsal plate of various forms, much broader than median sclerite. Aedeagal membrane with spinous microsetae. Cercus small, spherical in lateral view, with several hairs.

Pupa. Body length ca. 2.5 mm. ***Head.*** Integument yellowish brown, sparsely or moderately covered with cone-shaped tubercles of various sizes (Fig. 16); antennal sheath also sparsely covered with groups of many cone-shaped tubercles; face with 1 simple (or occasion-

ally bifid), stout trichome on each side (Fig. 17), while frons with 2 slenderer trichomes, of which 1 is much shorter, on each side (Fig. 18). ***Thorax.*** Integument yellowish brown, moderately covered with cone-shaped tubercles of various sizes, with 3 long, stout trichomes mediodorsally (Fig. 19), 2 (1 long, stout, and 1 medium, slender) trichomes mediolaterally (Fig. 20), 1 medium, stout trichome posterolaterally (Fig. 21), and 3 (2 medium, stout and 1 short, slender) trichomes ventrolaterally (Fig. 22), all simple (or occasionally bifid on tip). Gill (Fig. 23) with 4 slender filaments arranged in pairs, each pair with very short stalk, arising from very short basal common stalk; all filaments yellowish brown to dark brown, subequal in length to one another (1.7-2.0 mm) (or 2 filaments of dorsal pair slightly shorter than those of ventrolateral pair), and 2 filaments of dorsal pair slightly thicker than those of ventrolateral pair, at least near base; cuticular surface with distinct annular ridges and furrows, and densely covered with minute tubercles. ***Abdomen.*** Terga 1 and 2 yellowish brown, moderately tuberculate; tergum 1 with 1 medium, slender seta on each side; tergum 2 with 1 medium, slender seta and 5 short, spinous setae (1 or 2 occasionally much stouter than others). Terga 3-8 each with yellowish brown area of varying sizes anteriorly; terga 3 and 4 each with 4 hooks and a few spinous setae on each side; tergum 5 bare; terga 6-9 each with many groups of minute spines directed backward on each side; tergum 6 with 0-3 spine-combs, which are, if present, much smaller than those on tergum 8; tergum 7 with 0-8 spine-combs; tergum 8 with 7 or more spine-combs in transverse row on each side; tergum 9 yellowish brown, with a pair of distinct, cone-shaped terminal hooks (Fig. 24). Sternum 4 with 1 simple or bifid hook submedially, which is subequal in size to those on sterna 5-7, and a few slender setae (1 of which is often somewhat longer and stouter) on each side; sternum 5 with a pair of bifid hooks submedially and a few slender setae on each side; sterna 6 and 7 each with 1 bifid hook submedially and 1 simple hook laterally, and a few slender setae, on each side. ***Cocoon.*** Wall-pocket-shaped, usually with narrow anteroventral connection, compactly woven without open spaces in web, with anterior margin thickly woven, and only slightly extending ventrolaterally; 2.8-3.0 mm long ×1.2-1.4 mm wide.

Mature larva. Body length 5.0-5.3 mm. Body grayish dorsally, mottled to varying extents with dark violet brown color on each thoracic and abdominal segment. Cephalic apotome (Photo. 1) pale on anterior 2/5, dark yellow to yellowish brown on posterior 3/5, with posterior margin somewhat darkened, and with distinct positive head spots. Cervical sclerite composed of 2 small rod-like pieces, not fused to occiput, widely separated medially from each other. Antenna consisting of 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 1st, 2nd and 3rd segments 1.0:1.1:0.7-0.8. Labral fan with 34-36 main rays. Mandible (Fig. 25) with a few supernumerary serrations, as well as 2 usual mandibular serrations; comb-teeth composed of 3 teeth, of which 1st tooth is largest, 2nd is smallest and 3rd is intermediate. Hypostomium (Fig. 26) with a row of 9 apical teeth, of which median and corner teeth are moderately developed, and 3 intermediate teeth on each side are small and subequal in size to one another; lateral serrations moderately developed; 5 or 6 hypostomal bristles lying slightly divergent posteriorly from lateral margin on each side. Postgenal cleft (Fig. 27) medium, miter-shaped, with apex pointed or occasionally rounded, variable in length, 1.0-1.8× as long as postgenal bridge. Thoracic cuticle bare. Abdominal cuticle bare except last segment covered with colorless short setae on each side of anal sclerite. Rectal papilla of 3 simple lobes, without secondary lobules. Anal sclerite of usual X-form, with posterior arms ca. 1.4× as long as anterior ones; basal portion of arms and anterior arms widely sclerotized. Accessory sclerite absent. Ventral papillae small, laterally placed, then, often indiscernible when the larva is viewed laterally. Posterior circlet with ca. 90 rows of up to 15 hooklets per row.

SPECIMENS EXAMINED. HAHAJIMA IS. (E142°07′-E142°11′, N26°36′-N26°43′), OGASAWARA (BONIN) ISLANDS, JAPAN: 1 female (genitalia dissected and mounted on glass slide), pinned, probably light-trapped, 26.IV-9.VI.1958, F.M. Snyder (BMNH); 3 females, 3 males, all reared from pupae (all dissected and mounted on glass slides), 14 pupae, 10 pupal exuviae, 5 mature larvae, 3.VII.1973, K. Saito; 2 pupal exuviae, Kitamura, 20.IV.1996, H. Suzuki; 2 females, light-trapped, 10.V.1996, M. Yoshihara; 5 females, 27.V.1996, M. Yoshihara; 6 females (1 blood-engorged), light-trapped, 17.VI.1996, M. Yoshihara; 3 females, 17.VII.1996, M. Yoshihara; 3 females, 12.VIII.1996, M. Yoshihara; 1 female, netted, 10.VII.1997, K. Takehara. CHICHIJIMA IS. (E142°11′-E142°14′, N27°02′-N27°06′), OGASAWARA (BONIN) ISLANDS: 2 females, light-trapped, Sakaiura, 15.IV.1996, H. Suzuki; 3 pupae, 2 pupal exuviae, 9 mature larvae, Sakaiura, 17.IV.1996, H. Suzuki; 5 females (all blood-engorged), 2 males, light-trapped, 16.IV.1996, H.



Figures 1-15. Female and male adult characters of *Simulium* (*Nevermannia*) *bonninense*. 1, fronto-ocular area of female; 2, 3rd segment with sensory vesicle of female maxillary palp; 3 and 4, basitarsus and 2nd tarsal segment of hind leg, showing calcipala and pedisulcus (3, female; 4, male); 5, tarsal claw of female foreleg; 6, female genitalia (ventral view, female loaned from BMNH), showing 8th sternite, anterior gonapophyses, genital fork, spermatheca with weakly sclerotized tubal base and right paraproct and cercus; 7, right paraproct and cercus (lateral view); 8, spermatheca with pale tubal base (female reared from pupa); 9, male genitalia (ventral view), showing coxite, style of right side, ventral plate, parameres, aedeagal membrane and median sclerite; 10, ventral plate (lateral view); 11, style (ventromedial view); 12-14, dorsal plates in different shapes; 15, male 10th abdominal segment (lateral view) showing small hairy round cercus.

Suzuki; 3 females, 1 male, all reared from pupae, 9 pupae, 2 pupal exuviae, 17 mature larvae, Shigure-dam, 16.IV.1996, H. Suzuki; 4 mature larvae, Ohgiura, 18.IV. 1996, H. Suzuki.

BIOLOGICAL NOTES. Female and male adults of *S.* (*N.*) *bonninense* were captured by light trap from April to August, and some of the females were fully blood-fed, indicating that its life cycle is multivoltine, and its ovarian development is anautogenous. Host animals are unknown. One female adult was netted while flying around the knee of man during day-time. It remains uncertain as to whether this species feeds on man, although villagers often claimed so.

Immature stages of *S.* (*N.*) *bonninense* were found in small, swift-flowing streams partially shaded by shrubbery. Stream beds were mostly rocky and widths were about 50 cm or less. The pupae and larvae attached to grasses trailing in the water from the banks. The larvae collected comprised a final instar and many other earlier instars including a first instar. No other simuliid species was found in all the streams sampled.

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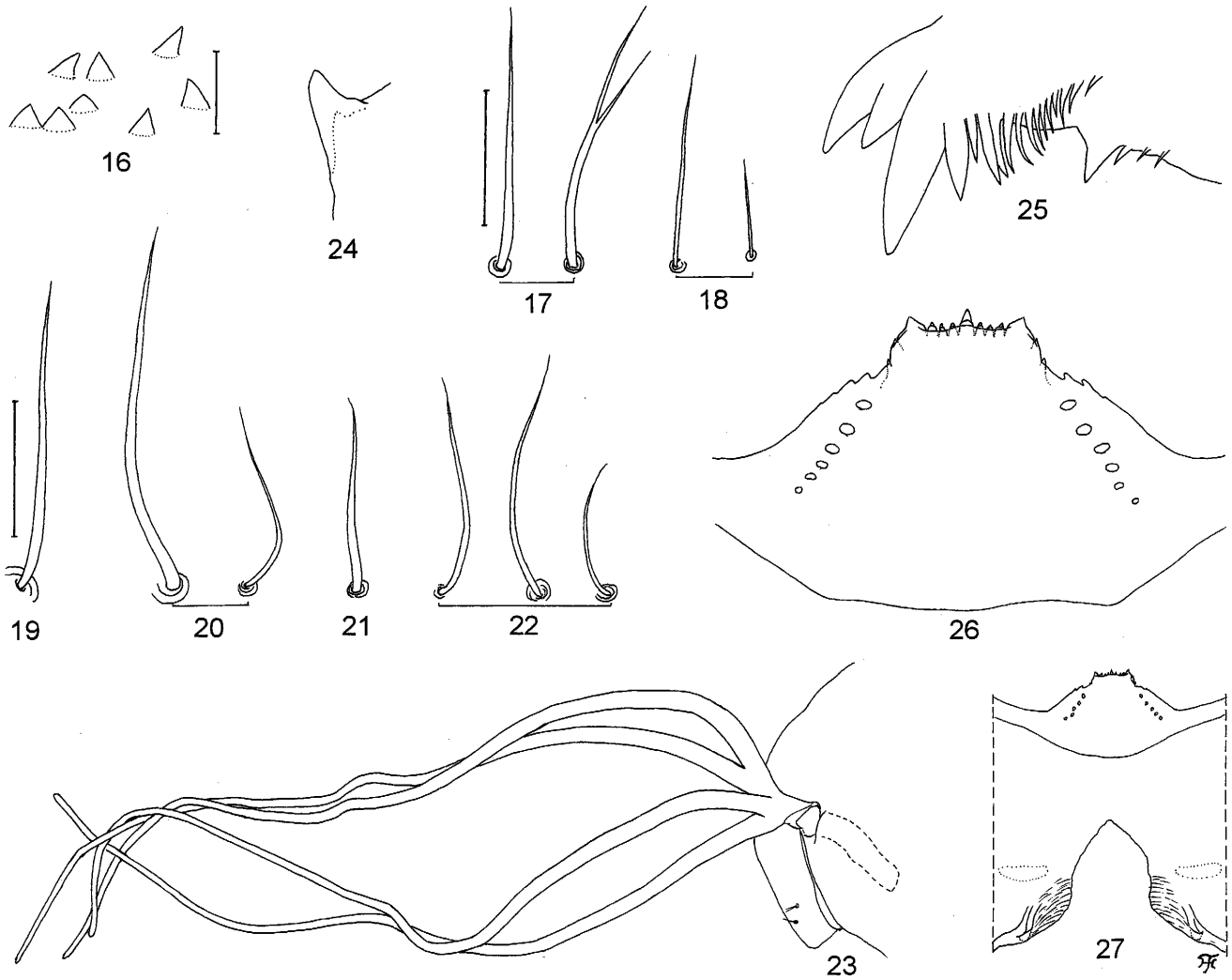

Figures 16-27. Pupal and larval characters of *Simulium* (*Nevermannia*) *bonninense*. 16-24, pupa; 25-27, larva. 16, tubercles on frons (oblique view); 17, facial trichomes (left, of usual simple form; right, of occasional bifid form); 18, frontal trichomes; 19, mediodorsal thoracic trichome; 20, mediolateral thoracic trichomes; 21, posterolateral thoracic trichome; 22, ventrolateral thoracic trichomes; 23, gill filaments (lateral view); 24, terminal hook (lateral view); 25, apical part of mandible; 26, hypostomium (ventral view); 27, head capsule (ventral view) showing postgenal cleft. Scale bars: 0.02 mm for Fig. 16; 0.04 mm for Figs. 17 and 18, and Figs. 19-22.

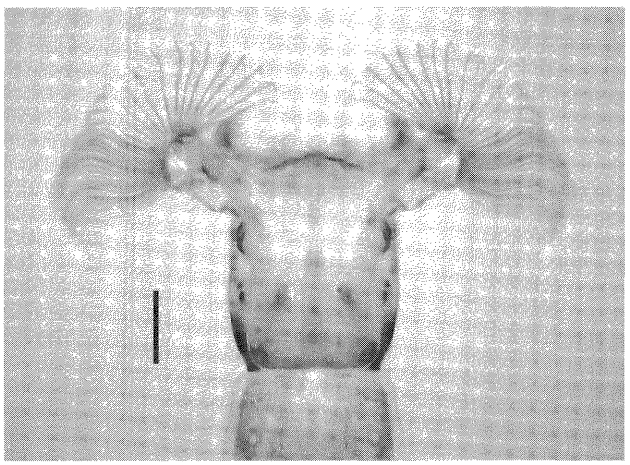

Photo. 1 Larval head (dorsal view) of *Simulium* (*Nevermannia*) *bonninense*. Scale bar: 0.2 mm.

the Ogasawara (Bonin) Islands, Japan.

PRMARKS. Within the subgenus *Simulium* (*Nevermannia*), *S.* (*N.*) *bonninense* is assigned to the *vernum*-group (formerly *latipes*-group, as defined by Crosskey in 1969), by the combination of the following characters: male genitalia with a simple, lamellate ventral plate, an elongate style with a large, inwardly-twisted apex, a single parameral hook per side, and an inverted Y-shaped median sclerite; pupal gill with four slender filaments per side; and larval mandible with supernumerary serrations.

It is worthwhile to note that *S.* (*N.*) *bonninense* has the katepisternum haired in both sexes of adults, female genital fork with a prominent projection directed forwards on each arm, and pupal frons with two trichomes on each side, all of which are rare in the *vernum*-group.

There are five other known species belonging to the *vernum*-group in Japan: *S.* (*N.*) *acmeria* (Ono), *S.* (*N.*) *boldstemta* (Ono) (Ono, 1978), *S.* (*N.*) *larvipilosum* Okazawa (Okazawa, 1984), *S.* (*N.*) *subcostatum* (Takahasi) and *S.* (*N.*) *uchidai* (Takahasi) (Takaoka, 1976). The first two species resemble *S.* (*N.*) *bonninense* by having the forwardly-directed projection on each arm of the female genital fork and two pairs of trichomes on the pupal frons, although there are great differences in many other characters including the shape of the ventral plate and the coloration of the hind legs.

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TAXONOMIC AND ECOLOGICAL NOTES ON *SIMULIUM* (*GOMPHOSTILBIA*) *PALAUENSE* (DIPTERA: SIMULIIDAE) FROM PALAU, MICRONESIA, WITH REDESCRIPTIONS OF ADULTS AND DESCRIPTIONS OF THE PUPA AND MATURE LARVA

HIROYUKI TAKAOKA[1] AND DOUGLAS A. CRAIG[2]

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Abstract: Redescriptions for the female and male, and descriptions for the pupa and mature larva, of *Simulium* (*Gomphostilbia*) *palauense* Stone are given based on reared adults and immature stages recently collected from Palau, Micronesia. *S. palauense* has several remarkable pupal and larval characters, which have not (or very rarely) been found in any other species of the subgenus *Gomphostilbia*, such as, in the pupa, the reduced number (i.e., four) of the gill filaments and the absence of spine-combs on the abdomen; in the larva, the presence of serrations on the lateral margins of the hypostomium, supernumerary mandibular serrations, and accessory sclerites, and the absence of a postgenal cleft. Ecological notes on immature stages are also given.

Key words: black fly, *Simulium*, Simuliidae, Palau, Micronesia

Stone (1964) described three species of Simuliidae from Micronesia. He described *Simulium* (*Gomphostilbia*) *palauense* (then, under the subgenus *Eusimulium*) from five female and 12 male adult specimens from Palau. This species was transferred to the subgenus *Inseliellum* by Crosskey (1989), but has been recently assigned in the subgenus *Gomphostilbia* after one of its paratype males preserved in The Natural History Museum, London (BMNH), was examined (Takaoka and Davies, 1995). The other two species were from Guam, and Chuuk (Truk) State, Federated States of Micronesia.

Larvae of *S. palauense* were collected for the first time by P. S. Cranston at Ngardmau Falls, Babeldoab, Palau in 1997. More larvae and pupae, for the first time, were collected from the same locality by us (D.A. Craig and R.E.G. Craig) in 1998 and adults successfully reared from pupae. In this paper we redescribe the adult female and male, describe the pupa and mature larva from these new specimens, and note briefly on several unusual pupal and larval characters found in this species. Ecological notes on immature stages are also given.

Simulium (*Gomphostilbia*) *palauense* Stone

Simulium (*Eusimulium*) *palauense* Stone, 1964: 633.
Simulium (*Inseliellum*) *palauense:* Crosskey, 1989: 224.
Simulium (*Gomphostilbia*) *palauense*: Takaoka and Davies, 1995: 161; Crosskey and Howard, 1997: 34.

Female. Body length 1.8 mm. ***Head.*** Slightly narrower than thorax. Frons black, shiny, densely covered with whitish recumbent pubescence, interspersed with several brown hairs, except median longitudinal portion narrowly bare; frontal ratio (i.e., greatest width at vertex: narrowest width near antennal base: height) 1.7-1.9:1.0: 1.8-2.4. Frons-head ratio (greatest width of frons: greatest width of head) 1.0:4.0-4.6. Fronto-ocular area (Fig. 1) deep, rounded apically. Clypeus black, shiny, densely covered with whitish recumbent pubescence, interspersed with brown hairs. Antenna composed of 2+9 segments, brownish black except scape, pedicel and

1 Department of Infectious Disease Control, Oita Medical University, Hasama, Oita 879-5593, Japan
2 Department of Biological Sciences, University of Alberta, Edmonton, T6G 2E9, Canada

base of 1st flagellar segment yellow. Maxillary palp composed of 5 segments, dark brown to brownish black, proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:2.3; sensory vesicle (Fig. 2) small, ellipsoidal, ca. 0.25× as long as 3rd segment. Maxillary lacinia with 8-11 inner teeth and 10-12 outer teeth. Mandible (Fig. 4) with ca. 20 small inner teeth and no outer teeth though outer margin is very weakly erose. Cibarium (Fig. 5) medially forming a thinly sclerotized plate folded forward from posterior margin, and with Y-shaped, heavily sclerotized medial projection. ***Thorax.*** Scutum brownish black, shiny, densely covered with brown recumbent pubescence, intermixed laterally and posteriorly with whitish yellow one. Scutellum brownish black, with brown recumbent pubescence and with brown upright hairs near posterior margin. Postscutellum brownish brown, shiny, bare. Pleural membrane bare. Katepisternum dark brown to brownish black, longer than deep, shiny, with many brown hairs. ***Legs.*** Brownish black except fore coxa dark yellow, base of mid tibia white of yellowish white, hind trochanter yellow, basal 2/5 or hind tibia white or yellowish white, basal 2/3 of hind basitarsus and basal 1/2 of 2nd hind basitarsus white; mid and hind tibiae covered with whitish hairs brightly shiny in light on basal 1/3 and basal 3/5 of posterior and outer surface, respectively. Fore basitarsus somewhat dilated, ca. 5.0× as long as its greatest width. Hind basitarsus (Fig. 6) slender, parallel-sided, ca. 6.6× as long as wide, and much narrower than hind femur and tibia, of which greatest breadths are ca. 2.3× and 1.7× as wide as basitarsus, respectively. Calcipala and pedisulcus (Fig. 6) well developed. All femora and tibiae densely covered with scale-like hairs (Fig. 8) on outer surface. Claws (Fig. 9) each with large basal tooth. ***Wing.*** Length 1.5 mm. Costa with spinules and hairs. Subcosta fully haired. Hair tuft on stem vein brown. Basal portion of radial vein fully haired. Basal cell absent. ***Abdomen.*** Basal scale brownish black, with a fringe of pale long hairs; segment 2 dark yellow to light brown except narrow portion near posterior margin brownish black, with light brown tergal plate, white-pruinose and shiny when illuminated, other segments brownish black to black, with dark short hairs; terga 6, 7 and 8 shiny. ***Genitalia*** (Figs. 10 and 11). Sternite 8 bare medially, and with 16-18 dark stout hairs on each side. Anterior gonapophysis triangular, thin, membraneous, densely covered with microsetae, with a few short setae; inner margin well sclerotized and darkened. Genital fork of usual inverted-Y form, with arms curved medially with distinct projection directed forward; base of arms broad with narrow notch inbetween. Paraproct somewhat produced ventrally beyond ventral margin of cercus, covered with 17-23 hairs on outer and ventral surface. Cercus semicircular, with dark hairs. Spermatheca ellipsoidal, well sclerotized except small area at tubal juncture and tube unsclerotized, without internal setae.

Male. Body length 1.6 mm. ***Head.*** Much wider than thorax. Upper eye consisting of 12 vertical columns and 13 horizontal rows of large facets. Face black, white-pruinose. Clypeus black, shiny, white-pruinose, moderately covered with dark hairs, intermixed with whitish yellow scale-like pubescence laterally except median longitudinal portion somewhat widely bare on lower 3/5 of clypeus. Antenna composed of 2+9 segments, yellow except apical 5 or 6 flagellar segments grayish brown; 1st flagellar segment somewhat elongate, ca. 1.5× as long as 2nd flagellar segment. Maxillary palp composed of 5 segments, grayish brown, proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.3; sensory vesicle (Fig. 3) small, ellipsoidal, ca. 0.2× as long as 3rd segment. ***Thorax.*** As in female. ***Legs.*** Light brown to brownish black except fore coxa dark yellow, base of mid tibia white, hind trochanter yellow, basal 1/3 of hind tibia yellow, basal 2/3 and a little more of hind basitarsus and basal 1/2 of 2nd hind tarsal segment white; mid and hind tibiae covered with whitish hairs, which are brightly shiny in light on basal 1/3 and basal 1/2 or a little more of posterior and outer surface, respectively. Fore basitarsus somewhat dilated, ca. 5.1× as long as its greatest width. Hind basitarsus (Fig. 7) slender, parallel-sided, ca. 5.6× as long as wide, and much narrower than hind femur and tibia, of which greatest breadths are ca. 2.3× and 1.9× as wide as basitarsus, respectively. Calcipala and pedisulcus (Fig. 7) well developed. All femora and tibiae densely covered with scale-like hairs on outer surface. ***Wing.*** As in female except length 1.3 mm, and subcosta with a few hairs. ***Abdomen.*** Basal scale brownish black, with a fringe of light brown long hairs. Dorsal surface of abdominal segments black, with brown short hairs; segment 2 with a pair of light brown, large, dorsolateral patches, which are silvery shining when illuminated; segments 5, 6 and 7 each with a pair of shiny, large, dorsolateral patches. ***Genitalia*** (Figs. 12-15). Coxite large, ca. 1.9× as long as wide. Style much shorter than coxite, gradually tapered toward apex, with single apical spine. Ventral plate transverse, much shorter than wide, remarkably produced ventrally, densely covered with microsetae on ventral and posterior surface; basal arms directed outward, then forward. Parameres narrow, each with 2 long parameral hooks

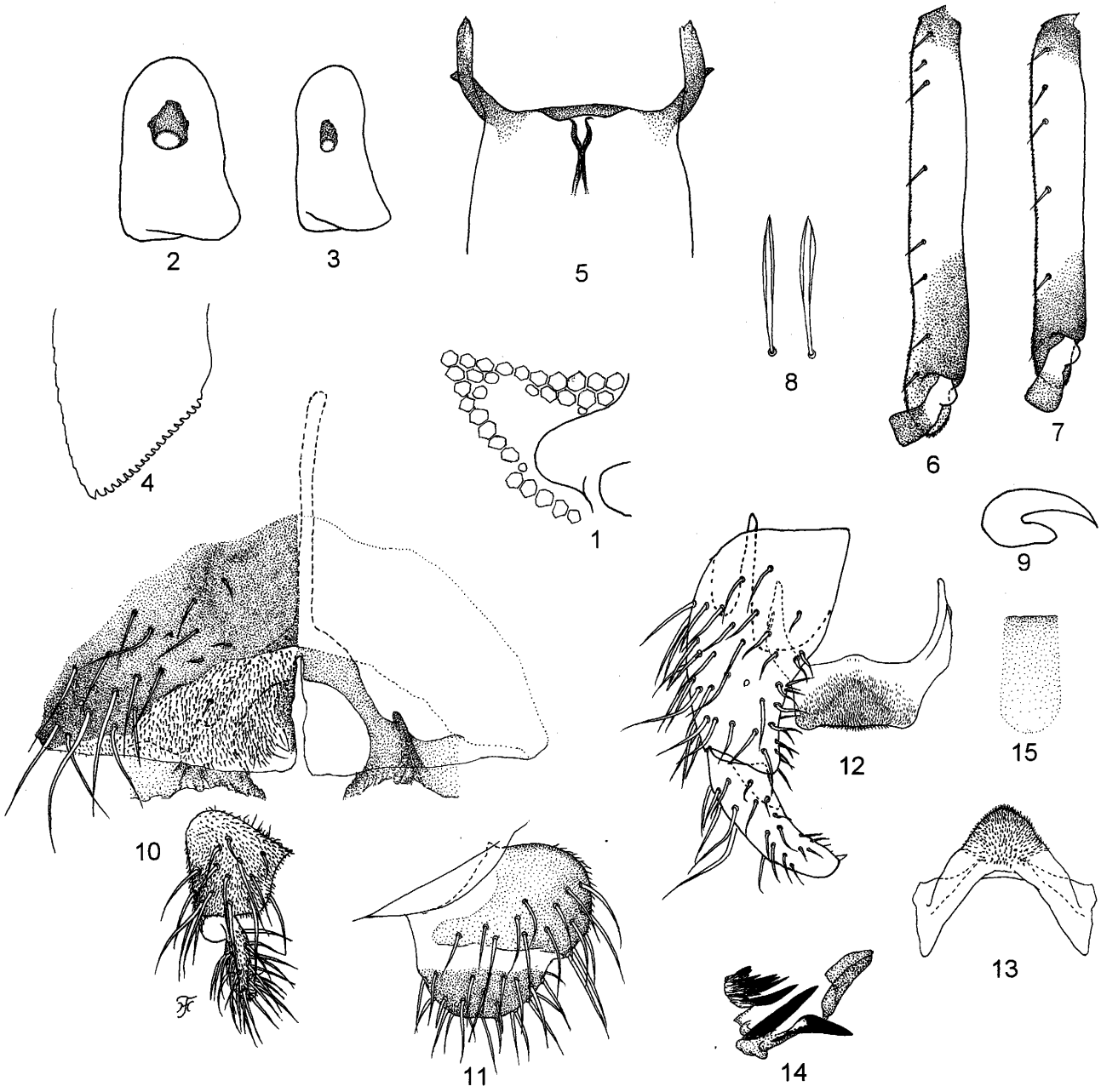
and several shorter ones. Median sclerite broad, nearly parallel-sided, and with apex rounded. Aedeagal membrane uniformly covered with microsetae. Dorsal plate wide, thin, weakly sclerotized, covering aedeagal membrane.

Pupa. Body length ca. 2.0 mm. ***Head*** and ***Thorax.*** Integument light to dark brown, moderately or somewhat sparsely covered with round tubercles; antennal sheath bare. Head with 1 facial and 3 frontal pairs of long, simple trichomes (Fig. 16). Thorax with 11 simple trichomes on each side (Fig. 17): 3 long mediodorsal trichomes, 2 dorsolateral ones (1 long and 1 medium) situated anteriorly, 1 medium dorsolateral one situated posteriorly, 2 short anterolateral ones just below gill base, and 3 mediolateral ones (1 long and 2 short). Gill (Figs. 18 and 19) with 4 simple, slender filaments on each side, arranged in pairs, arising from short basal common stalk; ventral pair of filaments with short stalk, and distinctly longer and thicker than dorsal pair of filaments which are sessile; inner filament in each pair is slightly longer and thicker than outer one; filaments somewhat variable in length by individual pupae, with longest filament (i.e., inner filament of ventral pair) 1.1-1.6 mm, and shortest one (i.e., outer filament of dorsal pair) 0.7-1.2 mm; basal portion of inner filament of ventral pair subequal in thickness to interspiracular trunk; all filaments light to dark brown, gradually tapered toward apex, furnished with annular ridges and furrows, and densely covered with minute tubercles on outer surface. ***Abdomen.*** Terga 1, 2 and 9 grayish brown, well sclerotized, other terga also sclerotized (and also dark-colored) to varying extent. Tergum 1 with 1 medium, simple seta on each side; tergum 2 with 1 medium, simple seta and 5 short, simple setae on each side; terga 3 and 4 each with 4 hooked spines directed forward and 2 short, simple setae on each side; tergum 5 with several short simple setae near posterior margin and comb-like groups of very minute spines anterolaterally on each side; terga 6-8 each with 1 short, simple seta near posterior margin and comb-like groups of very minute spines transversely near anterior margin; tergum 9 with a pair of distinct, simple, terminal hooks (Figs. 20 and 21) somewhat bent anterodorsally, and with apex rounded, and comb-like groups of very minute spines transversely near anterior margin; terga 5-9 without spine-combs. Sternum 3 with a few short simple setae on each side; sternum 4 with 1 simple hook and 3 short simple setae (1 of them somewhat longer and stouter) on each side; sternum 5 with a pair of bifid or trifid hooks submedially on each side; sterna 6 and 7 each with a pair of bifid or trifid submedial hook and bifid or simple lateral hook, and 2 short simple setae, on each side; sternum 8 usually darkened to some extent, with 1 short simple seta on each side; sterna 6-8 each with comb-like groups of very minute spines; sternum 9 darkened, bare; segment 9 with 3 grapnel-shaped hooklets laterally on each side (Fig. 22). ***Cocoon.*** Simple, wall-pocket-shaped (or slipper-shaped), somewhat roughly woven, with several small open spaces in webs, with anterior margin not thickly woven, and not extending ventrolaterally (though 1 of 12 cocoons examined extends slightly); floor woven on posterior 1/2; individual threads distinct; 2.3-2.5 mm long × 1.0-1.3 mm wide.

Mature larva. Body length 3.8-4.3 mm. Body color grayish green, often mottled with violet brown markings on posterior abdominal segments, in most of larvae, or yellowish or yellowish brown in some. Cephalic apotome (Photo. 1) pale except narrow area along posterior margin somewhat darkened, with distinct positive head spots; ventral and lateral surfaces darkened except eye spot areas. Cervical sclerites (Photo. 1), which are absent in earlier instars, rod-like, laterad and not fused to occiput in last instar. Antenna composed of 3 segments and apical sensillum, much longer than stem of labral fan; proportional lengths of 1st, 2nd and 3rd segments 1.0:0.6:1.1. Labral fan with stem slightly flattened, posterobasal corner notched, with 36-40 rays, 0.86 mm in length, medium brown, width of medial rays 0.012 mm, microtrichia of similar length, pattern of longer microtrichia with 3-4 slightly smaller ones between. Mandible (Fig. 23) with comb-teeth decreasing in size from 1st to 3rd; mandibular serrations composed of 1 large and 1 small teeth; supernumerary serrations present. Hypostomium (Fig. 24 and Photo. 2) with a row of 9 apical teeth, of which median and corner teeth are largest, subequal in length to each other, and 3rd and 7th teeth are smallest; lateral serrations well developed; hypostomal bristles 5 or 6 in row, diverging posteriorly from lateral margin on each side. Postgenal cleft (Photo. 2) absent. Thoracic and abdominal cuticle almost bare except small areas on both sides of anal sclerite covered with pale short setae. Rectal papilla compound, each of 3 lobes with 0-2 (mostly 1) finger-like secondary lobules ventrally. Anal sclerite (Fig. 25) X-shaped, posterior arms longer than anterior ones; accessory sclerites present, extending ventrally and continuous to each other (in earlier stages, accessory sclerites are present likewise but they are usually discontinuous at the ventral midline). Ventral papillae present ventrolaterally. Posterior circlet of hooks with

ca. 104 rows of up to 13 hooks per row.

SPECIMENS EXAMINED. Immature larvae, collected at Ngardmau Falls, NW Babeldoab Is., Palau, 14.VII. 1997, P.S. Cranston. Reared adults (7 females and 1 male), all associated with their pupal exuviae and cocoons, 25 pupae, and 10 mature larvae, many other mature and immature larvae collected from first rapid

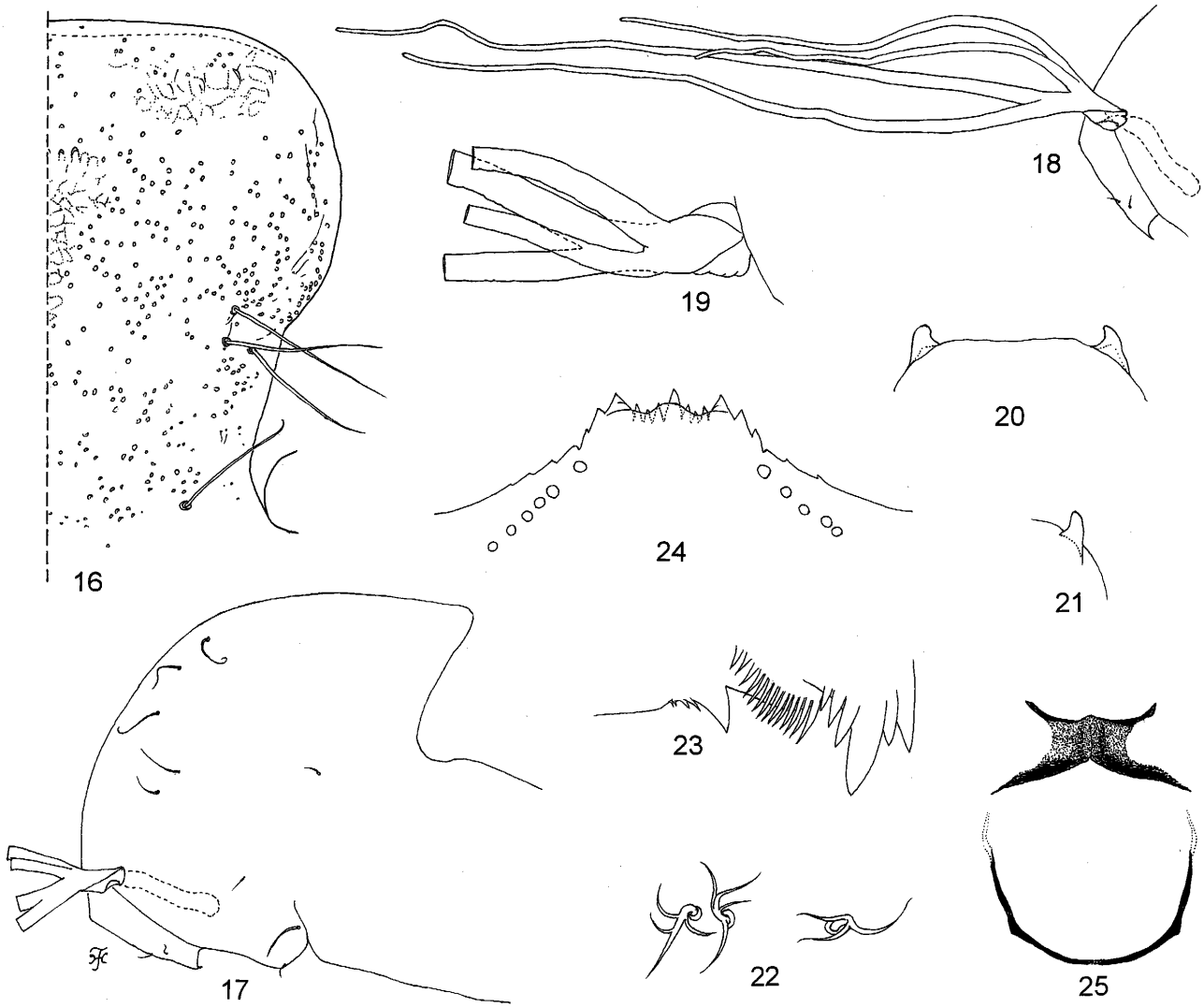


Figs. 1-15 Characters of the female and male of *Simulium* (*Gomphostilbia*) *palauense*. 1, fronto-ocular area of female; 2 and 3, 3rd segments of maxillary palp showing sensory vesicle (2, female and 3, male); 4, apex of female mandible; 5, cibarium of female; 6 and 7, basitarsi and 2nd tarsal segments of hind legs (6, female and 7, male); 8, scale-like setae of femora and tibiae of female; 9, female claw; 10, female genitalia *in situ* (ventral view) showing 8th sternite, anterior gonapophyses, genital fork, paraproct and cercus; 11, paraproct and cercus (lateral view); 12, male genitalia *in situ* (ventral view) showing left coxite and style, and ventral plate; 13, ventral plate (end view); 14, paramere (dorsal view); 15, median sclerite.

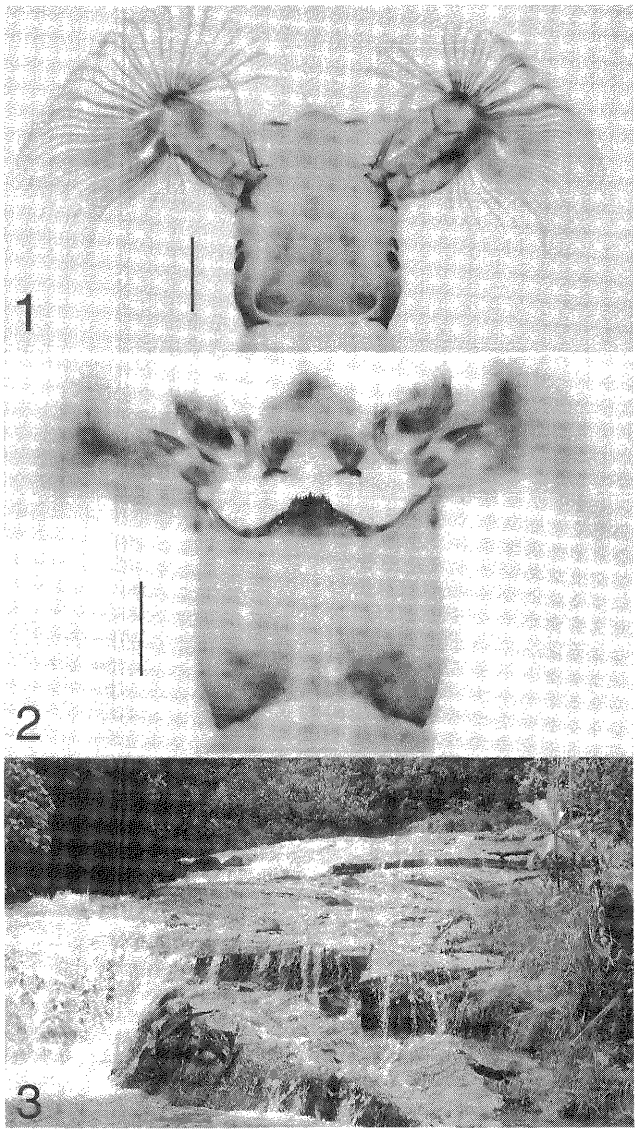
on the main tributary (Photo. 3) of the Diongradid River, near Ngardmau Falls, NW Babeldoab Is., Palau, 4.XII.1998, D.A. Craig and R.E.G. Craig.

DEPOSITION OF MATERIAL. 4 reared adults (3 females, 1 male), together with their pupal exuviae and cocoons, 5 pupae, 5 mature larvae (Bernice P. Bishop Museum, Honolulu), 2 female reared adults, together with their pupal exuviae and cocoons, and 5 pupae, 5 mature larvae (H. Takaoka, Oita Med. Univ.), 2 female reared adults, together with their pupal exuviae and cocoons, 5 pupae, and many other mature and immature larvae (D.A. Craig, Univ. Alberta). All these specimens are preserved in 80% ethanol.

ECOLOGICAL NOTES. The locality, known generally as Ngardmau Falls is a major tourist attraction in Palau. Since road travel is impossible if it has been raining, the falls are best reached by boat, from Koror, up the westernside of Babeldoab, a one-hour voyage to



Figs. 16-25 Characters of the pupa and mature larva of *Simulium* (*Gomphostilbia*) *palauense*. 16-22, pupa; 23-25, mature larva. 16, frons and part of face (left half only); 17, thoracic integument (lateral view) showing arrangement of 11 trichomes; 18, gill filaments (lateral view); 19, basal portion of gill filaments (dorsal view); 20 and 21, terminal hooks (20, end view and 21, lateral view); 22, grapnel-shaped hooklets; 23, apex of mandible; 24, anterior half of hypostomium; 25, anal sclerite with a well developed accessory sclerite.



Photos. 1-3 1 and 2, head capsule of mature larva of *Simulium* (*Gomphostilbia*) *palauense* (1, dorsal view; 2, ventral view; scale bars=0.2 mm); 3, habitat of *S.* (*G.*) *palauense* larvae: first rapid, main tributary of the Diongradid River, near Ngardmau Falls, NW Babeldoab Is., Palau.

the Ucherael jetty and then by vehicle past Ngetbong Village. A one-hour walk up the Diongradid River passes the Medallaiechad waterfall, which is the main attraction, and shortly thereafter reaches a major rapid at N7°35′267″; E134°35′304″. The river here is at 10 m altitude, 8 m wide, between 1-7 cm depth and flowing extremely rapidly, with velocities of 125-300 cm/s. Air temperature was 31.5°C and water temperature 27.0°C under overcast conditions. Since the substrate was black basalt, water temperatures are likely to be much higher in full sunshine. Conductivity was 40μS and pH 8.0. Particulate material in the water was 1.30-1.90 mg/l.

Larvae and pupae were found in large numbers on the rock in full flow and also on leaves that were trapped in shallow water which flows over them. The leaves appeared to be the preferred habitat. Larvae of *S. palauense* do not twist their bodies longitudinally, similar to larvae of the madicolous-flow inhabiting Tahitian *S.* (*Inseliellum*) *cataractarum* (Craig, 1997). From the same rapid, collected was a single immature larva of the other simuliid species. This second species lacks a postgenal cleft like *S. palauense*, but has well-developed ventral papillae placed ventrally, no supernumerary serration on the mandible, and no accessory sclerite around the posterior circlet of hooks. No other invertebrates were taken except a few Chironomidae larvae and aquatic pyralid lepidopteran larvae.

Since this is the only locality sampled for immature stages of simuliids in Palau, and *S. palauense* larvae appear specialized for fast flow, it is possible that smaller streams, yet unsampled, will have the second or other species.

REMARKS. Female and male adults reared from pupae collected by un (D.A.C. and R.E.G.C.) are conspecific to those of *S. palauense* described by Stone (1964), according to the comparison of the genitalia, and the shape and coloring of the body and legs. This result confirms that the association of both sexes of adults of *S. palauense* made by Stone was correct. The morphological characters of the present adult specimens agree with the original descriptions and illustrations given by Stone (1964) except a few characters. In the original, terga 8 and 9 of the female abdomen were stated to be shiny, but in fact terga 6, 7 and 8 (and also 9) are all shiny; in addition, gray-pruinose areas were mentioned to be present at least on sides of tergum 2 of the male abdomen, but these are found on terga 2, 5, 6 and 7; the female calcipala is large but not reaching the apex of the second tarsal segment, though noted so in the original description.

It is noteworthy that *S. palauense* has several remarkable pupal and larval characters which are not (or very rarely) possessed by any other species of the subgenus *Gomphostilbia*. In the pupa, the absence of spine-combs on the abdomen has not been recorded previously in this subgenus, though this is one of the key characters of the subgenus *Wallacellum* (Takaoka, 1983); the reduced number (i.e., four) of the gill filaments has been known only in a few species of the *ceylonicum*-group collected from Sulawesi and Flores, Indonesia (Takaoka, unpublished data) and in one

species from the Solomon and Bougainville Islands (Takaoka, 1994, 1995). In the larva, three characters, i.e., the absence of a postgenal cleft, the presence of serrations on the lateral margins of hypostomium, and the presence of supernumerary mandibular serrations, have not been found; in addition, this species has accessory sclerites on the last abdominal segment, a character being reported only in a few *Gomphostilbia* species collected from Papua New Guinea (Smart and Clifford, 1965) and Irian Jaya (Takaoka, unpublished data).

Although having such unusual characters, *S. palauense* belongs in the subgenus *Gomphostilbia* by having the following combinations of characters: adult thorax with the bare pleural membrane and haired katepisternum, wing with the basal portion of radial vein fully haired, male genitalia with a ventral plate hairy on its ventral and posterior surface, and with several parameral hooks, pupal head with three pairs of frontal trichomes, pupal abdomen with grapnel-shaped hooklets on the sides of the last segment, and larval abdomen with ventral papillae. This species is further assigned in the *batoense*-group, defined by Takaoka and Davies (1996), by having the antenna of both adult sexes 11-segmented, the female tarsal claw with a large basal tooth, and the male hind basitarsus parallel-sided, though the pupal gill filaments of this species are four in place of eight.

Larvae of *S. palauense* show considerable morphological convergence with the madicolous-flow inhabiting larvae of *S. cataractarum* of Tahiti (Craig, 1997). While there is not the same reduction in number of labral fan rays, the fan stem is similarly slightly flattened and possesses a posterobasal notch. Craig and Currie (1999) suggest that this fan-stem shape is used to manipulate madicolous flow into the fans. The postgenal cleft in *S. cataractarum* while present, is reduced. Body shape is also similar, in that both have amphora-shaped posterior abdomens, expanded rapidly at the 5th abdominal segment and smoothly decreased posteriorly; probably an adaptation for fast flow.

Both species possess a sclerite that is continuous around the circlet of hooks. In *S. cataractarum*, this appears to be an extension of the accessory sclerite, which remains very obvious, and is connected dorsally with the anal sclerite proper. However, the extension is often discontinuous at the ventral midline. In *S. palauense* this sclerite is constructed differently and is not connected to the anal sclerite proper, but is continued strongly completely around the circlet. The only evidence of the accessory sclerite, as known in other subgenera, is a paler dorsolateral area (Fig. 25).

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