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Review

ETHICS OF TROPICAL MEDICINE: PEOPLE-CENTERED HEALTH PROMOTION
REFLECTIONS FROM 20 YEARS EXPERIENCES OF MEDICAL COOPERATION IN BANGLADESH

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I would like to approach this giant theme from my own experience in Bangladesh and the ethical reflections for the last 20 years for tuberculosis control. I would especially address to young people who are interested in studying for the health promotion in the tropics. My talk can be summarized with the following 4 key terms: 1) Go to the people, 2) Health by the people, 3) DOTS and HSSR, and 4) Institutional Capacity in Japan.

Go to the People

To make an effective work or study, we need always to have a close contact with the fields where people live, and to keep learning from the fields. For example, if we want to do any study in the tropics and prepare a questionnaire in Japan, we need to know that our knowledge may be limited and needs to be largely changed according to the real needs of the people. Our attitude should be that we are trying to listen to the people and to find the problems and share the findings together with the people. In other words, our work or study should not be 'our program-oriented', but 'people's problem oriented'.

People's health awareness becomes higher in the improvement of life style (Fig. 1)

I once made a health awareness survey in a southern district of Bangladesh, asking people about their health problems in their villages with a semi-open questionnaire. The answers were recorded as they said, and classified later as 'preventive', 'curative' or 'no interest'. For example, 'no toilet' or 'no safe drinking water' was classified as preventive, and 'no doctor' or 'no drugs' was as curative. They were analyzed according to community group activity with or without health volunteer. It was then found that people in a group activity with health volunteer had more preventive idea than those without health volunteer. People not in a group had the least preventive ideas. I have learned that health awareness grows more in the people's own activities for improvement of their living condition or life style.

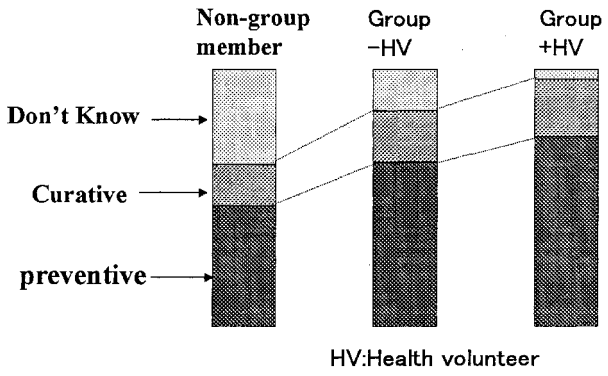

Figure 1 What is the health problems in your village? Health awareness rises by group activity and health volunteer.
(Ishikawa *et al*.: Promotion of PHC in Rural Bangladesh, NATAB, 1995)

TB prevalence survey in Manikganj Thana (1984)

I have once assisted a survey for a Japanese bacteriologist to conduct a tuberculosis prevalence survey by the bacteriological examination in rural Bangladesh. The study area (Manikganj Sub-district) was typically rural, 50 km away from Dhaka and had a population of 150,000. I made a sampling of 50 villages, and 7,000 adult population was selected randomly. Sputum specimens were collected from people who were screened by a symptomatic survey. The bacteriologist made both

smear and culture examination of the specimen for acid fast bacilli, especially *Mycobacterium tuberculosis*. It was then estimated that the sputum smear AFB positive rate was 0.3-0.5%, and the culture positive tuberculosis rate was 0.4-0.6%. Nearly 30% of them had a resistance to any anti-TB drug, and all of them had a previous treatment history. Among the cultured specimen, Atypical Mycobacteria (AM or MOTT) grew in high proportion possibly due to contamination. They were of course excluded from the analysis. The findings were of great value and are useful still now as there has been no other study like this since then. The study result was first reported in Japanese local journal, but the bacteriologist lost his interest in publishing it internationally as he was a laboratory oriented researcher and disappointed too much with the partial contamination in the field survey. I myself was also not much motivated in the publication simply because I was then busy in primary health project though assisting the survey as a collaborator. Another weakness was that there was no local ownership for this survey. The results should have been shared more in Bangladesh and internationally as well. In this sense, we made a big mistake.

Health by the People

After the survey in Manikganj people asked us how we would deal with the detected 24 TB patients. They requested us to make a better system of treating TB patients. I proposed them that I would be ready to help them if they wanted to develop a system of TB control in the community with active participation of the community. This was a great chance for me too, as I had wanted to try a community based TB program somewhere. A chance often comes before us when we are really looking for it.

Community based TB program (Table 1)

The Manikganj sub-district consists of 147 villages with 150,000 population. The medical services were very limited and the majority of people were using unqualified practitioners for the medical care. A local NGO called BRAC (Bangladesh Rural Advancement Committee) was actively organizing group activities and about 200 health volunteers (HVs) were already working as bare-foot doctors in the BRAC health program. But nothing had been yet done for TB. We planned to make a system of utilizing the HVs. They could screen the suspects with a chronic cough and give drugs to the patients. With my minimum advice, this program started in 1984 and it has been continuously sustained in the sub-district even after I left the country in 1986. Since then I visited there only twice a year, and my local assistant made monthly supervisory visits (Phase I). I never thought that the expansion to other areas in different settings was possible without the guidance by a specialist like me. However BRAC, using the experience in Manikganj applied the approach to 10 sub-districts (Phase II), and then to 60 sub-districts covering over 600,000 population currently as a part of national program (Phase III). As of 1998, the number of the treated TB patients counts over 13,000, and that of the HVs involved is about 13,000. This approach has been very useful in rural and remote areas where the access to the government health facilities is limited. WHO has recognized it and the achievements have been published in Lancet by BRAC staff two times. Though I could not publish any paper on this topic with my name, I am very proud that the local people have gained the ownership of the program. We could say ultimately that the local people are the subjects for their health improvement.

Table 1 Community based TB Program Development (BRAC: 1984-98)

	Phase I 1984-89	Phase II 1990-94	Phase III 1995-(98)
Thana	1	10	60
Pop.(×1,000)	150	1,800	15,000
VHV	200	1,500	10,000
TB patients	280	3,500	13,000
Cure%	66-79%	81%	86%

DOTS (national TB program package) and HSSR

The current global strategy for TB control called as Directly Observed Treatment, short course (DOTS) has been derived from the analysis of the successful programs in the world. This package program has an excellence as policy science in that the government commitment is given the highest importance; cost effective idea for the available technology is applied; for example, diagnosis is made simply by microscopic examination of sputum smear, but most costly but effective short course regimen of anti-TB drugs are used; a regular monitoring system is included in the program and a simplified 'cure rate' is used for the criteria of successful program. The DOTS however needed an operational research on how it could be applied in Bangladesh.

Model area development through participatory action research

When I went to see Dr. Ahsan Ali, then the director

of national TB program in 1991 for my regular visit to Bangladesh, he asked for my help to conduct a research, as he was a professor of medicine. Then I proposed him to do an operational research through a pilot model area development, as the new TB project had been prepared under the World Bank and WHO. This big scale project was being introduced by WHO initiative but no proper pilot area had been tried.

Two thanas (sub-districts) near Dhaka were then chosen for the model trial and TB services were introduced at the thana health center. Participatory action research (PAR) was used for the duration of 2 years. This is a kind of operational research method, used for finding a suitable way of proposed program and it is also useful to make the staff and people involved lively through their active participation. The process and outcomes in this PAR was excitingly greater than we expected. Some new ideas were introduced in the main project, and above all, the central managerial staff gained the experience of supervising the periphery centers and understanding the local reality more deeply through periodical workshops.

Health research and empowerment

From the above experience, I come to realize that the health systems and services research (HSSR) is very important to make a real impact to the country. At the same time the real empowerment of the people both in the government and community can be made through health research.

Institutional capacity in Japan

The last key term is institutional capacity. This means the need for strengthening the institutional capacity in Japan as a base for the field activities. In my case, the Research Institute of Tuberculosis has been a base for my work in Bangladesh. But generally speaking, this capacity is still weak in Japan.

Ethics of Tropical Medicine

Ethics is an attitude to think of the one's own deeds by standing on the other's standpoint. It can be also an attitude of the dialogue or interaction between the subject and the object of any action (The Ethics of Knowledge, by Y. Kobayashi and Y. Funabiki, Tokyo University Press, 1996). Here lies a basis for hope, peace or social justice. I would like to discuss what tropical medicine is through ethical reflections based on my own experiences in Bangladesh in the area of tuberculosis control.

Where is the place for tropical medicine?

The experiment in the laboratory or analysis at the study room is one, and the field experiment or field study is the other. Some people spent most of the time in the laboratory under the name of tropical medicine, and others are too busy working in the fields under the same name or international health. But I would say that both are equally needed for tropical medicine.

Who is the subject of tropical medicine?

The subject or actor of tropical medicine is usually a researcher outside the tropics or community. In this case, local people in the tropics are just an object of the study. However a local researcher, workers and even people should be the subject of any research as well. The external researcher and local people need to cooperate and collaborate equally. There are various kinds of local people, including local researchers, supervisors, community level workers, and community people. We need to clarify for whom and with whom we are working, and to whom we should feed back the outcomes of the research. And we need to be aware that the local rich or local researcher cannot often represent the local people.

Table 2 Perspectives of Tropical Medicine

	Present	Future adoption/correction
Scientific ground:	・Positivist Science	・Applied, Practical Science
Purpose:	・Creation of new knowledge ・Publication	・Health promotion ・Health system improvement
Methods:	・positivism ・Qualitative	・Action oriented ・Social science ・Qualitative
Owner:	・Researcher (Authorship)	・People (Ownership)

Who is the owner of the research outcome of tropical medicine? (Table 2)

Conventionally tropical medicine has been positivist science. This characteristic is basically important but we need to know its limitation as well. It has a danger that the importance of the knowledge often lies only in the authorship. A new horizon for tropical medicine to overcome this is to strengthen the aspects of applied science, social science or qualitative analysis, and to change of our awareness that the knowledge belongs to the people or the people have the ownership of the knowledge.

Conclusion

In summary, the aims of tropical medicine are; to work or conduct a study for better health collaboratively with the people in the tropics; to share equally the study results; to support the people in the tropics to work and study by themselves for their own health promotion; and to share internationally the health problems in the tropics through our work in the laboratory or publications. For this we need to strengthen more the institutional capacity in Japan.

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PRELIMINARY REPORT OF FAUNISTIC SURVEYS ON BLACK FLIES (DIPTERA: SIMULIIDAE) IN SUMATRA, INDONESIA

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Abstract: Collections of pupae and larvae of black flies (Diptera: Simuliidae) were carried out in 1992 and 1994 at 59 sites in four provinces of Sumatra, Indonesia. As a result, 22 simuliid species including 12 species newly recorded from Sumatra were identified, bringing the total number of simuliid species recorded from Sumatra to 26. All these species were assigned to the genus *Simulium* s. l. and placed into three subgenera, i.e., *Gomphostilbia* (12 spp.), *Nevermannia* (3 spp.), and *Simulium* s. str. (11 spp.). Further groupings were made at the species-group level within each subgenus. The simuliid fauna of Sumatra is Oriental in character, being very closely related to those of Peninsular Malaysia and Java. Infections with mermithids, microsporidians and/or fungi due to *Coelomycidium* sp. were found in the larvae of 10 of the 22 black-fly species examined.

Key words: Simuliidae, fauna, Sumatra, mermithid, microsporidian fungus

The simuliid fauna of Sumatra, Indonesia was first studied by Edwards (1925, 1934), who reported nine black-fly species. Later in 1977 Dr. Rolf Glatthaar collected several simuliid species including a new species from North Sumatra, which was described later as *Simulium glatthaari* by Takaoka and Davies (1995a).

In 1992 and 1994 we made surveys on black flies at various localities of four provinces in Sumatra, and collected 22 species, of which three new taxa were already described (Takaoka and Sigit, 1997).

In this paper we report the results of preliminary identification of these 22 black-fly species including 12 ones newly recorded from Sumatra, together with those of the examinations of larval specimens for infections with mermithids, microsporidians and fungi due to *Coelomycidium* sp.

COLLECTION METHODS AND CLASSIFICATION

The methods of collecting simuliid pupae and larvae in flowing waters and rearing pupae until adults emerged were mentioned in Takaoka (1983). The classification of each black-fly species was done at the generic and subgeneric levels by the definitions and keys made by Crosskey (1967, 1969), and at the species-group level by those proposed by Takaoka and Davies (1996).

The black-fly larvae collected were examined under a dissecting microscope for mermithid, microsporidian and fungal infections. These infections were diagnosed in appearance by the signs of symptoms of the larval body characteristic for each group of pathogens (Crosskey, 1990).

COLLECTION DATA

For each site surveyed, collection data such as a brief description of flowing water, water temperature, altitude, its location, and date, are given below by provinces (only available data are shown). All samples were collected by H. Takaoka.

Lampung

Surveys were carried out at two sites (L-1 and L-2) near Mutaralam, and five sites (L-3 to L-7) along the road between Liwa and Gunungkemala in the Barisan Range.

L-1. An outlet small stream 0.3 cm wide slowly running from the pond, shaded, water temperature 23°C,

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altitude ca. 900 m, at Batu Kebayan, west of Mutaralam, 30.VII.1992.

L-2. An irrigation ditch ca. 1.0 m wide, exposed to the sun, slowly flowing in a paddy field, water temperature 23°C, altitude ca. 900 m, at Batu kebayan, nearer to Mutaralam than L-1, 30.VII.1992.

L-3. A brook ca. 0.5 m wide, shaded, slowly flowing, water temperature 20°C, just south-west of Liwa, 31.VII.1992.

L-4. A brook 0.3-1.0 m wide, partially shaded, fast flowing down on the rocky streambed, just south-west of L-3, 31.VII.1992.

L-5. A rivulet 0.2-0.3 m wide, shaded, moderately flowing on the rocky streambed, water temperature 21°C, near L-4, 31.VII.1992.

L-6. A creek 2.0-3.0 m wide, partially shaded, rapidly or moderately flowing down among large stones, near L-5, water temperature 22°C, 31.VII.1992.

L-7. An irrigation ditch 0.3 m wide, exposed to the sun, slowly flowing in a paddy field, water temperature 23°C, 31.VII.1992.

Bengkulu

Surveys were maded at six sites around the northern shore of the Lake Ranau.

B-1. An outlet river 10-20 m wide, originated from the Lake Ranau, exposed to the sun, moderately flowing, water temperature 26°C, at Bandingagung, 29.VII.1992.

B-2. A small stream 0.3-0.5 m wide, partially shaded, running in a paddy field, water temperature 22°C, at Desa Surabaya, near Bandingagung, 31.VII.1992.

B-3. A rivulet 0.5-1.0 m wide, shaded, slowly flowing down in a palm forest and a cultivated land for coffee plantation, water temperature 26°C, altitude ca. 350 m, at Dusun Pelawi, along the road from Simpangsender to Muaradua, 29.VII.1992.

B-4. A small stream 0.2 m wide, shaded, moderately flowing in the bush down to the Lake, water temperature 23.5°C, altitude ca. 500 m, near Simpangsender, along the road from Simpangsender to Liwa, 29.VII.1992.

B-5. A ditch 0.2-0.3 m wide, exposed to the sun, slowly flowing, water temperature 24°C, altitude ca. 500 m, near B-4, 29.VII.1992.

B-6. A creek, called Air Mujin, 2.0-3.0 m wide, partially shaded, moderately flowing down to the Lake, water temperature 24°C, altitude ca. 500 m, at Tanjungjati, along the road between Simpangsender and Liwa, 29.VII.1992.

West Sumatra

Surveys were carried out in two areas, one around Padang and the other around Bukittinggi. In the Padang area, collections were made at three sites (W-1 to W-3) along the road between Painan and Taratak running along the Indian Ocean coast, at seven sites (W-4 to W-10) along the road going up from Lubukbargalung to Lubuksulasih, at one site (W-11) along the road from Lubuksulasih to Talang, and at six sites (W-12 to W-17) along the road between Lubuksulasih and Alahanpanjang, and at five sites (W-18 to W-22) between Alahanpanjang and Surian.

W-1. A small stream 0.3-1.0 m wide, shaded, fast running down on the rocky streambed, water temperature 24°C, altitude ca. 120 m, just north of Painan, 10.VIII.1994.

W-2. A ditch 0.2-0.4 m wide, slowly flowing along the road, shaded, water temperature 25°C, altitude ca. 140 m, approximately half way between Painan and Pasarkuok, 10.VIII. 1994.

W-3. A brook 1.0 m wide, exposed to the sun, slowly flowing, water temperature 30°C, altitude ca. 120 m, at Tigosakato, Tanjung Kandis, 10.VIII.1994.

W-4. An irrigation ditch 0.8 m wide, slowly running along the road, exposed to the sun, water temperature 22°C, altitude ca. 260 m, about half way from Lubukbargalung to Lubuksulasih, 11.VIII.1994.

W-5. A rivulet 0.3 m wide, shaded, moderately running in the bush, water temperature 19°C, altitude ca. 960 m, just north of W-4, 8.VIII.1994.

W-6. A rivulet 0.3-0.5 m wide, shaded, slowly or moderately flowing in a pine forest, water temperature 19°C, altitude ca. 1,060 m, just before the mountain pass, back of the restaurant, 8.VIII.1994.

W-7. A ditch 0.3 m wide, shaded by bush, slowly flowing, water temperature 19°C, altitude ca. 1,100 m, near the mountain pass, 8.VIII.1994.

W-8. A creek 1.0-3.0 m wide, shaded, moderately flowing in the forest, water temperature 20°C, altitude ca. 1,000 m, just over the mountain pass, 8.VIII.1994.

W-9. A stream ca. 1.0 m wide, exposed to the sun, moderately flowing, water temperature 19°C, altitude ca. 980 m, near Lubuksulasih, 8.VIII.1994.

W-10. A stream ca. 1.0 m wide, partially shaded, moderately flowing in an open land, water temperature 20°C, altitude ca. 950 m, at Lubuksulasih, 8.VIII.1994.

W-11. A river 4.0-5.0 m wide, exposed to the sun, moderately flowing in a paddy field, water temperature 20°C, altitude 970 m, near Talang, 9.VIII.1994.

W-12. A ditch 0.2 m wide, slowly flowing along the road, exposed to the sun, water temperature 21°C, alti-

tude ca. 1,230 m, near Lubuksulasih, along the road to Alahanpanjang, 9.VIII.1994.

W-13. A ditch 0.3 m wide, exposed to the sun, slowly flowing, 19.5°C, altitude ca. 1,230 m, 9.VIII.1994.

W-14. An irrigation ditch 0.3 m wide, exposed to the sun, slowly or moderately running, water temperature 20°C, altitude 1,280 m, just south of W-13, 9.VIII.1994.

W-15. A stream 2.0-5.0 m wide, exposed to the sun, slowly flowing in a swampy land, water temperature 18°C, altitude 1,450 m, at Dusun Rawang Godang, Lembang Jaya, Solak, 11.VIII.1994.

W-16. A ditch 0.2-0.4 m wide, exposed to the sun, slowly flowing, water temperature 23°C, altitude ca. 1,500 m, at Sungai Silah, Lembang Jaya, Solak, 22.VIII.1994.

W-17. A stream 0.4-0.6 m wide, shaded, moderately running in a natural forest, water temperature 16°C, altitude ca. 1,580 m, near W-16, just south of the Lake Dibawah, 21.VIII.1994.

W-18. A stream 3-4 m wide, exposed to the sun, moderately running in a paddy field, water temperature 21°C, altitude ca. 1,460 m, just south of Alahanpanjang, along the road to Surian, 12.VIII.1994.

W-19. A small stream 0.3-0.5 m wide, exposed to the sun, slowly running down from a forest and joining W-18, water temperature 20°C, altitude ca. 1,470 m, 12. VIII.1994.

W-20. A stream 0.4-1.0 m wide, exposed to the sun, moderately flowing, water temperature 20°C, altitude ca. 1,090 m, south-east of W-18, 11.VIII.1994.

W-21. A stream ca. 1.0 m wide, shaded, moderately flowing, water temperature 22°C, altitude ca. 1,110 m, south-east of W-20, 11.VIII.1994.

W-22. A ditch 0.2-0.3 m wide, exposed to the sun, moderately running along the roadside, water temperature 23°C, altitude ca. 1,110 m, at Surian, 11.VIII.1994.

In the Bukittinggi area, collections were made at eight sites (W-23 to W-30) in and around the Mt. Singkarak, at four sites (W-31 to W-34) along the road from Payakumbuh to Kotoalam, and at four sites (W-35 to W-38) along the road from Bukittinggi to Lubuksikaping.

W-23. An irrigation channel 0.4-0.6 m wide, exposed to the sun, slowly flowing in a paddy field, water temperature 25°C, altitude ca. 190 m, at Pasir, Kekambil, Kayutanam, 14.VIII.1994.

W-24. A river ca. 10 m wide, exposed to the sun, moderately or rapidly flowing, water temperature 20°C, altitude ca. 560 m, at Airmancuur, along the road from Kayutanam to Padangpanjang, 14.VIII.1994.

W-25. An irrigation channel 0.2-0.3 m wide, partially shaded by bush, moderately flowing, water temperature 17°C, altitude ca. 1,400 m, at Sawararas, near Koto Rarang, on the northern slope of Mt. Singkarak, 15.VIII.1994.

W-26. A small stream 0.3 m wide, shaded, moderately flowing down in a natural forest, ca. 300 m south from W-25, water temperature 16°C, altitude ca. 1,460 m, 15.VIII.1994.

W-27. A brook 2.0-3.0 m wide, exposed to the sun, moderately flowing, water temperature 19°C, altitude ca. 1,400 m, in the Mt. Singkarak, at Ganting Singgalang, Tanahdatar, 19.VIII.1994.

W-28. A creek 4.0-6.0 m wide, exposed to the sun, moderately flowing, water temperature 20°C, altitude ca. 1,100 m, on the way from Kotabaru to the peak of Mt. Singkarak, 16.VIII.1994.

W-29. An irrigation ditch 0.5-0.7 m wide, exposed to the sun, slowly flowing, water temperature 26°C, altitude ca. 1,000 m, near W-28, 16.VIII.1994.

W-30. A ditch 0.3-0.4 m wide, exposed to the sun, slowly flowing along the roadside, at the eastern shore of the Lake Maninjau, just south of Maninjau, altitude ca. 500 m, 12.VIII.1994.

W-31. A ditch 0.2-0.3 m wide, exposed to the sun, slowly flowing along the roadside, water temperature 23°C, altitude ca. 530 m, near Kotoalam, 17.VIII.1994.

W-32. A rivulet 0.4-0.6 m wide, shaded, slowly flowing in a natural forest, water temperature 22°C, altitude ca. 570 m, just south of W-31, 17.VIII.1994.

W-33. A brook 1.0 m wide, exposed to the sun, slowly flowing, water temperature 21°C, altitude ca. 600 m, 17.VIII.1994.

W-34. A rivulet 0.5 m wide, exposed to the sun, slowly flowing, water temperature 21°C, altitude 820 m, half way between Payakumbuh and Kotoalam, south of W-33, 20.VIII.1994.

W-35. A river 10-15 m wide, partially shaded, moderately or fastly flowing in a natural forest, water temperature 23°C, altitude ca. 340 m, in Pasaman, half way between Bonjol and Lubuksikaping, 18.VIII.1994.

W-36. A ditch 0.3 m wide, exposed to the sun, slowly flowing along the roadside, water temperature 23.5°C, altitude ca. 240 m, at Bonjol, Pasaman, 18.VIII. 1994.

W-37. A ditch 0.3 m wide, exposed to the sun, very slowly flowing in a paddy field, water temperature 29°C, altitude ca. 300 m, at Tanjung Bunga, just south of W-36, 18.VIII.1994.

W-38. A rivulet 0.3-0.4 m wide, exposed to the sun, moderately flowing, water temperature 24°C, altitude ca. 300 m, at Tanjung Bunga, near W-37, 18.VIII.1994.

North Sumatra

Surveys were carried out at eight sites along the north-eastern shore of the Lake Toba.

N-1. A creek 4.0-6.0 m wide, shaded, moderately flowing in a forest, water temperature 22°C, altitude ca. 880 m, at Bandar Baru, Sibolangit, 25.Ⅷ.1994.

N-2. A creek 6.0-8.0 m wide, exposed to the sun, moderately flowing, water temperature 24.5°C, altitude ca. 650 m, foot of the Mt. Meriah, at Deliserdang, near Sondi, along the road from Pematangsiantar to Pematang Purba, 26.Ⅷ.1994.

N-3. A small stream 1.0 m wide, exposed to the sun, slowly flowing, water temperature 22.3°C, altitude ca. 680 m, near N-2, 26.Ⅷ.1994.

N-4. An irrigation ditch 0.4 m wide, exposed to the sun, slowly flowing in the paddy rice field, water temperature 25°C, altitude ca. 960 m, near Seribudolok, 26.Ⅷ.1994.

N-5. A creek 2.0-3.0 m wide, exposed to the sun, moderately flowing, water temperature 19°C, altitude 1,130 m, at Aek Nauli, near Parapat, 27.Ⅷ.1994.

N-6. A brook 1.0 m wide, partially shaded, moderately flowing in a pine forest, water temperature 20°C, altitude ca. 950 m, at Sualan, near Prapat, 27.Ⅷ.1994.

N-7. An irrigation ditch 0.5 m wide, exposed to the sun, fastly flowing, water temperature 19°C, altitude ca. 1,100 m, at Junggadolok, along the road between Porsea and Prapat, 26.Ⅷ.1994.

N-8. A creek ca. 4.0 m wide, exposed to the sun, moderately flowing in the paddy rice field, water temperature 21°C, altitude ca. 920 m, at Lunbanjulu, Siruur, Porsea, 26.Ⅷ.1994.

Results of Collections of Black Flies and Examinations of Pathogens in the Larvae

Overall, 22 black-fly species including 12 species newly recorded from Sumatra were identified; and these were all assigned in the genus *Simulium* Latreille s. l., and further placed into three subgenera, i.e., *Gomphostilbia* Enderlein, *Nevermannia* Enderlein and *Simulium* Latreille s. str., as treated below. Brief remarks on taxonomy, distribution and infections were given for each species following the numbers of specimens examined. The present results bring the total number of black-fly species recorded from Sumatra to 26, of which seven are indigenous to Sumatra, six, six, and one are common to Java, Peninsular Malaysia, and Thailand, respectively, four are common to all these three areas, and two are common to the latter two areas. The simuliid fauna of Sumatra is thus very similar to those of neighbouring areas, in particular, to those of Peninsular Malaysia and Java, both of which are known to be Oriental in elements (Takaoka and Davies, 1995b, 1996).

Infections with mermithids, microsporidians and fungi were detected in 10 of the 22 black-fly species examined. These are first records of these pathogens in these 10 black-fly species from Indonesia. Of these, four simuliid species harboured all these three categories of pathogens, three had both microsporidians and fungi, two had either of the latter two pathogens, as noted below. Most of infected larvae were at the immature stage, but some were at the mature stage.

Abbreviations used below are as follows: ♀ for the reared female adult and its pupal exuvia; ♂ for the reared male adult and its pupal exuvia; P for pupa; PE for pupal exuvia; ML for mature larva; IL for immature larva. Localities, where black-fly samples were collected, are shown by the initial of the province's name with the site number, which correspond to those shown in the collection data. Numbers of larva(e) infected, if present among the total larvae examined, are shown in parenthesis: larva(e) infected with mermithid(s), microsporidians and fungi due to *Coelomycidium* sp. are abbreviated as NE, SP, and FU, respectively.

1. *Simulium* (*Gomphostilbia*) *cheongi* Takaoka and Davies, 1995

Simulium (*Gomphostilbia*) *cheongi* Takaoka and Davies, 1995b: 37 (female, male, pupa and larva).

Specimens examined. L-4: 2P, 1PE, 3ML.

Remarks. This species was described from Peninsular Malaysia (Takaoka and Davies, 1995b). This is the first record of this species from Sumatra.

No infection was found.

2. *Simulium* (*Gomphostilbia*) *duolongum* Takaoka and Davies, 1995

Simulium (*Gomphostilbia*) *duolongum* Takaoka and Davies, 1995b: 19 (female, male, pupa and larva).

Specimens examined. N-2: 1 ♂, 1P, 5ML, 13IL; N-3: 1PE; N-5: 12 ♀, 13 ♂, 2PE, 9ML, 19IL; W-8: 1IL.

Remarks. *S. duolongum* was also described from Peninsular Malaysia (Takaoka and Davies, 1995b). This is the first record of this species from Sumatra.

No infection was found.

3. *Simulium* (*Gomphostilbia*) *friederichsi* Edwards, 1934

Simulium (*Eusimulium*) *friederichsi* Edwards, 1934: 118 (male).

Simulium (*Gomphostilbia*) *friederichsi*: Crosskey, 1973: 425; Datta, 1983: 229; Crosskey, 1987: 450; Takaoka and Davies, 1995b: 161; Takaoka and Davies, 1996: 26.

Specimens examined. W-3: 1 ♀; W-10: 12 ♀, 18 ♂, 6ML, 9IL; W-15: 2 ♀, 2 ♂, 2ML, 4IL; W-21: 1 ♂; W-36: 9 ♀, 15 ♂, 26ML, 31IL.

Remarks. *S. friederichsi* was described from a single male adult collected from Java (Edwards, 1934). The female, pupa and larva of this species have remained unknown for a long time. The male of this species is characterized by the presence of the unique ornamentation on the scutum, i.e., an elongate median black vitta and two submedian black spots on the greyish pruinose ground color. The male specimens reared from the pupae collected in this survey show several such scutal patterns, of which one is almost the same as illustrated for *S. friederichsi* (Takaoka, 1991). However, some other scutal patterns are also in a good agreement with those found in *S. siamense* from Thailand (Takaoka and Suzuki, 1984) and *S. yaeyamaense* from Japan (Takaoka, 1991). The pupae and mature larvae collected from Sumatra are also very similar to those of *S. siamense*. Under these situations, it should be remembered that the present identification is tentative until additional samples of *S. friederichsi* from the type locality in Java become available for detailed comparison.

No infection was found.

4. *Simulium* (*Gomphostilbia*) *gyorkosae* Takaoka and Davies, 1996

Simulium (*Gomphostilbia*) *gyorkosae* Takaoka and Davies, 1996: 28 (female, male, pupa and larva).

Specimens examined. W-15: 1 ♂; W-18: 1 ♀; W-25: 13 ♀, 10 ♂, 3PE, 12ML, 21IL; W-27: 9 ♀, 6 ♂, 29ML, 89IL (1SP); L-2: 2P, 1ML, 3IL.

Remarks. *S. gyorkosae* was originally described from Java (Takaoka and Davies, 1996). This is the first record of *S. gyorkosae* from Sumatra. This species is very similar to *S. asakoae* described from Peninsular Malaysia (Takaoka and Davies, 1995b) and there is a possibility that the latter species is mixed in the present specimens. Further studies are now under way.

Only 1 of the 89 immature larvae collected at W-27 was infected with microsporidians.

5. *Simulium* (*Gomphostilbia*) *padangense* Takaoka and Sigit, 1997

Simulium (*Gomphostilbia*) *padangense* Takaoka and Sigit, 1997: 69 (female, male, pupa and larva).

Specimens examined. W-6: 1 ♀, 3 ♂, 1P, 21ML, 38IL.

Remarks. This species was fully described from the female, male, pupal and larval specimens collected from West Sumatra by Takaoka and Sigit (1997). Its distribution is now restricted to the type locality in West Sumatra.

No infection was found.

6. *Simulium* (*Gomphostilbia*) *parahiyangum* Takaoka and Sigit, 1992

Simulium (*Gomphostilbia*) *parahiyangum* Takaoka and Sigit, 1992: 135 (female, male, pupa and larva); Takaoka and Davies, 1995b: 50.

Specimens examined. W-9: 3ML, 19IL; W-15: 6 ♀, 5 ♂, 3P, 4ML, 1IL; W-38: 1P.

Remarks. Edwards (1934) incorrectly associated the larva of this species collected from South Sumatra with *S. varicorne,* which was described based upon the unique male specimen collected at the same locality (Edwards, 1925). *S. parahiyangum* was originally described from Java (Takaoka and Sigit, 1992), and was later recorded from Peninsular Malaysia (Takaoka and Davies, 1995b).

No infection was found.

7. *Simulium* (*Gomphostilbia*) *sheilae* Takaoka and Davies, 1995

Simulium (*Gomphostilbia*) *sheilae* Takaoka and Davies, 1995b: 60 (female, male, pupa and larva).

Specimens examined. N-1: 1 ♀; N-5: 1 ♂, W-2: 2 ♀, 1 ♂, 1PE, 5ML; W-13: 1 ♀, 1 ♂; W-15: 3 ♀, 2 ♂, 3ML, 4IL; W-16: 1 ♀, 1ML; W-35: 1 ♀; L-4: 5 ♀, 5 ♂, 3P, 7ML, 4IL.

Remarks. *S. sheilae* was originally described from Peninsular Malaysia (Takaoka and Davies, 1995b). This is the first record of *S. sheilae* from Sumatra.

No infection was found.

8. *Simulium* (*Gomphostilbia*) *sundaicum* Edwards, 1934

Simulium (*Eusimulium*) *sundaicum* Edwards, 1934: 122 (female, male, pupa and larva).

Simulium (*Gomphostilbia*) *sundaicum*: Crosskey, 1973: 425; Datta, 1983: 230; Takaoka and Davies, 1995b: 161; Takaoka and Davies, 1996: 19 (female, male, pupa and larva).

Specimens examined. N-1: 2 ♂, 1ML; N-2: 2ML, 1IL; N-3: 1 ♀, 1 ♂, 1PE, 5IL; N-4: 1 ♂, 1P, 1PE, 2ML, 45IL; N-5: 2PS, 1ML, 5IL; N-6: 1P, 1ML; N-7: 2 ♀, 1 ♂; W-1: 28 ♀, 20 ♂, 21ML, 88IL; W-3: 8 ♀, 3 ♂, 2P, 2ML,

4IL; W-4: 2 ♀, 11 ♂, 9ML, 49IL (1NE); W-10: 1 ♂, 2ML, 13IL; W-12: 2 ♀, 1 ♂, 2ML, 5IL; W-14: 9 ♀, 7 ♂, 28ML, 13IL; W-15: 10 ♀, 10 ♂, 27ML, 134IL (2SP); W-18: 1 ♀, 1 ♂; W-19: 1 ♀, 3IL; 1PE; W-20: 7 ♀, 6 ♂, 14ML, 42IL (1NE, 1SP); W-21: 2 ♂, 2P, 3ML, 19IL (2NE); W-22: 20 ♀, 20 ♂, 10ML, 30IL; W-27: 3ML, 6IL; W-29: 1 ♀, 1 ♂; W-30: 1 ♂, 1ML; W-33: 2 ♀, 1 ♂, 4ML, 17IL; W-34: 1ML, 1IL; W-36: 1 ♀, 4 ♂, 9ML, 34IL; W-37: 2IL; W-38: 2P, 8IL; B-2: 5 ♀, 4 ♂, 30ML, 20IL (2NE); B-3: 23 ♀, 18 ♂, 2P, 43ML, 87IL (8NE, 5FU); B-5: 8 ♀, 13 ♂, 11ML, 46IL (3NE); B-6: 3 ♀, 3 ♂, 1ML, 14IL (3NE); L-2: 5 ♀, 5 ♂, 6ML, 10IL; L-5: 2 ♀, 3 ♂, 3ML, 7IL; L-6: 1PE, 1ML; L-7: 6 ♀, 9 ♂, 14ML (1NE), 21IL (2NE).

Remarks. *S. sundaicum* was originally described from Java and South Sumatra (Edwards, 1934). This is one of the common species in Sumatra.

Mermithid, microsporidian and fungal infections were found in several sites.

9. *Simulium* (*Gomphostilbia*) *whartoni* Takaoka and Davies, 1995

Simulium (*Gomphostilbia*) *whartoni* Takaoka and Davies, 1995b: 32 (female, male, pupa and larva).

Specimens examined. W-17: 1 ♀, 1P, 7ML, 13IL; W-27: 1 ♀; W-30: 2PE, 4ML, 15IL; W-35: 4 ♀, 4 ♂, 1P, 4ML, 11IL; L-4: 1 ♀, 1 ♂, 2PE, 2ML.

Remarks. This species was described from Peninsular Malaysia (Takaoka and Davies, 1995b). This is the first record of this species from Sumatra.

No infection was found.

10. *Simulium* (*Nevermannia*) *aureohirtum* Brunetti, 1911

Simulium aureohirtum Brunetti, 1911: 283 (male); Edwards, 934: 134 (female, pupa and larva).

Simulium (*Nevermannia*) *aureohirtum*: Ogata, 1956: 61; Ogata, 1966: 129; Crosskey, 1987: 459; Takaoka and Roberts, 1988: 194; Crosskey and Lowry, 1990: 204; Takaoka and Davies, 1995b: 87; Takaoka and Davies, 1996: 5.

Simulium (*Eusimulium*) *aureohirtum*: Puri 1933: 1; Ogata and Sasa, 1954: 325; Ogata, Sasa and Suzuki, 1956: 73; Crosskey, 1973: 423; Takaoka, 1976: 170; Takaoka, 1979: 382 (female, male, pupa and larva); Datta, 1983: 225; Takaoka and Suzuki, 1984: 11; Davies and Györkös, 1987: 1485.

Eusimulium aureohirtum: Orii, Uemoto and Onishi, 1969: 1.

Simulium (*Eusimulium*) *tuaranense* Smart and Clifford, 1969: 40. Syn. by Crosskey 1973.

Simulium (*Eusimulium*) *philippinense* Delfinado 1962: 47. Syn. by Takaoka 1983.

Specimens examined. N-3: 1 ♀, 1P, 1ML, 18IL; N-4: 1IL; W-3: 4ML, 2IL; W-12: 8 ♀, 7 ♂, 5ML, 44IL; W-14: 25 ♀, 10 ♂, 24ML (1FU), 43IL (1SP); W-15: 1 ♀, 17IL; W-21: 3IL; W-22: 4 ♀, 3 ♂, 8ML, 10IL; W-31: 2 ♀, 3 ♂, 2ML, 8IL; W-35: 1ML; W-37: 2 ♀, 1 ♂, 1P, 2ML, 29IL; B-1: 1PE; B-2: 1IL; B-5: 4 ♀, 8 ♂; L-1: 6 ♀, 1 ♂, 40ML, 98IL; L-2: 1IL.

Remarks. *S. aureohirtum* is known to be widely distributed in the Oriental and the southern part of the Palaearctic Regions (Takaoka and Davies, 1995b). This species extends its distribution eastwards to the Halmahera Island in the Australasian Region (Takaoka, 1996). This species is distinctive among the Oriental Simuliidae by its autogenous ovarian development (Takaoka, 1989). This physiological feature of this species was also confirmed in the populations of Sumatra by dissecting female flies kept alive in small plastic tubes with 5% sugar solution for 3-5 days after they emerged from the pupae (data not shown).

Microsporidian and fungal infections were found, each in one immature larva.

11. *Simulium* (*Nevermannia*) *feuerborni* Edwards, 1934

Simulium feuerborni Edwards, 1934: 129 (male, pupa and larva).

Simulium (*Eusimulium*) *feuerborni*: Crosskey, 1969: 64; Datta, 1973: 367; Datta, 1983: 226.

Simulium (*Nevermannia*) *feuerborni*: Crosskey, 1987: 458; Crosskey and Lowry, 1990: 213; Takaoka and Davies, 1995b: 86; Takaoka and Davies, 1996: 10 (female, male, pupa and larva).

Specimens examined. N-6: 1 ♂, 1P, 20IL (1FU); N-7: 3 ♀, 1 ♂, 2ML, 16IL; W-14: 1 ♀, 1IL; W-25: 15 ♀, 26 ♂, 1P, 12ML, 24IL.

Remarks. This species was originally described based on the male, pupal and larval specimens collected from East Java and Bali by Edwards (1934), and the female was later described from specimens collected from West Java by Takaoka and Davies (1996). This species was also recorded from West Malaysia (Takaoka and Davies, 1995b) and from Thailand (Kuvangkadilok *et al.*, 1998). The present specimens are indistinguishable morphologically from the Javanese ones. This is the first record of this species from Sumatra.

A fungal infection was found in one immature larva.

12. *Simulium* (*Simulium*) *argyrocinctum* de Meijere, 1913

Simulium argyrocinctum de Meijere, 1913: 332 (female and male); Edwards, 1934: 108 (female, male, pupa and larva).

Simulium (*Simulium*) *argyrocinctum*: Crosskey, 1969: 115; Takaoka and Davies, 1996; 44 (female, male, pupa and larva).

Specimens examined. W-24: 7 ♀, 8 ♂, 1P, 7PE, 7ML, 7IL; W-28: 5IL (1SP, 3FU); B-6: 4 ♀, 5 ♂, 8ML, 13IL.

Remarks. This species was described from adult specimens collected from Java (de Meijere, 1913), and its pupa and larva were described based on the specimens collected from Java and South Sumatra (Edwards, 1934). It is noted that some pupae collected from West and South Sumatra in this survey showed some variations in the branching schemes of the ten respiratory filaments on each side. The pupal respiratory filaments of this species are arranged in five pairs in most pupae, but in two pairs plus two triplets in some, and even in four pairs plus two individuals in a few others.

Microsporidian and fungal infections were found in one site.

13. *Simulium* (*Simulium*) *bishopi* Takaoka and Davies, 1995

Simulium (*Simulium*) *bishopi* Takaoka and Davies, 1995b: 111 (female, male, pupa and larva).

Specimens examined. L-3: 2IL; L-4: 3 ♀; L-6: 1 ♂, 2ML.

Remarks. This species was originally described from Peninsular Malaysia (Takaoka and Davies, 1995b). The present specimens agree morphologically with the original descriptions. This is the first record of *S. bishopi* from Sumatra.

No infection was found.

14. *Simulium* (*Simulium*) *brevipar* Takaoka and Davies, 1995

Simulium (*Simulium*) *brevipar* Takaoka and Davies, 1995b: 132 (female, male, pupa and larva); Takaoka and Saito, 1996: 168.

Specimens examined. L-5: 1 ♀, 1 ♂, 2P, 3ML, 20IL.

Remarks. This species was originally described from Peninsular Malaysia (Takaoka and Davies, 1995b) and also recorded from Thailand (Takaoka and Saito, 1996). Our survey shows that this species extends its distribution to South Sumatra.

No infection was found.

15. *Simulium* (*Simulium*) *fenestratum* Edwards, 1934

Simulium fenestratum Edwards, 1934: 110 (male, pupa and larva).

Simulium (*Simulium*) *fenestratum*: Crosskey, 1969: 115; Takaoka and Saito, 1996: 166.

Specimens examined. N-1: 1 ♀, 1P, 1PE, 1ML, 9IL (1SP); N-2: 2IL; N-6: 11 ♀, 13 ♂, 1PE, 15ML, 71IL (1FU); N-7: 1IL (1FU); N-8: 5 ♀, 4 ♂, 6ML, 33IL; W-6: 1 ♀; W-8: 1 ♀, 3 ♂, 1PE, 2ML, 1IL; W-9: 3 ♀, 7 ♂, 1PE, 12ML, 41IL (2SP, 1FU); W-10: 18 ♀, 12 ♂, 3P, 1PE, 28ML, 72IL (2NE, 1SP); W-11: 14 ♀, 10 ♂, 5P, 5ML, 58IL (1NE, 1SP, 3FU); W-13: 2 ♀, 3 ♂, 1PE, 3ML, 13IL (4FU); W-14: 1 ♂, 1ML; W-15: 1 ♂, 1IL; W-19: 1 ♀, 4ML, 27IL (5FU); W-20: 5 ♀, 8 ♂, 5P, 20ML, 52IL (1NE, 2SP); W-21: 2 ♂, 2ML, 32IL (2NE, 1SP); W-24: 1 ♀, 1 ♂, 3IL (1FU); W-25: 4 ♀, 8 ♂, 2PE, 3ML, 2IL; W-27: 18 ♀, 21 ♂, 33ML, 236IL (3NE, 2SP); W-28: 1 ♀, 1PE, 1ML, 14IL (3NE); W-29: 1 ♀, 1ML, 8IL (1NE); W-33: 1 ♀, 1 ♂, 2ML, 24IL; W-38: 4 ♀, 5 ♂, 2PE, 2ML, 76IL (2SP); L-2: 8 ♀, 8 ♂, 13ML, 58IL (35NE): L-4: 1P, 2PE, 1IL; L-6: 3 ♂, 1P, 4ML, 23IL; L-7: 2IL.

Remarks. *S. fenestratum* was described from West and North Sumatra (Edwards, 1934), and later recorded from Thailand (Takaoka and Saito, 1996). The present larval specimens show bimorphism with regard to the dorsal protuberances on the abdomen, which are present in one type, but absent in the other type. This bimorphism was found even in the same populations. In addition, some males had bicolored hind basitarsi while most of the other males examined in this survey had almost darkened ones. Therefore, the possibility is not ruled out that more than one species are involved in the present specimens, representing a species complex. Further studies are now under way.

Larvae of this species were frequently infected with mermithid, microsporidians and fungi. It is noteworthy that as high as 60% of the immature larvae examined were infected with mermithid parasites at L-2.

16. *Simulium* (*Simulium*) *iridescens* de Meijere, 1913

Simulium iridescens de Meijere, 1913: 333 (female and male); Edwards, 1934; 112 (female, male, pupa and larva).

Simulium (*Simulium*) *iridescens*: Crosskey, 1973: 427; Crosskey, 1987: 475; Takaoka and Davies, 1996: 67 (female, male, pupa and larva).

Specimens examined. N-1: 4 ♀, 6 ♂, 1P, 2PE, 3ML (1NE), 18IL (8NE); N-2: 3ML, 1IL; W-8: 1 ♀; W-9: 8 ♀, 5 ♂, 2P, 8ML, 56IL (2NE, 2FU); W-10: 3 ♀, 1 ♂,

13ML, 63IL (13SP, 6FU); W-11: 2 ♀, 4 ♂; W-17: 1 ♂; W-20: 2P, 2ML, 1IL; W-21: 1P, 2ML, 10IL; W-22: 1 ♀; W-24: 6 ♀, 9 ♂, 3P, 2PE, 7ML, 14IL; W-28: 1PE; W-35: 3 ♂, 3PE, 2ML, 1IL; W-38: 1P, 3ML, 6IL; L-3: 26ML; L-4: 1 ♀, 1 ♂, 3P, 4ML, 3IL; L-5: 1IL; L-6: 1 ♀, 5 ♂, 2ML.

Remarks. This species appears to be one of the widely distributed species in Sumatra since many samples were collected from North and West Sumatra, as well as from South Sumatra.

Mermithid, microsporidian and fungal infections were found.

17. *Simulium* (*Simulium*) *minangkabaum* Takaoka and Sigit, 1997

Simulium (*Simulium*) *minangkabaum* Takaoka and Sigit, 1997: 76 (female, male, pupa and larva).

Specimens examined. W-11: 6 ♀, 6 ♂, 2PS, 1ML, 18IL; W-15: 1 ♀, 1 ♂; W-18: 5 ♀, 5 ♂, 8P, 28ML, 151IL (1SP).

Remarks. This species was already described based on the female, male, pupal and larval specimens collected from West Sumatra by Takaoka and Sigit (1997).

The microsporidian infection was found in one larva.

18. *Simulium* (*Simulium*) *nebulicola* Edwards, 1934

Simulium nebulicola Edwards, 1934: 114 (male).

Simulium (*Simulium*) *nebulicola*: Takaoka and Davies, 1996: 71 (female, male, pupa and larva).

Specimens examined. W-5: 1 ♂, 1PE, 1IL; W-6: 1 ♀, 8IL; W-7: 1P, 1IL; W-13: 1 ♂; W-26: 1P, 6IL.

Remarks. This species was originally described from the male specimen collected from East Java by Edwards (1934). The female, pupa and mature larva were described by Takaoka and Davies (1996). The present specimens collected from West Sumatra agree well morphologically with the descriptions given by Takaoka and Davies (1996). This represents the new distributional record of this species from Sumatra.

No infection was found.

19. *Simulium* (*Simulium*) *nobile* de Meijere, 1907

Simulium nobile de Meijere, 1907: 206 (male); Edwards, 1934: 115 (female, male, pupa and larva).

Simulium (*Simulium*) *nobile*: Crosskey, 1973: 428; Crosskey, 1988: 475; Takaoka and Davies, 1995b: 123; Takaoka and Davies, 1996: 61 (female, male, pupa and larva).

Specimens examined. N-1: 2IL; N-6: 1 ♀, 1 ♂, 1PE; W-: 2 ♀, 1 ♂, 1P, 1ML, 1IL; W-4: 3P, 2ML; W-10: 1 ♀, 1 ♂, 1ML; W-11: 1 ♀, 1P, 1ML, 3IL; W-20: 1ML; W-21: 7IL (2NE); W-23: 4 ♀, 5 ♂, 11ML, 27IL; W-24: 1 ♀, 1 ♂; W-28: 10 ♀, 11 ♂, 76ML, 95IL (1NE, 1SP, 2FU); W-29: 2 ♀, 3 ♂, 1PE, 3ML, 16IL (2NE); W-35: 1 ♀, 2 ♂, 2PE, 1ML, 3IL; W-36: 1 ♂, 2ML, 1IL; W-38: 1 ♀, 2P, 2PS, 2ML, 6IL; B-1: 7 ♀, 5 ♂, 3P, 2PE, 1ML, 14IL; B-6: 18 ♀, 17 ♂, 14ML, 19IL; L-2: 2 ♀, 1 ♂, 3ML.

Remarks. *S. nobile* was originally described from Java (de Meijere, 1907), and later recorded from South Sumatra (Edwards, 1934). This species was also recorded from Peninsular Malaysia and Sabah (Crosskey, 1973). This is one of the common black-fly species in Sumatra.

Mermithid, microsporidian and fungal infections were found.

20. *Simulium* (*Simulium*) *sumatraense* Takaoka and Sigit, 1997

Simulium (*Simulium*) *sumatraense* Takaoka and Sigit, 1997: 72 (female, male, pupa and larva)

Specimens examined. W-17: 1 ♀, 1 ♂, 1P, 3ML, 19IL; W-19: 1 ♀, 1ML, 3IL; W-26: 1P, 6IL; W-27: 4 ♀, 1 ♂, 1PE; W-30: 1 ♂, 1PE, 9IL.

Remarks. *S. sumatraense* was described based on the female, male, pupal and larval specimens collected from West Sumatra by Takaoka and Sigit (1997). This species is very similar to *S. nebulicola* but differs from the latter species by the longer pupal respiratory filaments and the presence of the terminal hooks on the pupal abdomen, as mentioned by Takaoka and Sigit (1997).

No infection was found.

21. *Simulium* (*Simulium*) *tani* Takaoka and Davies, 1995

Simulium (*Simulium*) *tani* Takaoka and Davies, 1995b: 137 (female, male, pupa and larva).

Specimens examined. W-9: 1 ♀; W-11: 1PS, 1ML; W-19: 1 ♀, 1ML, 2IL; W-27: 1IL; W-33: 1ML.

Remarks. This species was originally described from Peninsular Malaysia (Takaoka and Davies, 1995b) and also recorded from Thailand (Takaoka and Saito, 1996). It is noted that there are slight differences in the thickness of six pupal respiratory filaments on each side between the present specimens and Malaysian ones. This is the first record of this species from Sumatra.

No infection was found.

22. *Simulium* (*Simulium*) sp. nr. *eximium* Edwards, 1934

Specimens examined. N-6: 1P; W-24: 2 ♀, 3 ♂, 2P, 1PE, 5ML, 46IL (1SP); W-27: 1IL; W-35: 4 ♀, 8 ♂, 4P, 8ML, 82IL; B-6: 15 ♀, 15 ♂, 1PE, 11ML, 39IL (1FU).

Remarks. This species is almost indistinguishable morphologically from *S. eximium* reported from Java (Takaoka and Davies, 1996) except a few characters including the adult male abdomen with five pairs of dorsolateral silvery spots each on segments 2, 4, 5, 6, and 7 in this species (c.f. four pairs of such spots each on segments 2, 5, 6, and 7 in *S. eximium*). Interestingly, the adlut male specimens collected from Flores in the Lesser Sunda Islands have the same spot pattern as found in this species from Sumatra (Takaoka, unpublished data). Hadi *et al.* (1996) have given a cytotaxonomical evidence that this species from Sumatra is a sibling species differing from *S. eximium* from Java. Further morphotaxonomic studies on the species status of this species are now in progress.

Two immature larvae were infected with microsporidians and fungi, respectively.

A Summarized List of Black-Fly Species in Sumatra

The 22 black-fly species identified in this survey were further placed in the species-groups within each subgenus, as follows. The four species previously known from Sumatra and not collected in this survey (shown by an asterix) were also included in this list.

Genus *Simulium* Latreille
Subgenus *Gomphostilbia* Enderlein
batoense species-group
1. *S. cheongi*
2. *S. duolongum*
3. *S. friederichsi*
4. *S. flavocinctum**
5. *S. whartoni*
6. *S. padangense*
7. *S. parahiyangum*
8. *S. sundaicum*
9. *S. zonatum**
ceylonicum species-group
10. *S. gyorkosae*
11. *S. sheilae*
varicorne species-group
12. *S. varicorne**
Subgenus *Nevermannia* Enderlein
feuerborni species-group
13. *S. feuerborni*
ruficorne species-group
14. *S. aureohirtum*
15. *S. glatthaari**
Subgenus *Simulium* Latreille s. str.
eximium species-group
16. *S.* sp. nr. *eximium*
melanopus species-group
17. *S. bishopi*
18. *S. iridescens*
multistriatum species-group
19. *S. fenestratum*
nobile species-group
20. *S. nobile*
striatum species-group
21. *S. argyrocinctum*
tuberosum species-group
22. *S. brevipar*
23. *S. tani*
unplaced speices
24. *S. minangkabaum*
25. *S. nebulicola*
26. *S. sumatraense*

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TAXONOMIC NOTES ON SIMULIIDAE (DIPTERA) FROM THAILAND: DESCRIPTION OF A NEW SPECIES AND NEW DISTRIBUTIONAL RECORDS OF NINE KNOWN SPECIES

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Abstract: *Simulium* (*Gomphostilbia*) *chumpornense* sp. nov. is described from reared adults, pupae and mature larvae collected from southern Thailand. This new species is assigned to the *varicorne* species-group within the subgenus *Gomphostilbia* by having the adult antennae composed of 2+8 segments in place of usual 2+9 segments. In addition, nine known simuliid species, most of which were described from Peninsular Malaysia, are recorded for the first time from Thailand, bringing the total number of simuliid species in this country to 40.

Key words: Simuliidae, black fly, Thailand, new species, fauna

Since Takaoka and Suzuki (1984) reported 19 black-fly species (Diptera: Simuliidae) from Thailand, seven more species were added (Takaoka and Saito, 1996; Takaoka and Adler, 1997), totaling the number of simuliid species from this country to 26 consisting of 22 named and four unnamed species.

In 1996–1999, one of us (CK) made faunistic and ecological surveys on Simuliidae in various provinces of Thailand, and collected many species including five new species, nine newly recorded ones, and two unnamed ones (i.e., *Simulium* sp. A and *S.* sp. B, *sensu* Takaoka and Suzuki, 1984). In our recent cytogenetic papers (Kuvangkadilok *et al.,* 1998, 1999a, 1999b), we have applied two Malaysian known species names, i.e., *S. caudisclerum* and *S. feuerborni,* for *S.* sp. A and *S.* sp. B, respectively. In addition, we have already described four of the five new black-fly species, of which three were assigned to the subgenus *Simulium* s. str. and the one remained unplaced in any subgenera (Takaoka and Kuvangkadilok, 1999).

We herein describe the remaining one new species which apparently belongs to the subgenus *Gomphostilbia,* and give brief notes on the nine known species which were newly recorded from Thailand.

The morphological features and terms used herein follow mostly those of Crosskey (1969), and partially those of Takaoka (1983). All type specimens of a new species will be deposited in the Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand.

DESCRIPTION OF NEW SPECIES

Simulium (*Gomphostilbia*) *chumpornense* Takaoka and Kuvangkadilok sp. nov.

Female. Body length 1.8 mm. ***Head***. As wide as thorax. Frons (Fig. 1) brownish black, dull, moderately covered with yellowish white, scale-like, recumbent hairs (Fig. 2) (except median longitudinal portion narrowly bare on upper 1/2), and with 1 dark hair on upper part of right side; lower narrow juncture to clypeus also fully covered with yellowish white, scale-like, recumbent hairs; frontal ratio 1.5:1.0:1.7; frons-head ratio 1.0:4.0. Fronto-ocular area (Fig. 3) well developed, narrow. Clypeus brownish black, dull, densely covered with yellowish white, scale-like, recumbent hairs interspersed with several dark hairs along each lateral margin. Antenna (Fig. 4) composed of 2+8 segments, apical flagellar segments compressed dorsoventrally; scape, pedicel, 2nd and 4th flagellar segments entirely yellow, and 1st and 3rd flagellar segments light or medium

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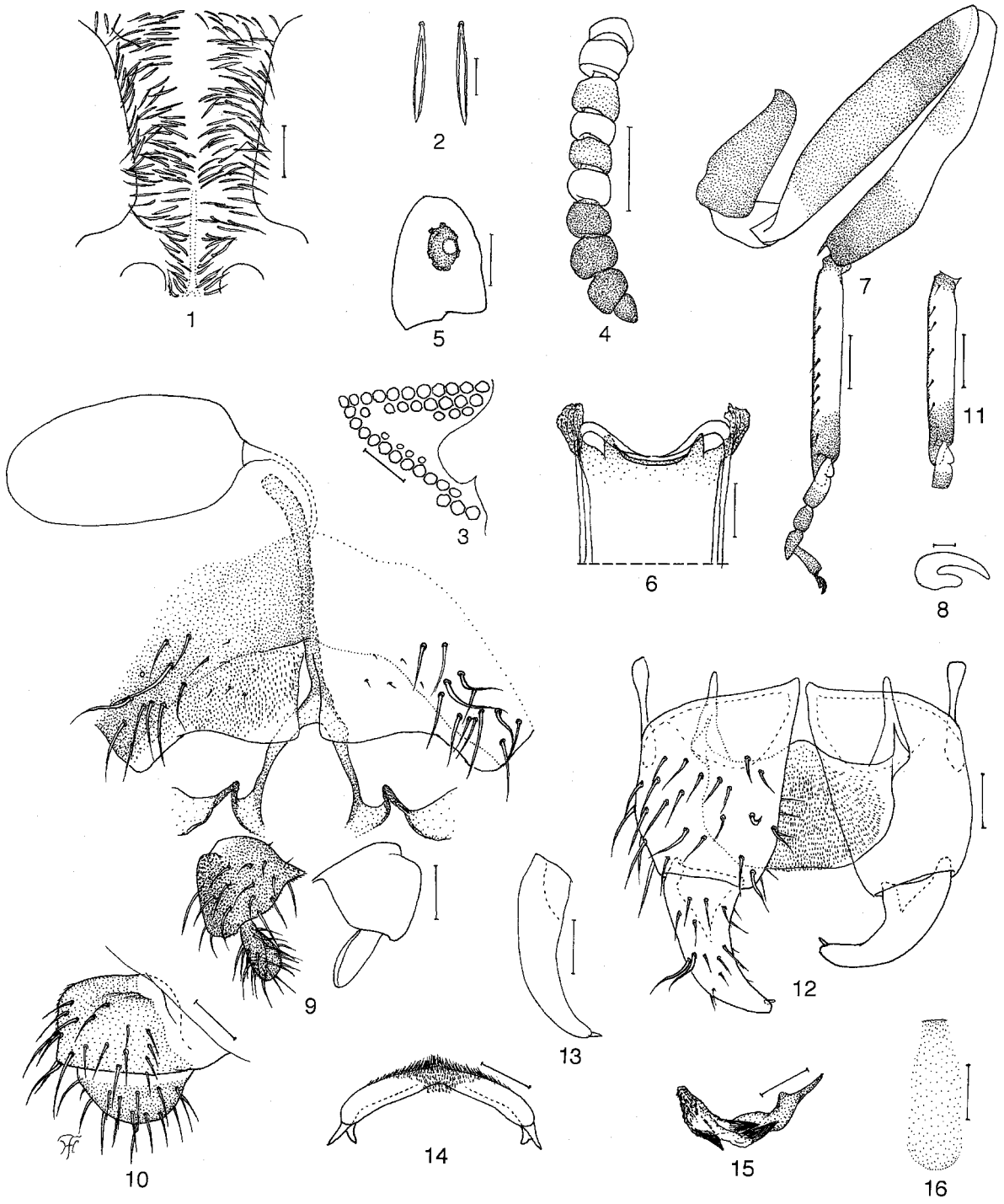
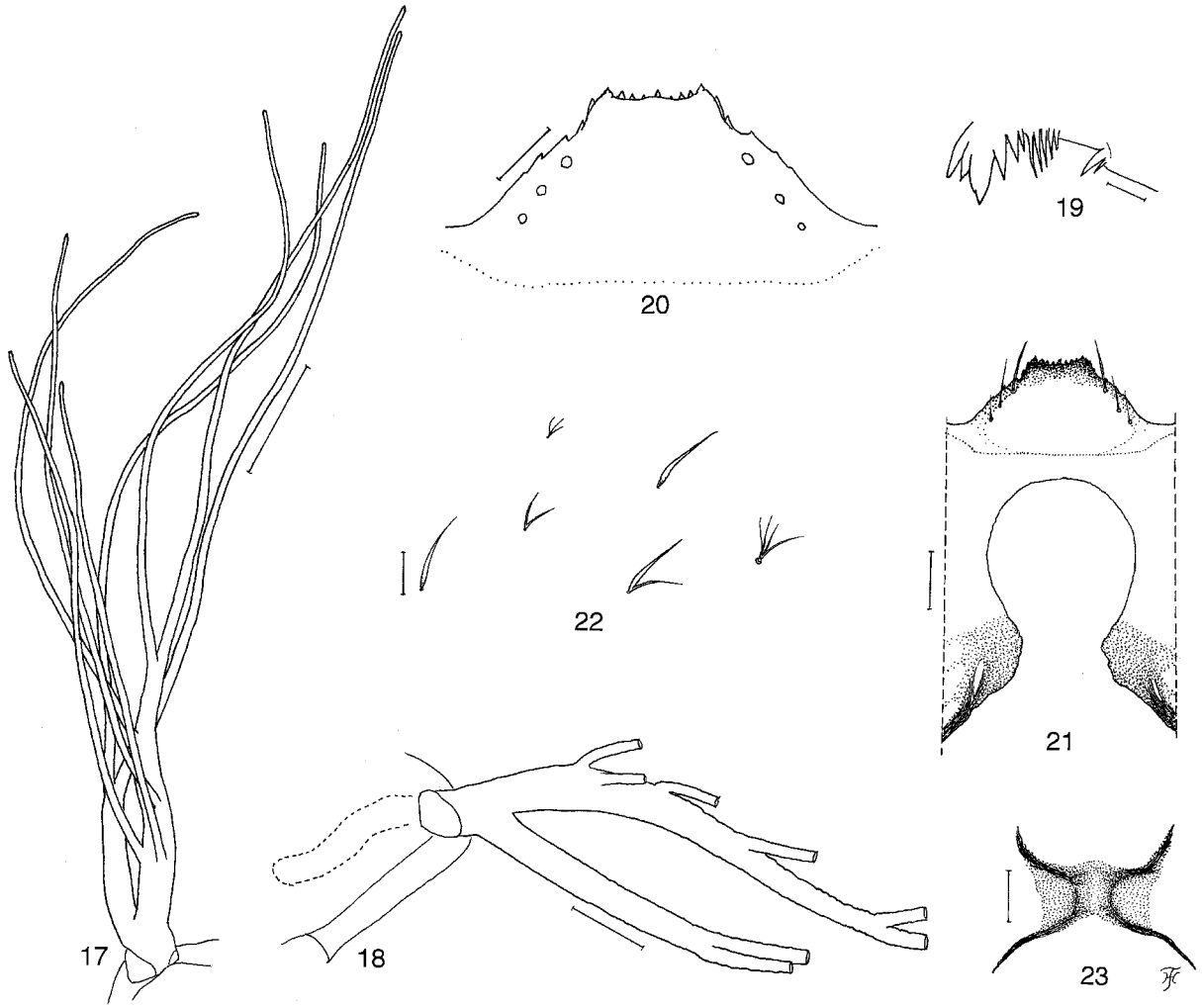
brown except base of 1st flagellar segment yellow, and 5th to 8th flagellar segments brownish black. Maxillary palp composed of 5 segments, light brown, proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:2.5, 3rd segment (Fig. 5) widened distally; sensory vesicle (Fig. 5) of moderate size, nearly ellipsoidal, 0.36× as long as 3rd segment, with medium opening. Maxillary lacinia with 9 inner teeth and 13 or 14 outer teeth. Mandible with 22–24 small inner teeth and lacking outer ones. Cibarium (Fig. 6) with a pair of triangular, well sclerotized projections at posterior margin; any processes or tubercles absent. ***Thorax***. Scutum brownish black with anterolateral calli somewhat paler, subshiny, thinly white pruinose, with 3 dark longitudinal vittae (1 medial and 2 submedial), densely covered with yellowish white, scale-like, recumbent hairs, interspersed with dark, similar hairs; scutellum brownish black, covered with short, yellowish white, scale-like hairs as well as long, upright, dark hairs along posterior margin. Postscutellum brownish black, bare. Pleural membrane bare. Katepisternum brownish black, longer than deep, with fine, short, yellowish white hairs. ***Legs***. Foreleg: coxa and trochanter pale yellowish white; femur dark grey; tibia pale yellowish white, with basal knee black, and with subbasal and subapical dark grey bands; tarsus brownish black with basal knee of basitarsus yellow, with sparse dorsal hair crest; basitarsus nearly cylindrical, ca. 5.9× as long as its greatest width. Midleg: coxa brownish black; trochanter pale yellowish white; femur dark grey with apex paler; tibia pale yellowish white on basal 2/5 with dark grey or light brown subbasal band, and medium brown on distal 3/5; tarsus light to medium brown with basal 2/3 of basitarsus, and base of 2nd tarsal segment yellowish white. Hind leg (Fig. 7): coxa medium brown, trochanter pale yellowish white; femur light brown with base pale yellowish white and apical cap medium brown; tibia yellowish white on basal 2/3 with dark grey subbasal band, and brownish black on distal 1/3; tibia much angulate posteriorly at distal 1/3 and also at basal 1/3 when viewed laterally; tarsus brownish black except basal 2/3 or a little more of basitarsus, basal 1/2 of 2nd tarsal segment, and base of 3rd tarsal segment yellowish white; basitarsus narrow, nearly parallel-sided, ca. 6.5× as long as wide, and ca. 0.50× and ca. 0.48× as wide as greatest width of tibia and femur, respectively; calcipala ca. 1.3× as long as wide, and ca. 0.6× as wide as distal portion of basitarsus. All femora, tibia and parts of tarsus densely covered with pale or dark, scale-like hairs. Claws (Fig. 8) each with large basal tooth, 0.5× as long as claw. ***Wing***. Length 1.6 mm. Costa with dark spinules as well as dark hairs except basal hair tuft pale. Subcosta almost bare. Hair tuft on stem vein pale. Basal portion of radius fully haired. ***Abdomen***. Basal scale light brown, with fringe of pale hairs. Abdomen brownish black except base of 2nd segment somewhat paler, sparsely or moderately covered with short yellowish white hairs, intermixed with dark hairs; tergites of segments 2, 6, 7 and 8 shiny; sternal plate on segment 7 undeveloped. ***Genitalia*** (Figs. 9 and 10). Sternite 8 bare medially, with 10–12 long hairs and a few short hairs on each side. Anterior gonapophyses nearly triangular, thin, membraneous, covered densely with microsetae (though posteromedial corner somewhat widely bare), interspersed with 2–4 short setae; inner margins narrowly sclerotized, moderately separated from each other. Genital fork of usual inverted-Y form; arms slender, folded medially, forming a distinct projection directed forward. Paraproct somewhat produced ventrally, with 16–18 long hairs on outer surface, and with 3 sensilla on inside surface. Cercus ca. 0.5× as long as wide, rounded posteriorly. Spermatheca oblong, ca. 1.8× as long as wide, well sclerotized, even near tubal juncture, with several internal setae.

Male. Body length probably 2.0 mm. ***Head***. Lost. ***Thorax***. Scutum brownish black, shiny, whitish grey-pruinose except central large area unpruinose, densely covered with yellow, scale-like, recumbent hairs, interspersed with dark ones. Scutellum brownish black, covered with yellow, scale-like, short hairs and several dark hairs near posterior margin. Postscutellum brownish black, bare. Pleural membrane bare. Katepisternum as in female. ***Legs***. Colorings and shapes as in female except relative sizes as follows: fore basitarsus ca. 6.2× as long as greatest width; hind basitarsus (Fig. 11) ca. 6.1× as long as wide, and ca. 0.55× and ca. 0.51× as wide as greatest width of tibia and femur, respectively. Calcipala ca. 1.2× as long as wide. ***Wing***. As in female. ***Abdomen***. Basal scale light brown, with fringe of pale hairs. Dorsal surface of abdomen brownish black except basal 3/4 of 2nd segment yellow to light brown, covered with dark hairs; segments 2, 5, 6 and 7 each with a pair of shiny dorsolateral patches, those on segment 2 silvery iridescent in certain angles of light. ***Genitalia*** (Figs. 12–16). Coxite in ventral view, subquadrate, ca. 1.5× as long as wide. Style ca. 0.85× as long as coxite, gradually tapered toward apex, curved inward, with apical spine. Ventral plate transverse, densely covered with microsetae on most area of ventral surface and central area of posterior surface; basal arms directed forward, expanded laterally and dorsally near base.



Figures 1–16 Morphological characters of *S. chumpornense* sp. nov. 1–10, female, and 11–16, male. 1, frons; 2, scale-like hairs on frons; 3, fronto-ocular area; 4, antenna; 5, 3rd segment of maxillary palp with sensory vesicle inside; 6, cibarium; 7, hind leg; 8, claw of hind leg; 9, genitalia *in situ* (ventral view), showing 8th sternite, anterior gonapophyses, genital fork, paraprocts, cerci and spermatheca; 10, paraproct and cercus (lateral view); 11, hind basitarsus and 2nd tarsal segment; 12, genitalia *in situ* (ventral view) showing coxites, styles, and ventral plate; 13, right style (medial view); 14, ventral plate (end view); 15, left paramere (end view); 16, median sclerite (end view). Scale bars; 0.1 mm for figs. 4, 7 and 11; 0.05 mm for figs. 1 and 3; 0.03 mm for figs. 5, 6, 9, 10, 12–16; 0.01 mm for figs. 2 and 8.



Figures 17–23 Morphological characters of *S. chumpornense* sp. nov. 17 and 18, pupa, and 19–23, larva. 17, left gill filaments (dorsal view); 18, basal 1/3 of right gill and interspiracular trunk (lateral view) showing an arrangement of filaments; 19, apical portion of mandible; 20, hypostomium (bristles omitted); 21, ventral surface of head capsule showing hypostomium and postgenal cleft; 22, various setae on dorsal surface of abdomen; 23, anal sclerite. Scale bars: 0.2 mm for fig. 17; 0.1 mm for fig. 18; 0.05 mm for figs. 21 and 23; 0.03 mm for fig. 20; 0.01 mm for figs. 19 and 22.

Parameres of moderate size, each with 3 long hooks and several short incomplete ones. Median sclerite thin, plate-like, wide, with apex rounded. Cerci small, rounded, each with 10 or 11 short hairs.

Pupa. Body length 2.0 mm. ***Head***. Integument yellowish brown, moderately or somewhat sparsely covered with round tubercles; antennal sheath normal, with no spinous projections, and almost bare; face with 1 pair of simple, long trichomes with coiled or uncoiled apex, and frons with 3 pairs of simple, long trichomes with uncoiled apex; 3 frontal trichomes on each side arising close together, subequal in length to one another and also to facial one. ***Thorax***. Integument yellowish brown, moderately covered with round tubercles, with 3 pairs of simple, long trichomes dorsally, with 2 pairs of simple, long trichomes anterolaterally, with 1 pair of simple, medium trichomes posterolaterally, and with 3 pairs of simple, medium trichomes ventrolaterally. Gill (Figs. 17 and 18) composed of 8 slender filaments, with somewhat swollen, transparent organ ventrally at base; gill divided vertically near base into 2 main stout filaments, of which upper one is much thicker than lower one, and with 5 slender filaments each arising independently from its dorsal or outer surface; lower main stout filament further divided into 2 slender filaments, of which outer one is somewhat thinner at least near bifurcation than the inner one; all filaments light yellow-

ish brown, subequal in length to one another (1.2–1.6 mm), slightly tapered toward apical tips; cuticular surface of filaments with distinct annular ridges and furrows gradually becoming indistinct apically, and densely covered with minute tubercles; annular ridges on basal portion of upper and lower main filaments very distinct forming reticulate pattern, with relatively larger tubercles on ridges and smaller ones on interridges. ***Abdomen***. Terga 1 and 2 pale yellow, almost bare; tergum 1 with 1 simple, slender seta on each side; tergum 2 with 1 simple, slender, short seta and 5 short, somewhat spinous setae, submedially on each side; terga 3 and 4 pale, each with 4 hooked spines and 1 short, somewhat spinous seta on each side; tergum 5 lacking spine-combs; terga 6–9 each with distinct spine-combs in transverse row, together with comb-like groups of minute spines on each side; tergum 9 with a pair of distinct, conical terminal hooks, which have round apex. Sternum 4 with 1 simple hook (slightly smaller in size than those on sternum 7) and a few simple, slender, minute setae on each side; sternum 5 with a pair of bifid hooks submedially on each side; sterna 6 and 7 each with a pair of bifid inner and simple or bifid outer hooks somewhat spaced from each other, and a few, simple, short, slender minute setae on each side. Each side of segment 9 with 3 grapnel-like hooklets. ***Cocoon***. Simple, wall-pocket-shaped, neatly and compactly woven without open spaces in webs, moderately extending ventrolaterally; anterior margin somewhat thickly woven; posterior 1/2 with floor roughly woven; individual threads indistinct; 2.8 mm long × 1.5–1.8 mm wide.

Mature larva. Body length 3.8–4.0 mm. Body pale yellowish, with dark greyish transverse band on 1st thoracic segment and on each abdominal segment. Cephalic apotome pale yellow, moderately covered with short colorless setae; head spots indistinct. Antenna with 3 segments (of subequal length) and apical sensillum, longer than stem of labral fan. Labral fan with 28–31 main rays. Mandible (Fig. 19) with comb-teeth decreasing in size from 1st to 3rd; mandibular serration consisting of 2 teeth (1 large and 1 small); large tooth directed anteroventrally, making an acute angle distally with ventral margin of mandible. Hypostomium (Fig. 20) with an anterior row of 9 teeth, of which median tooth subequal in length to each corner tooth, and longer than 3 intermediate teeth on each side; lateral margin with 1 tooth or 2; hypostomal bristles 3 or 4 in number, slightly diverging posteriorly from lateral margin on each side. Postgenal cleft (Fig. 21) very deep, leaving narrow bridge. Thoracic segment 3 with 2 pairs (dorsal and dorsolateral) of conical protuberances; thoracic cuticle sparsely covered with simple, bifid or trifid setae (similar to those on abdomen) on dorsal surface. Abdominal segments 1–5 each with 2 pairs (dorsal and dorsolateral) of conical protuberances, of which dorsal pair is somewhat more prominent; abdominal cuticle covered dorsally and dorsolaterally with simple, bifid, trifid and quadrifid setae (Fig. 22) sparsely on segments 1–4, and moderately on segments 5–8; last segment moderately covered with simple and bifid setae on each side of anal sclerite. Rectal gill compound, each of 3 lobes with 8 finger-like secondary lobules. Anal sclerite (Fig. 23) X-shaped, with anterior arms ca. 0.8× as long as posterior ones. Accessory sclerite absent. Ventral papillae well developed, conical in shape. Posterior circlet with ca. 60 rows of up to 11 hooklets per row.

TYPE SPECIMENS. Holotype: female, reared from pupa, collected from Ka Po Waterfall (altitude 40 m), Chumporn Province, southern Thailand, 4.VI.1999, by C. Kuvangkadilok and C. Boonkemtong. Paratypes: 1 male, reared from pupa, 2 pupae, and 39 larvae, same data as holotype.

ECOLOGICAL NOTES. The pupae and larvae of this new species were collected from trailing grasses in a stream, 5–6 m wide, together with *S. nobile*. The water temperature and acidity of the stream were 26°C and pH 9.3, respectively.

ETYMOLOGY. The species *chumpornense* refers to the province, Chumporn, where this species was collected.

REMARKS. This new species is assigned to the *varicorne* species-group within the subgenus *Gomphostilbia,* defined by Takaoka and Davies (1996), by having the antenna with 2+8 segments in place of the usual 2+9 segments.

The pupa of *S. chumpornense* is characterized by the arrangement of the eight gill filaments, as shown in Figs. 17 and 18. All the four known speices of the *varicorne* species-group, i.e., *S. varicorne* Edwards from Indonesia and Peninsular Malaysia, *S. shogakii* Rubtsov from Japan, Korea, and China, *S. burtoni* Takaoka and Davies, and *S. novemarticulatum* Takaoka and Davies, both from Peninsular Malaysia, have the ordinary arrangement of eight gill filaments (i.e., 3+3+2 filaments) (Bentinck, 1955; Takaoka and Davies, 1995; unpublished data). However *S. chumpornense* seems to be most closely related to *S. varicorne* since the pupal gill of the latter species is first divided into two rather

stout filaments near its base, as in *S. chumpornense*.

The adults of *S. chumpornense* are easily separated from *S. novemarticulatum* by the antenna consisting of 2+8 segments (c.f., 2+7 segments in the latter). Among the other three related species which have 2+8 antennal segments, this new species seems to be more closely related to *S. varicorne* than to *S. burtoni* and *S. shogakii* by having the similar flat ventral plate in the male genitalia. The ventral plates of the latter two species are produced ventrally near its posterior margin (Bentinck, 1955; Takaoka and Davies, 1995).

The female of *S. chumpornense* is easily distinguished from *S. shogakii* by having the ellipsoidal sensory vesicle (Fig. 5) {c.f., globular in the latter according to Bentinck (1955)}, and from *S. burtoni* by having the different color pattern of the antenna (Fig. 4). In the antenna of *S. burtoni,* first and second flagellar segments are dark yellow, and third to eighth ones are dark brown, though most of the inside surface of the fourth segment is yellowish (Takaoka and Davies, 1995). The female of *S. varicorne* has not been known as yet.

Notes on Newly Recorded Species

Simulium (*Gomphostilbia*) *angulistylum* Takaoka and Davies, 1995

Simulium (*Gomphostilbia*) *angulistylum* Takaoka and Davies, 1995: 42–46 (female, male, pupa and larva).

Specimens Examined. 30 larvae, Haew Suwat Waterfall (altitude 630 m), Khao Yai National Park, Nakorn Ratchasima Province, northeastern Thailand, 10.VI.1998, by C. Kuvangkadilok and C. Boonkemtong; 1 female, 2 males and 25 larvae, Huai Yang Waterfall (altitude 75 m), Prachuap Kirikuan Province, central Thailand, 4.VI. 1999, by C. Kuvangkadilok and C. Boonkemtong; 25 larvae, Sa Nangmanora Waterfall (altitude 150 m), Sa Nangmanora Forest, Park, Phangnga Province, southern Thailand, 3.VII.1999, by C. Kuvangkadilok and C. Boonkemtong; 1 male, 2 pupae and 78 larvae, Khao Phra Narai Waterfall (altitude 120 m), Ranong Province, southern Thailand, 4.VII.1999, by C. Kuvangkadilok and C. Boonkemtong; 1 female, 1 male and 29 larvae, Muang Tuad Waterfall (100 m), Tai Rom Yen National Park, Suratthani Province, southern Thailand, 3.VIII.1999, by C. Kuvangkadilok and C. Boonkemtong; 2 pupae and 23 larvae, Ngao Waterfall (altitude 50 m), Ranong Province, southern Thailand, 4.VII.1999, by C. Kuvangkadilok and C. Boonkemtong; 2 pupae, Huai Luang Waterfall (altitude 300 m), Phu-Jong Na-Yoy National Park, Ubon Ratchathani Province, northeastern Thailand, 16. XI.1998, by C. Kuvangkadilok and C. Boonkemtong.

Ecological Notes. The larvae and pupae of this species were collected from fallen leaves and/or trailing grasses in several streams of various widths 0.5–5.0 m. The water temperature and acidity of the streams were 24.0–26.5°C and pH 7.6–9.4, respectively. This species was collected together with *S. grossifilum, S. nakhonense, S. nobile, S. parahiyangum, S. quinquestriatum, S. sheilae, S. siamense,* and *S. tani.*

Remarks. *S. angulistylum* was originally described from Peninsular Malaysia (Takaoka and Davies, 1995). This species is characterized by the eight short slender pupal gill filaments and the male genitalia with an abruptly-bent style and a posteriorly-attenuated ventral plate. The Thai specimens are in good agreement at all stages with the original descriptions.

Simulium (*Gomphostilbia*) *sheilae* Takaoka and Davies, 1995

Simulium (*Gomphostilbia*) *sheilae* Takaoka and Davies, 1995: 60–65 (female, male, pupa and larva).

Specimens Examined. 1 male, 4 pupae and 255 larvae, Boripat Waterfall (altitude 50 m), Thalay Bun National Park, Songkhla Province, southern Thailand, 1.VI.1999, by C. Kuvangkadilok and C. Boonkemtong; 3 females, 1 male, 6 pupae and 32 larvae, Tone Phrew Waterfall (altitude 125 m), Khao Bun Tad Wildlife Sanctuary, Trang Province, southern Thailand, 2.VI.1999, by C. Kuvangkadilok and C. Boonkemtong: 1 male, Huai Yang Waterfall (altitude 75 m), Prachuap Kirikuan Province, western Thailand, 4.VI.1999, by C. Kuvangkadilok and C. Boonkemtong.

Ecological Notes. The pupae and larvae of this species were collected from fallen leaves and/or small plant roots in several streams 0.5–3.0 m wide. The water temperature and acidity of the streams were 24–26°C, and pH 7.0–8.4, respectively. This species was collected together with *S. fenestratum, S. grossifilum, S. nakhonense* and *S. tani.*

Remarks. *S. sheilae* was originally described from Peninsular Malaysia, and was assigned to the *ceylonicum* species-group by Takaoka and Davies (1995). This species is separated from other related

species of the same group by the enlarged oblong female sensory vesicle (ca. 0.7 times as long as 3rd maxillary palpal segment) and the almost brown male hind basitarsus (though basal 1/3 or a little less somewhat paler). The reared adult female and male specimens, as well as pupal and larval ones examined in this study, are morphologically almost the same as those originally described.

***Simulium* (*Gomphostilbia*) *dentistylum* Takaoka and Davies, 1995**

Simulium (*Gomphostilbia*) *dentistylum* Takaoka and Davies, 1995: 51–55 (male, pupa and larva).

SPECIMENS EXAMINED. 1 male reared from pupa, Huai Luang Waterfall (altitude 300 m), Phu-Jong Na-Yoy National Park, Ubon Ratchathani Province, northeastern Thailand, 16.XI.1998, by C. Kuvangkadilok and C. Boonkemtong; 1 pupa, Yong Waterfall (altitude 120 m), Nakorn Srithammarat Province, southern Thailand, 19.IV.1999, by C. Kuvangkadilok and C. Boonkemtong.

ECOLOGICAL NOTES. The pupae of this species were collected from fallen leaves in streams 1.0–5.0 m wide. The water temperature and acidity of the streams were 24.0–26.0°C and pH 7.4–7.5, respectively. This species was collected together with *S. asakoae, S. chainarongi, S. nakhonense, S. nodosum, S. parahiyangum, S. siamense* and *S. tani*.

REMARKS. *S. dentistylum* was originally described from Peninsular Malaysia (Takaoka and Davies, 1995). This species is very similar to *S. parahiyangum* by having the eight short very slender pupal gill filaments on each side, and the very deep larval postgenal cleft, but differs from the latter by the arrangement of the pupal gill, the fewer tubercles on each round ridge of the pupal antennal sheaths, and the absence of the dorsal protuberances on the larval abdomen. The two pupae and one reared adult male from Thailand agree well morphologically with the original descriptions.

***Simulium* (*Gomphostilbia*) *gombakense* Takaoka and Davies, 1995**

Simulium (*Morops*) *gombakense* Takaoka and Davies, 1995: 82–84 (larva).

Simulium (*Gomphostilbia*) *gombakense*: Takaoka, 2000: (male and pupa).

SPECIMENS EXAMINED. 18 larvae, Mae Klang Waterfall (altitude 940 m), Doi Inthanon National Park, Chiang Mai Province, northern Thailand, 18.V.1997, by C. Kuvangkadilok, S. Phayuhasena and C. Boonkemtong; 1 female reared from pupa, Riang Thong Waterfall (175 m in altitude), Khao-Pu Khao-Ya National Park, Pattalung Province, southern Thailand. 2.VI.1999, by C. Kuvangkadilok and C. Boonkemtong.

ECOLOGICAL NOTES. The pupa and larvae of this species were collected from fallen leaves in two streams 0.5 m and 1.0–2.0 m wide, respectively. The water temperature and acidity were 25.5°C and pH 8.4 in one stream, and 26.0°C and pH 9.4 in the other. This species was collected together with *S. asakoae* and *S. tani* or with *S. nakhonense*.

REMARKS. This species was originally described from pharate pupal and larval specimens from Peninsular Malaysia and was tentatively assigned to the subgenus *Morops* (Takaoka and Davies, 1995). However, it was transferred to the subgenus *Gomphostilbia* when its adult male reared from a pupa was obtained and studied (Takaoka, 2000). The pupa of this species is characterized by its gill of much inflated form with six finger-like projections and with eight slender thread-like filaments (Takaoka, 2000). The pupa and larvae collected from Thailand agree well morphologically with the redescriptions (Takaoka, 2000).

***Simulium* (*Simulium*) *grossifilum* Takaoka and Davies, 1995**

Simulium (*Simulium*) *grossifilum* Takaoka and Davies, 1995: 105–110 (female, male, pupa and larva).

SPECIMENS EXAMINED. 1 male reared from pupa, and 8 larvae, Muang Tuad Waterfall (100 m in altitude), Tai Rom Yen National Park, Nakorn Srithammarat Province, southern Thailand, 3.VI.1999, by C. Kuvangkadilok and C. Boonkemtong; 56 larvae, Ngao Waterfall (altitude 50 m), Ranong Province, southern Thailand, 4.VIII.1999, by C. Kuvangkadilok and C. Boonkemtong; 1 female reared from pupa and 1 pupa and 3 larvae, Mae Yai Waterfall (100 m in altitude), Khao Sok National Park, Suratthani Province, southern Thailand, 2.VIII.1999, by C. Kuvangkadilok and C. Boonkemtong.

ECOLOGICAL NOTES. The pupae and larvae of this species were collected from trailing grasses and fallen leaves in streams 0.5–2.0 m wide. The water temperature and acidity of the streams were 24.8–26.0°C, and pH 8.0–8.3, respectively. This species was collected

together with *S. angulistylum, S. asakoae, S. decuplum, S. malayense, S. nakhonense, S. parahiyangum, S. quinquestriatum, S. tani* and *S. yongi*.

REMARKS. *S. grossifilum* was originally described from Peninsular Malaysia, and was assigned to the *griseifrons* species-group (Takaoka and Davies, 1995). This species is very distinctive among the subgenus *Simulium* s. str. by its swollen pupal gill filaments covered with minute setae, the female anterior gonapophyses with a dorsal projection, and the male lamellate ventral plate. The female, male, pupal and larval specimens collected from Thailand are morphologically in good agreement with the original descriptions.

***Simulium* (*Simulium*) *malayense* Takaoka and Davies, 1995**

Simulium (*Simulium*) *malayense* Takaoka and Davies, 1995: 120–123 (female, pupa and larva).

SPECIMENS EXAMINED. 1 male reared from pupa, Mae Yai Waterfall (altitude 100 m), Khao Sok National Park, Suratthani Province, southern Thailand, 2.Ⅷ.1999, by C. Kuvangkadilok and C. Boonkemtong.

ECOLOGICAL NOTES. The one pupa of this species was collected from a fallen leaf in a small stream 1 m wide. This species was collected together with *S. grossifilum* and *S. yongi*.

REMARKS. *S. malayense* was described from female, pupal and larval specimens collected from Peninsular Malaysia, and was assigned to the *multistriatum* species-group (Takaoka and Davies, 1995). This species is easily separated from the other related species of the same group by its simple wall-pocket-shaped cocoon without anterolateral windows. Morphological characters of the pupa collected from Thailand are almost the same as those in the original description.

***Simulium* (*Simulium*) *nobile* de Meijere, 1907**

Simulium nobile de Meijere, 1907: 206 (male); Edwards, 1934: 115 (female, male, pupa and larva).

Simulium (*Simulium*) *nobile*: Crosskey, 1973: 428; Crosskey, 1988: 475; Takaoka and Davies, 1995: 123; Takaoka and Davies, 1996: 61 (female, male, pupa and larva).

SPECIMENS EXAMINED. 8 females, 6 males, 60 pupae and ca. 700 larvae, Ka Po Waterfall (altitude 40 m), Chumporn Province, southern Thailand, 5.Ⅶ.1999, by C. Kuvangkadilok and C. Boonkemtong; 6 pupae and 114 larvae, Sa Nangmanora Waterfall (altitude 50 m), Sa Nangmanora Forest Park, Phangnga Province, southern Thailand, 3.Ⅶ.1999, by C. Kuvangkadilok and C. Boonkemtong; 7 females, 5 males, 85 pupae and 833 larvae, Tone Sai Waterfall (altitude 30 m), Phuket Province, southern Thailand, 2.Ⅷ.1999, by C. Kuvangkadilok and S. Phayuhasena.

ECOLOGICAL NOTES. The pupae and larvae of this species were collected from fallen leaves in streams 1.0–3.0 m wide. The water temperature and acidity were 25.0–27.0°C, and pH 7.8–9.4, respectively. This species was collected together with *S. angulistylum, S. chumpornense, S. nakhonense* and *S. siamense*.

REMARKS. *S. nobile* was originally described from Java (de Meijere, 1907), and later recorded from South Sumatra (Edwards, 1934), Peninsular Malaysia and Sabah (Crosskey, 1973). This species is a type species of the *nobile* species-group, and is readily distinguished, by its six short slender pupal gill filaments, from *S. nodosum,* the only species of the same group so far known from Thailand. The Thai specimens examined in this study are morphologically almost the same as the Javanese specimens redescribed by Takaoka and Davies (1996).

***Simulium* (*Simulium*) *rudnicki* Takaoka and Davies, 1995**

Simulium (*Simulium*) *rudnicki* Takaoka and Davies, 1995: 155–157 (female).

SPECIMENS EXAMINED. 20 pupae and 216 larvae, Mae Ya Waterfall (altitude 520 m), Doi Inthanon National Park, Chiang Mai Province, northern Thailand, 21.Ⅷ.1996, by C. Kuvangkadilok, S. Phayuhasena and C. Boonkemtong: 83 larvae, Vachiratharn Waterfall (altitude 700 m), Doi Inthanon National Park, Chiang Mai Province, northern Thailand, 15.Ⅱ.1997, by C. Kuvangkadilok, S. Phayuhasena and C. Boonkemtong; 13 larvae, Tad Deuan Waterfall (altitude 170 m), Srisatchanalai National Park, Sukothai Province, northern Thailand, 26.I.1999, by C. Kuvangkadilok and C. Boonkemtong.

ECOLOGICAL NOTES. The pupae and larvae of this species were collected from rock and stone surfaces in streams 1.0–2.0 m wide. The water temperature and acidity of the streams were 16–22°C and pH 8.4–9.0,

respectively. This species was collected together with *S. nakhonense, S. quinquestriatum* and *S. siamense*.

REMARKS. *S. rudnicki* was originally described only from two females collected from Langkawi Island, Malaysia, and remained ungrouped within *Simulium* s. str. (Takaoka and Davies, 1995). Two pharate females dissected out of the pupae collected from Thailand conform to the original description in most characters including the yellowish leg colorings and the anterior gonapophyses with a narrow transparent portion along inner and posterior margins. The pupae collected from Thailand are not different from those of *S. rudnicki* recently collected from Langkawi Island (unpublished data). They have six slender gill filaments on each side.

***Simulium* (*Simulium*) *yongi* Takaoka and Davies, 1997**

Simulium (*Simulium*) *yongi* Takaoka and Davies, 1997: 11–16 (female, male, pupa and larva).

SPECIMENS EXAMINED. 1 female reared from pupa, Mae Yai Waterfall (altitude 100 m), Khao Sok National Park, Suratthani Province, southern Thailand, 2.Ⅷ.1999, by C. Kuvangkadilok and S. Phayuhasena.

ECOLOGICAL NOTES. The only one pupa of this species was collected from a fallen leaf in a small stream ca. 1.0 m wide. This species was collected together with *S. grossifilum* and *S. malayense*.

REMARKS. The reared female and its pupal exuvia examined in this study agree well morphologically with those originally described from Peninsular Malaysia by Takaoka and Davies (1997). The female of this species is very similar to *S. rudnicki,* but differs from the latter by the darker leg colorings.

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ROLE OF *ANOPHELES SUBPICTUS* AS A PRIMARY VECTOR OF MALARIA IN AN AREA IN INDIA

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Abstract: Sibling species A of *Anopheles subpictus* (fresh water form) has been incriminated and established as a primary vector of malaria for the first time in an area (Tarakeswar, West Bengal) in Indian subcontinent with a natural sporozoite (*Plasmodium vivax*) rate of 0.32% (2/621). Moderate anthropophilic index (41.0) and high survival rate per gonotrophic cycle (0.58) indicate its efficiency as vector. Coincidence of higher malaria cases and higher density of *An. subpictus* in the study area is of high relevance. No natural sporozoite infection was detected in other anophelines available like *An. vagus, An. annularis* and *An. barbirostris*.

Key words: *Anopheles subpictus*, primary vector, malaria, India

INTRODUCTION

Anopheles subpictus Grassi is a recognised primary vector of malaria in Australasian Zone (Russel *et al*., 1963), Celebes (Van Hell, 1952) and in Portuguese timor (Ferreira and Breda, 1962) and a secondary vector in Sri Lanka (Hearth *et al*., 1983). The role of *An. subpictus* as a vector of malaria in India is obscure. The existence of two sibling species of *An. subpictus* namely, species A (fresh water form) and species B (brakish water form) have been described in India (Suguna, 1982). Sibling species B has been incriminated as a vector in some coastal areas of South India (Russel and Jacob, 1939, Russel and Rao, 1940; Panicker *et al*., 1981) and *An. subpictus* (sibling species not defined) has been incriminated in Central India with very low sporozoite rate (0.02%) in the presence of other recognised primary vector (Kulkarni, 1983). Present study has been performed in Tarakeswar area of West Bengal, India (175 km away from Bay of Bengal). According to data recorded in Tarakeswar Block Hospital average annual incidence of *Plasmodium vivax* and *P. falciparum* was 962 and 65, respectively for the last ten years (1988–1997) where the total population was 70,000, seventy nine percent of the cases were recorded during monsoon months (Jul.–Oct.).

MATERIALS AND METHODS

Indoor resting mosquitoes were collected by aspirators (WHO, 1964) from fixed 5 human habitations and 5 cattlesheds of each of selected 4 major villages (Bajitpur, Baligori, Mirzapur and Bhanjipur) of Tarakeswar area of Hooghly district, West Bengal, India. Collections were done both from human habitations and cattlesheds because there were a large number of cattlesheds very close to human habitations. One insect collector collected mosquitoes for 12 min per habitation/shed i.e. for 120 min (2 hrs) from 10 habitations/sheds of each village in each month. Thus a total of 192 man-hour (2 hrs×4 villages×24 months) was employed for collection in two years from January '96 to December '97. Moreover, man-bait collections were made (following the method of Chandra and Hati, 1993) from 6 p.m. to 6 a.m. both at indoor and outdoor locations, once a month for one year from January '97 to December '97 (thrice in a year i.e. once in each season in each village) employing a total of 288 man-hour (12 hrs×2 locations×3 seasons×4 villages).

Salivary glands and guts of 621 *An. subpictus*, 621 *An. vagus*, 621 *An. barbirostris* and 65 *An. annularis* mosquitoes (indoor-resting and man-landing) were dissected and examined to detect sporozoites and/or oocysts of *Plasmodium*. Sporozoite species were identified by ELISA test. Sibling species of *An. subpictus* was ascertained on the basis of banding pattern of polytene chromosome as described by Suguna (1982). A total of 120 mosquitoes were examined for polytene chromosome observation (5 mosquitoes from each village in each season for two years i.e. 5 mos-

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quitoes×4 villages×3 seasons×2 years=120).

Blood meals of 480 indoor-resting engorged *An. subpictus* (collected in the morning hours from human habitations of all the villages in all the three seasons) were tested by Ouchterlony's gel diffusion technique (Ouchterlony and Nilson, 1973) against human, bovine, avian and goat antisera to determine the anthropophilic index.

Age composition of *An. subpictus* population was determined on the basis of the number of ovariolar dilatations (Polovodova, 1949). Ovarioles of 300 mosquitoes were examined which were collected in the morning hours from all the four villages in all the three seasons. Daily survival rate and daily mortality rate were calculated with the method of Davidson (1954) and Service (1976) respectively. Survival rate per gonotrophic cycle was determined with the help of log curve following the Method of Chandra *et al*. (1996). The gonotrophic cycle of blood feeding insects is determined by the time taken between successive blood meals and egg laying (WHO, 1975). The duration of each gonotrophic cycle of a laboratory-maintained *An. subpictus* colony was estimated by artificial blood feeding in three sets of experiments conducted during each season, namely summer, winter and rainy seasons at normal temperature. Though there is no evidence that the gonotrophic cycle of the wild population would be essentially the same as those of populations in cages (Chandra *et al*., 1996).

Eight small ponds, 8 submerged fields and 8 drains (2 from each village) were searched for *An. subpictus* larvae in the study area. Larvae were collected with 250 m*l* dipper. One hundred dips were given in each pond/field/drain in each season. Larvae were counted, examined and larval species were determined. Moreover, temporary water collections (available only during monsoons) were searched for *Anopheles* larvae.

RESULTS

During the two year study period altogether 4,717 (1,830 from human habitations and 2,887 from cattlesheds) indoor-resting *Anopheles* mosquitoes were collected of which *An. subpictus* predominated over the others, comprising 50% (2,356) of them with a density of 12.3 per man-hour. Other species were *An. annularis* (65; 1.4%), *An. barbirostris* (1,187; 25.2%) and *An. vagus* (1,109; 23.5%). Monthly collection of *An. subpictus* from January '96 to December '97 (two years) in 4 villages has been presented in Table 1. Seasonal prevalence of *An. subpictus* during rainy (Jul.-Oct.), winter (Nov.-Feb.) and summer (Mar.-Jun.) was 49.3%, 13.8% and 36.9% respectively.

During one year man-bait collection, the same 4 *Anopheles* species (Like indoor-resting population) were encountered and *An. subpictus* comprised of 48.3% (156) out of altogether 320 *Anopheles* mosquitoes caught. Density per man-hour and per man night of man-landing *An. subpictus* population was 0.54 and 13.0, respectively. Among the biting population, 43.6%, 14.7% and 41.7% came to bite in rainy, winter and summer seasons, respectively. Monthwise collection of man-

Table 1 Monthly collection of *An. subpictus* from human habitations(H) and cattlesheds(C) of 4 villages of Tarakeswar area during Jan. 1996 to Dec. 1997

Month	Name of the villages								Total	
	Bajitpur		Baligori		Mirzapur		Bhanjipur			
	H	C	H	C	H	C	H	C	No.	%
Jan.	2	10	1	7	1	9	1	7	38	1.62
Feb.	12	42	7	26	11	22	6	36	162	6.88
Mar.	28	51	20	45	18	40	16	32	250	10.61
Apr.	23	36	15	24	21	16	12	20	167	7.09
May	18	25	12	16	5	20	12	14	122	5.18
Jun.	35	70	28	51	30	55	20	41	330	14.00
Jul.	52	93	39	74	41	64	32	80	475	20.16
Aug.	38	61	38	46	35	39	22	29	308	13.07
Sep.	31	47	25	35	25	33	29	30	255	10.82
Oct.	7	36	5	37	1	12	4	22	124	5.26
Nov.	4	29	1	20	2	13	2	19	90	3.82
Dec.	0	13	1	12	1	4	2	2	35	1.49
Total	250	513	192	393	191	327	158	332	2,356	100

Table 2 Monthwise collection of *An. subpictus* off human baits during night (6 p.m. to 6 a.m.) extending from January 1997 to December 1997 in Tarakeswar area

Month	Indoor			Outdoor			Total		
	Number	Percent(%)	Per man-hour collection	Number	Percent(%)	Per man-hour collection	Number	Percent(%)	Per man-hour collection
Jan.	3	3.19	0.25	1	1.61	0.08	4	2.56	0.16
Feb.	7	7.45	0.58	6	9.68	0.50	13	8.33	0.54
Mar.	13	13.83	1.08	8	12.90	0.66	21	13.46	0.87
Apr.	9	9.58	0.75	5	8.06	0.41	14	8.98	0.58
May	8	8.51	0.66	6	9.68	0.50	14	8.98	0.58
Jun.	9	9.58	0.75	7	11.30	0.58	16	10.26	0.66
Jul.	14	14.89	1.16	10	16.13	0.83	24	15.38	1.00
Aug.	12	12.76	1.00	9	14.52	0.75	21	13.46	0.87
Sep.	9	9.58	0.75	5	8.06	0.41	14	8.98	0.58
Oct.	6	6.39	0.50	3	4.84	0.25	9	5.77	0.37
Nov.	2	2.12	0.16	1	1.61	0.08	3	1.92	0.12
Dec.	2	2.12	0.16	1	1.61	0.08	3	1.92	0.12
Total/ *Average	94	100.00	*0.65	62	100.00	*0.43	156	100.00	*0.54

landing *An. subpictus* has been presented in Table 2.

Natural sporozoite infection was detected in the salivary glands of one man-landing *An. subpictus* (out of 156 dissected) and one indoor-resting *An. subpictus* (out of 465 dissected) with the sporozoite rates of 0.64% and 0.21%, respectively. Overall sporozoite rate of *An. subpictus* (combining indoor-resting and man-landing population) was 0.32%. The sporozoite species was detected to be *P. vivax* in both the cases. All the other species of *Anopheles* dissected, were negative for sporozoite infection. No gut infection with oocysts was detected in any anopheline species available in the study area.

Out of 480 indoor-resting *An. subpictus* subjected to precipitin test, 197 (41.0%) were positive for human blood. Rests (59.0%) were positive for bovine blood. None of the engorged *An. subpictus* imbibed avian and goat blood.

Duration of gonotrophic cycle of *An. subpictus* was 98 hrs, 102 hrs and 88 hrs in rainy, winter and summer seasons respectively. Average duration of the cycle was calculated to be 96 hrs (4 days). Number of mosquitoes of different parous state, proportion parous, daily survival rate and daily mortality rate of natural population have been presented in Table 3. Survival rate per gonotrophic cycle averaged over two years was 0.58 which was obtained from log curve and the slope of the straight line was −0.234. Among the female *An. subpictus* population 14.5% passed 3 or more gonotrophic cycles in natural condition.

Larval density of *An. subpictus* in different types of breeding places in different seasons have been presented in Table 4. All the *An. subpictus* mosquitoes, collected in all the seasons form all the villages of the study area and examined for polytene chromosome, were detected to be as sibling species A (fresh water from).

Table 3 Seasonal proportion parous (PP), daily survival rate (DSR) and daily mortality rate (DMR) of *An. subpictus* population of the study area

Season	No. dissected	Mosquitoes of different parous					PP	DSR	DMR
		NP	P_1	P_2	P_3	P_4			
Summer (Mar.-Jun.)	100	67	25	5	2	1	0.33	0.76	24%
Rainy (Jul.-Oct.)	100	44	20	16	13	7	0.56	0.86	14%
Winter (Nov.-Feb.)	100	35	24	20	13	8	0.65	0.90	10%
Total	300	146	69	41	28	16	0.51	0.84	16%

Correlation coefficient between the number of dilatations and the number of females in each dilatation class=−0.9159.

Table 4 Larval density of *An. subpictus* in different types of breeding places in different seasons in the study area

Season	No. of dips	Pond		Drain		Submerged field	
		No.and(percent) collected	density per dip	No.and(percent) collected	density per dip	No.and(percent) collected	density per dip
Summer (Mar.–Jun.)	800	836 (17.25)	1.04	17 (24.28)	0.02	230 (6.87)	0.29
Rainy (Jul.–Oct.)	800	3,757 (77.54)	4.70	49 (70.0)	0.06	2,982 (89.14)	3.73
Winter (Nov.–Feb.)	800	252 (5.20)	0.31	4 (5.71)	0.005	133 (4.0)	0.17
Total	2,400	4,845 (100)	2.01	70 (100)	0.03	3,345 (100)	1.40

DISCUSSION

Information on the role of *An. subpictus* as the vector of malaria in India is limited. Sibling species B (brakish water form) of *An. subpictus* has been incriminated as vector in some coastal areas of South India (Russel and Jacob, 1939; Russel and Rao, 1940; Panicker *et al*., 1981) in the presence of some other recognised vectors. In the present study area of Tarakeswar, W.B., India, species A of *An. subpictus* is predominating over the other anophelines and has been incriminated for the first time as the vector of human malarial parasite in absence of any other recognised vector. Species B has not been available probably because the study area is nearly 175 km away from the sea (Bay of Bengal).

Anthropophilic index (41.0) of indoor-resting population is moderate but higher than other places recorded so far (Senior White, 1938; Roy, 1943; Bruce Chwatt, 1966; Kulkarni, 1983; Hati, 1986; Collins *et al*., 1989; Banerjee *et al*., 1991; Abhaywardana *et al*., 1996) indicating considerable extent of man vector contact. No preference to avian blood meal excludes the probability of transmitting avian malaria.

Proportion parous (0.51) and daily survival rate (0.84) are very high and survival rate per gonotrophic cycle (0.58) is consistent with high proportion parous. 14.5% of the female *An. subpictus* population have passed 3 or more gonotrophic cycles in nature which indicates that a considerable portion of the wild population can survive long enough in nature to develop sporozoites in them and thereby able to transmit malaria successfully.

The prevalence of indoor-resting population of *An. subpictus* was higher in the rainy season. Man-bait experiments also indicate that they came to bite more in the rainy season. The cause of such higher prevalence of this species may be due to greater breeding facilities during rainy season in the paddy fields, shallow ponds and also in the temporary water collections. When breeding places were searched, per dip density of *An. subpictus* larvae was always higher in the rainy season in all types of breeding places. This is worthy mentioning that they breed more, take rest in more number in human habitations and cattlesheds and also bite more coinciding with the higher number of malaria cases during rainy season in present study area.

Present findings help us to establish the role of *An. subpictus* as a primary vector of malaria in absence of any other recognised primary vector in an area (Tarakeswar, West Bengal) in Indian subcontinent. Natural infection of *P. falciparum* in the mosquitoes was not available, may be, due to lower incidence of *P. falciparum* malaria cases is the study area. Further studies are necessary to find out its role as vector in other parts of the country.

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THE EFFECT OF REPEATED FOUR MONTHLY TREATMENTS ON THE PREVALENCE AND WORM BURDEN OF *ASCARIS, TRICHURIS* AND HOOKWORM INFECTIONS IN AN ENDEMIC AREA

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Abstract: The effect of repeated 4 monthly treatment of single dose of 400 mg albendazole on the prevalence and worm burden of *Ascaris, Trichuris* and hookworm was studied in an endemic area. Repeated 4 monthly treatment, had significant effect in reducing the worm burden of *Ascaris* and not the prevalence. As for hookworm infection repeated 4 monthly treatment had significant impact in reducing both prevalence and worm burden in the community. However, repeated 4 monthly treatments had no significant impact in reducing the prevalence and worm burden of *Trichuris* infection in the community. *Trichuris* infection should be considered as a continuing infection rather than reinfection because of poor cure rate of albendazole at the onset of the study. Significant and strong predisposition ($p < 0.0001$) were detected after single and repeated 4 monthly treatment in *Ascaris* and *Trichuris* infection but not in hookworm infection. Stratification of data indicated that significant and strong predisposition was also seen in gender. This study concludes that changes in mean egg count per gram (mean EPG) is a sensitive indicator in measuring effect of treatment in an endemic area, and repeated 4 monthly treatments has successfully reduced the worm burden of *Ascaris* and hookworm infection. Subjects remained predisposed to *Ascaris* infection over 2 reinfection periods, and for hookworm infection evidence of predisposition was seen only over 1 reinfection period.

Key words: Predisposition, *Ascaris, Trichuris,* hookworm, reinfection

INTRODUCTION

In highly endemic areas of *Ascaris, Trichuris* and hookworm infections, reinfection can occur as early as 2 months after treatment (Norhayati *et al.,* 1995). By 4 months almost half and one-tenth of the population treated become reinfected with *Ascaris* and hookworm respectively (Norhayati *et al.,* 1997), and by 6 months the intensity of infection of *Ascaris* and *Trichuris* were similar to pre-treatment levels (Elkins *et al.,* 1988; Albinco *et al.,* 1995). Thus a 4 monthly targeted chemotherapy in highly endemic areas may be necessary in order to have an impact on the prevalence and intensity of infection of *Ascaris* and hookworm infections (Bundy *et al.,* 1990; Albinco *et al.,* 1995; Norhayati *et al.,* 1997). Predisposition to reinfection following single-treatment has been reported in hookworm infection (Schad and Anderson, 1985), *Ascaris* infection (Elkins *et al.,* 1986; Thein-Hlaing *et al.,* 1987; Haswell-Elkins *et al.,* 1987; Forrester *et al.,* 1990) and *Trichuris* infection (Haswell-Elkins *et al.,* 1987; Bundy *et al.,* 1987; Bundy and Cooper, 1988; Forrester *et al.,* 1990). Predisposition has also been reported following repeated treatments (2 reinfection period) in *Ascaris* infection (Holland *et al.,* 1989; Hall *et al.,* 1992; Chan *et al.,* 1992) and in *Trichuris* infection (Chan *et al.,* 1992).

This intervention study was conducted in a highly endemic area of *Ascaris*, *Trichuris* and hookworm infections. The objectives of this study were to evaluate the impact of 4 monthly 400 mg albendazole treatment for

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12 months on the prevalence and intensity of infection/worm burden (mean eggs per grasm-EPG) of *Ascaris*, *Trichuris* and hookworm infections and to examine the evidence of predisposition over 2 reinfection periods.

Materials and Methods

Study Area

This study was part of a bigger study conducted among residents of 6 Orang Asli (Aborigine) villages in the sub-district of Dengkil, Selangor, Malaysia. It was situated about 50 km from Kuala Lumpur, the capital of Malaysia. Each village comprised a very small population and most of the residents worked as palm oil estate labourers, rubber tappers, farmers or were engaged doing odd jobs such as fishing and selling forest products. Most of them lived in single-roomed houses made of bamboo and wood. Almost all the houses in the villages had no electricity, no pipe water nor toilet facilities. The residents used well or river water for daily use and defaecated in the open among the bushes.

Subjects and Parasitologic Surveys

In all 205 children (95 males and 110 females) aged 1-13 years old were included in this study. Stool samples were collected and those found infected were treated with 400 mg of albendazole periodically at 4 months interval for 1 year. The prevalence and worm burden of *Ascaris*, *Trichuris* and hookworm infection at pre-treatment (phase I), at 4 months after single treatment (phase II) and at 12 months after repeated treatments (four monthly intervals-phase III) were determined before each intervention and compared. To measure the efficacy of albendazole, stool samples were collected and examined at 1 month after single treatment. All samples were examined by the Kato-Katz technique and worm burden was measured indirectly as EPG (WHO, 1987). For *Ascaris*, EPG of 1-9,999, 10,000-49,999 and 50,000 and above is defined as mild, moderate or severe infection respectively. For *Trichuris* and hookworm EPG of 1-1,999, 2,000-9,999 and 10,000 and above is defined as mild, moderate or severe infection respectively (WHO, 1987).

The efficacy of albendazole in reducing the prevalence and worm burden was measured by cure rate and mean egg count reduction rate respectively. Cure rate is the percentage of cured cases at 1 month after the single treatment. The mean egg count reduction rate is the reduction between the mean EPG at 1 month after treatment and the mean EPG at pre-treatment, expressed as a percentage of the mean EPG at pre-treatment.

Statistical Analysis

Changes in the prevalence and worm burden was compared by 2 proportional test and by paired t-test after logarithmic transformation of data respectively. Correlation of egg counts were analysed using Kendall's Rank Correlation Test. Data were analysed using EpiInfo (EpiInfo version 6.02, 1994) and SPSS for Windows (SPSS, 1993).

Results

The overall pre-treatment prevalence of *Trichuris*, *Ascaris*, and hookworm infections in 205 children (95 boys and 110 girls) was 91.7%, 62.5% and 28.8% respectively. Almost two-third were infected with moderate and severe infections of *Trichuris*, 46.3% had moderate and severe infections of *Ascaris* and only 1.5% had moderate infection of hookworm. The cure rate of *Ascaris* infection was 97.4% and the mean egg count reduction rate was 99.9%; the cure rate of hookworm infection was 93.1% and the mean egg count reduction rate was 96.6%. The cure rate of *Trichuris* infection was low (5.5%), however the mean egg count reduction rate was more evident (49.1%).

Prevalence of Ascaris, Trichuris and hookworm infections

Table 1 shows the prevalence of *Ascaris*, *Trichuris* and hookworm infections at phase I, phase II and phase III. The overall prevalence of *Ascaris* infection at phase II and III decreased by 31.8% and 7.2% respectively, compared with phase I. However the difference was not statistically significant. The increase in the prevalence of *Trichuris* infection at phase II and III compared with phase I by 2.3% and 5.0% respectively was not statistically significant. For hookworm infection, the overall prevalence at phase II and III compared to phase I decreased by 63.9% and 69.8% respectively and the difference was statistically significant. Although the prevalence of *Ascaris* and *Trichuris* infections increased in phase III compared to phase II, the difference was not statistically significant. The decrease in the prevalence of hookworm infection between phase II and III was also not significant.

Intensity of Ascaris, Trichuris and hookworm infections

Table 2 shows the mean geometric EPG of *Ascaris*, *Trichuris* and hookworm infections at phase I, phase II and phase III. The mean EPG of *Ascaris* at phase II and III decreased by 53.3% and 75.0% respectively compared with phase I and the difference was statisti-

Table 1 Prevalence of *Ascaris*, *Trichuris* and hookworm infection at phase I, II and III

Infections	Prevalence (%)			Percent reduction	
Phase	I	II	III	I-II/I	I-III/I
Ascaris	62.5	42.6	58.0	−31.8(P>0.05)	− 7.2(P>0.05)
Trichuris	91.7	93.8	96.3	+ 2.3(P>0.05)	+ 5.0(P>0.05)
Hookworm	28.8	10.4	8.7	−63.9(P<0.05)	−69.8(P<0.05)

Table 2 Mean intensity of *Ascaris, Trichuris* and hookworm infection at phase I, II and III

Infections	Mean EPG (Geometric)			Percent reduction	
Phase	I	II	III	I-II/I	I-III/I
Ascaris	24,071	11,251	6,008	−53.3(P<0.05)	−75.0(P<0.05)
Trichuris	5,516	5,604	5,822	+ 1.6(P>0.05)	+ 5.5(P>0.05)
Hookworm	131	59	10	−55.0(P<0.05)	−92.4(P<0.05)

cally significant. For *Trichuris*, the increase in the mean EPG at phase II and III compared with phase I by 1.6% and 5.5% respectively was not statistically significant. For hookworm infection, there was a significant decrease of mean EPG at phase II and III compared to phase I. Although the mean EPG of *Trichuris* infection increased in phase III compared to phase II, the difference was not statistically significant. The decrease in the mean EPG of *Ascaris* and hookworm infections between phase II and III was statistically significant.

Correlation of egg counts

The correlation for overall and stratified EPG of *Ascaris*, *Trichuris* and hookworm infections according to gender at phase I, II and III are shown in Table 3. Analysis shows that in *Ascaris* infection, there is a strong and significant positive correlation of mean EPG between phase I and II and phase I and III. When the data was stratified according to gender, the level of significance remained strong and significant except in males between phase I and III.

In *Trichuris* infection, strong and significant overall correlation was detected in all phases and when the data was stratified according to gender. In hookworm infection significant correlation was detected between phase I and II and between phase II and III but with lower significant level. When the data was stratified according to gender, significant correlation was detected in males (phase I & II and phase I & III) and in females (phase I & II), although the level was lower.

DISCUSSION

In endemic areas of *Ascaris*, *Trichuris* and hookworm infections, where reinfections occur continuously, repeated treatments with effective antihelminthic were required to reduce worm burden (measured by mean EPG) in the community. The interval required varied. A study done in Burma suggests that 3 monthly repeated treatments give significant impact on the prevalence and worm burden of *Ascaris* (Thein-Hlaing *et al.*, 1987). In contrast, a study in Malaysia reports that 6 monthly repeated treatment has significant effect in reducing both prevalence and worm burden of *Ascaris* and *Trichuris* (Chan *et al.*, 1992). On the other hand, other studies show that by 6 months to 1 year, the prevalence and worm burden of *Ascaris* and *Trichuris* have reached

Table 3 Correlation of *Ascaris, Trichuris* and hookworm mean EPG between phase I and II and phase I and III

Group	Phase I and II	Phase I and III
Ascaris		
Overall	0.3517‡	0.2902‡
Gender		
Male	0.3531‡	0.2457†
Female	0.3522‡	0.3480‡
Trichuris		
Overall	0.5388‡	0.4474‡
Gender		
Male	0.5533‡	0.4198‡
Female	0.5327‡	0.4758‡
Hookworm		
Overall	0.3304†	0.1377
Gender		
Male	0.2730*	0.1173
Female	0.4007‡	−0.0564

* Significant correlation at $p<0.05$
† Significant correlation at $p<0.01$
‡ Significant correlation at $p<0.0001$

the pre-treatment level (Holland *et al.,* 1989; Elkins *et al.,* 1988; Albonico *et al.,* 1995).

We studied the impact of 4 monthly treatment (for 12 months) on the prevalence and worm burden of *Ascaris*, *Trichuris* and hookworm infections in an endemic area. The results of our study showed that repeated 4 monthly treatments had no significant impact in reducing prevalence and worm burden of *Trichuris* infection in the community and this was different from an earlier study done in Malaysia (Chan *et al.,* 1992). Poor cure rate of single dose of 400 mg albendazole against *Trichuris* infection and high prevalence of moderate and severe infections of the worm in this community compared to the study done by Chan *et al.* (1992) were the main reasons for the failure of repeated 4 monthly treatments in reducing prevalence and worm burden of *Trichuris* infection. In the present study, *Trichuris* infection should be considered as a continuing infection rather than reinfection because of poor cure rate at the onset. This factor explains the increases in prevalence and worm burden of *Trichuris* infection in this study.

Repeated 4 monthly treatments had significant effect in reducing worm burden of *Ascaris* but not the prevalence. The increase in *Ascaris* prevalence after repeated 4 monthly treatments can be explained by the continuing infection in the community. This factor suggests that the use of antihelminth can only control this infection by significantly reducing the worm burden in the community and not prevent people from becoming infected. As for hookworm infection, repeated 4 monthly treatments had significant impact in reducing both prevalence and worm burden in the community. Low prevalence, low worm burden and a drastic reduction in worm burden in the community following treatment may explain these findings. Besides that, the speed with which the worm burden bounces back to its pre-treatment level differs between species for example it is slower in hookworm and faster in *Trichuris* and *Ascaris* (Anderson, 1986). This finding also indicates that the mean egg count is a sensitive indicator in evaluating the progress of periodic chemotherapy in the control of *Ascaris* and hookworm infections. A similar finding was reported by Anderson and May (1985).

Based on this study, it is more relevant to suggest implementation of repeated 4 monthly treatments in the planning of periodic chemotherapy schedule in an endemic area. Such a suggestion is also supported by our earlier study in the same community, where after 4 months of treatment, the reinfection rate of *Ascaris* and hookworm was seen in 50.0% and 10.0% of the subjects respectively (Norhayati *et al.,* 1997). Only one-fifth of the subjects infected with *Ascaris* reached their pre-treatment intensity (Norhayati *et al.,* 1997). Our presest study also suggests that the mean EPG should be used as an indicator to monitor the progress of periodic chemotherapy in the community.

This study examines the evidence of predisposition over one and two reinfection periods of *Ascaris*, *Trichuris* and hookworm infections. For *Trichuris* infection evidence of predisposition was seen over one and two reinfections. This evidence was seen as a result of poor cure rate at the start.

As for *Ascaris* infection, evidence of predisposition was seen after one and two reinfection periods. This finding generally agreed with most of the studies done before (Holland *et al.,* 1989; Hall *et al.,* 1992; Chan *et al.,* 1992). For hookworm infection evidence of predisposition after one reinfection period was weak and after two reinfection periods, the evidence was negative. Predisposition following one reinfection period has been reported in hookworm infection by Schad and Anderson (1985). These findings indicate that subjects remained predisposed to *Ascaris* infection over 2 reinfection periods and for hookworm infection evidence of predisposition was only seen over 1 reinfection period.

It can be concluded that repeated 4 monthly treatments have successfully reduced the worm burden of *Ascaris* and hookworm infection in the Orang Asli community. The risk of acquiring similar worm burden as before treatment was only evident in *Ascaris* and not in hookworm infection.

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Research Note:

IDENTIFICATION OF *TOXOPLASMA GONDII* TACHYZOITES AND BRADYZOITES BY A QUANTITATIVE COMPETITIVE POLYMERASE CHAIN REACTION METHOD AFTER THE ACID TREATMENT

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Abstract: It was indicated that tachyzoites and bradyzoites of *Toxoplasma gondii* (*T. gondii*) defined by the susceptibility/resistance to acid treatment could be quantitatively analyzed by a quantitative competitive polymerase chain reaction method targeting SAG1 gene, specific for *T. gondii*. The tachyzoites were destroyed not only by pepsin-HCl but also by HCl or other acid solutions. The acid-induced destructions occurred under the pH conditions lower than pH 1.8. Tachyzoite DNA of *T. gondii* was easily destructed by acid treatment. These DNA destructions made it possible to differentiate between tachyzoites and bradyzoites by QC-PCR.

Key words: bradyzoite, HCl treatment, tachyzoite

Introduction

A physiological infection route of *Toxoplasma gondii* (*T. gondii*) to hosts including humans is the peroral infection of cysts or oocysts. Jacobs *et al.* (1960a) reported that tachyzoites were killed within a few minutes of incubation in peptic digestive fluid, whereas bradyzoites survived for up to 3 hrs. The difference of resistance to digestive enzymes is used as a criterion to differentiate between parasite stages (Frenkel, 1996; Gross *et al.,* 1996). Pepsin digestion technique has been used to define bradyzoites in animal tissues (Dubey *et al.,* 1997; Jacobs *et al.,* 1960b; Remington *et al.,* 1965). However, bradyzoites are not easy to detect microscopically in digested materials, they were commonly detected by inoculation of the digested materials into mice or cats. A quantitative competitive polymerase chain reaction (QC-PCR) method was developed to assay the number of *T. gondii,* an intracellular infective protozoan, by estimating the copy numbers of SAG1, a *T. gondii* specific gene (Luo *et al.,* 1995). In the present study, we found that *T. gondii* tachyzoites and bradyzoites could be defined and quantitatively measured by QC-PCR after the acid treatment of *T. gondii*.

Materials and Methods

Cysts of an avirulent strain of *T. gondii* (Kamei *et al.,* 1976) were prepared from B10.A(4R) mice. The cysts were then purified from infected brain tissue on 45% Percoll gradients according to the method of Cornellissen *et al.* (1981). RH strain tachyzoites were prepared according to the method described by Yano *et al.* (1989). The mouse cell lines L was maintained in RPMI 1640 supplemented with 1% fetal bovine serum (FBS) and 0.1% Nutridoma (Boehringer Mannheim, Mannheim, Germany).

The pepsin-HCl solution was prepared as follows; pepsin (Difco; 1:10,000) 0.2 g; NaCl, 0.9 g; HCl, 1.4 m*l*; and H_2O to make 100 m*l* of solution. The pepsin-HCl

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solution was mixed with an equal volume of parasite suspension containing tachyzoites or bradyzoites and incubated for 5, 15 or 30 min at 37°C. After treatment with digestive solution, parasites ($3\text{-}5\times10^3$ tachyzoites; $2\text{-}3\times10^3$ bradyzoites per each well) were inoculated into Falcon 24-well plates (No. 3047) previously seeded with L cells in RPMI 1640. One day after cultivation, to remove the free parasites, the medium was changed. Two days after cultivation, the medium was removed and the cells were washed twice with PBS. Then DNA was extracted as previously described (Luo *et al.,* 1995, 1997), and DNA was stored at −20°C until use for PCR.

T. gondii was determined and calculated the numbers by quantitative competitive-polymerase chain reaction (QC-PCR) targeting the *T. gondii* SAG1 gene (surface specific antigen gene 1) as previously described (Luo *et al.,* 1995, 1997).

Results

T. gondii tachyzoites and bradyzoites were treated with 0.1% pepsin-HCl solution for various times at 37°C. The parasites were examined their infectivities to *in vitro* cultured L cells after the treatment with pepsin-HCl. As shown in Table 1, none of the tachyzoites treated for more than 15 min were infected to the cells, as determined by QC-PCR. In contrast, the bradyzoites were not killed by pepsin-HCl. They survived even 180 min after the treatment (not shown in Table 1), and retained the infectivity against the cultured cells. In order to analyze precisely the susceptibility of tachyzoites to pepsin-HCl, when tachyzoites were treated with pepsin-HCl or HCl solutions, they lost the infectivity against L cells (Table 1). The tachyzoites treated with acetic acid or sulfuric acid for more than 15 min also lost the infectivities against L cells. It was suggested that the acids showed a destructive effect against *T. gondii* tachyzoites. The tachyzoites treated with pH 1.2 for more than 5 min, with pH 1.4 for more than 15 min and with pH 1.8 for more than 30 min lost the infectivity against L cells (Table 1). The infectivity of tachyzoites against cultured cells were affected with pH dependently. On the other hand, as shown in Table 2, when tachyzoites were treated with HCl solutions for one hour, DNAs of intracellular infecting tachyzoites as well as free tachyzoites were not detected by QC-PCR. It was found out that their DNAs were degraded when they were analyzed by QC-PCR. Thus, DNA destructions of *T. gondii* tachyzoites made it possible to differentiate between tachyzoites and bradyzoites by QC-PCR.

Table 1 Destructive effects of acid treatment on *T. gondii* tachyzoites determined by infectivities against L-cells

Treatment	Time for treatment (min) *		
	5	15	30
Tachyzoite			
Pepsin-HCl †	430	0	0
HCl			
pH 1.2 ‡	0	0	0
pH 1.4 ‡	292	0	0
pH 1.8 ‡	320	470	0
pH 2.2 ‡	295	298	296
pH 2.5 ‡	291	288	284
1 N CH_3COOH	320	0	0
0.01 N H_2SO_4	9	0	0
Non-treated	510	530	650
Bradyzoite			
Pepsin-HCl †	113	95	108
Non-treated	120	108	102

* *T. gondii* number per μg DNA measured by QC-PCR.
† 0.1% pepsin-0.084 N HCl.
‡ Adjusted by HCl solution.

Table 2 Destructive effects of HCl treatment on *T. gondii* free tachyzoites and intracellular tachyzoites

Treated with	Number of *T. gondii* (QC-PCR *)	
	Free tachyzoites	Itracellular tachyzoites
HCl	0	0
0.1% Pepsin-HCl	0	0
Non-treated	310	380

* Tachyzoites were treated with pepsin-HCl or HCl (0.084 N) solutions for 1 hr at 37°C, then *T. gondii* numbers per μg DNA were measured by QC-PCR.

Discussion

The inter-conversion of *T. gondii* between tachyzoites and bradyzoites is defined by their replication velocity, by morphological criteria, by the expression of bradyzoite-specific molecules and genes, and by oral infectivity against mice or cats (Gross *et al.,* 1996). Though identifications of tachyzoites or bradyzoites are important for diagnosis of toxoplasmosis, quantitative measuring systems for definition of stage of *T. gondii* have not been established. However, we described a useful method for identification of tachyzoites and bradyzoites, and for measuring the *T. gondii* number in tissues using QC-PCR.

In the present study, number of *T. gondii* tachyzoites and bradyzoites can be quantitatively measured by QC-PCR targeting SAG1 gene of *T. gondii* after acid treatment. *T. gondii* tachyzoites and bradyzoites contained SAG1 gene in their genome, though SAG1 was

generally expressed in tachyzoites. QC-PCR was developed to assay the number of *T. gondii* by estimating copy numbers of SAG1 (He *et al.,* 1997; Luo *et al.,* 1995). The truncated SAG1 was used as a competitor of the QC-PCR targeting SAG1 of *T. gondii*. The determined amount of competitor SAG1 DNA was coamprified with *T. gondii* genomic DNA. The band intensities of QC-PCR products were measured by comparison between competitor band and genomic SAG1 gene products of cDNA. From 1 to 10^4 copies of SAG1 can be quantitated in 2 μg genomic DNA. This method is more sensitive than any other cell counting methods for *T. gondii*.

Most of the tachyzoites degenerated within 15 min of acid pepsin digestion. Jacobs *et al.* (1960a) also reported that tachyzoites were damaged immediately by acid pepsin treatment. Pettersen (1979) reported that the destruction of tachyzoites in HCl-pepsin solution was due to HCl, not pepsin. We further analyzed acid types and pH conditions which destroy tachyzoites *in vitro*. We found that the tachyzoites were destroyed not only by HCl but also by other acid solutions. Tachyzoite destructions were occurred at pH conditions lower than pH 1.8 in HCl treatment. These lower pH conditions are found in the physiological life cycle of *T. gondii,* especially in the peptic digestive fluid of the stomach in humans (pH 1.2-2.3) (Geigy and Basle, 1956) and mice (pH 1.4-3.0) (Ogawa and Necheles, 1958). Pettersen (1979) suggested that tachyzoites had a higher permeability than bradyzoites, because tachyzoites were stained faster than bradyzoites in a methylene blue dye solution. The difference in acid-mediated destruction between tachyzoites and bradyzoites might be related to a difference in their surface membranes.

In the present study, the RH-strain of tachyzoites was easily degenerated by acid treatment. However, Dubey (1998) re-evaluated the effects of acid pepsin on *T. gondii* tachyzoites and bradyzoites. It was found that the RH strain of *T. gondii* tachyzoites occasionally survived for 2 hrs in acid pepsin solution. However, the transitional form between tachyzoites and bradyzoites might explain the inconsistencies regarding the resistance to acid treatment. The development of a criterion to supplement the well-known characteristic of bradyzoites and tachyzoites is necessary to differentiate the stages of *T. gondii*.

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CONTENTS

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