SNAIL TRANSMISSION OF SCHISTOSOMIASIS IN THE LOWER MEKONG BASIN

WITH OBSERVATIONS ON
OTHER WATERBORNE DISEASES

Office of International & Environmental Programs
Smithsonian Institution

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Background of the Study

The Mekong project seeks the development of the water and related resources of the Lower Mekong Basin in terms of hydro-electric power, irrigation, navigation improvement, flood control and other benefits. The Mekong project is directed by the Committee for Coordination of Investigations of the Lower Mekong Basin, which the four Lower Mekong Riparian Governments (Khmer Republic, Laos, Thailand and the Republic of Vietnam) established in 1957 as an autonomous inter-governmental agency under the aegis of the United Nations Economic Commission for Asia and the Far East (ECAFE). The Committee consists of a plenipotentiary representative from each of the four countries, and is authorized to promote, co-ordinate, supervise and control the planning and investigation of water resource projects in the Basin. The Mekong project seeks to advance the welfare of the people of the Basin without distinction as to nationality, religion or politics.

The Mekong Committee, interested as it is in the comprehensive, multipurpose development of water resources in the Basin, is giving considerable attention to the ecological changes resulting from the creation of reservoirs and other bodies of water associated with dam construction. With the increased storage of Mekong water which is planned by the Mekong project, and its gradual release over all seasons, changes are expected to occur in the populations of disease-carrying vectors, particularly snails.

Snails are the obligatory intermediate hosts of a number of waterborne diseases which infest many tropical regions, including Southeast Asia and the Mekong Basin. Chief among these in terms of public health importance is schistosomiasis.

The first case of human schistosomiasis of Southeast Asian origin was reported in 1957 by Vic-Dupont *et al.* in a Lao living in Paris. In 1959, Chaiyaporn *et al.* reported human cases from the south of Thailand. There followed a number of reports of what appeared to be schistosomiasis japonica in this new geographic location. In Thailand, studies have been made of geographic distribution (Harinasuta and Kruatrachue, 1960, 1964; Lee *et al.*, 1966; Desowitz *et al.*, 1967; Harinasuta *et al.*, 1970), pathogenicity (Harinasuta *et al.*, 1967), diagnosis (Harinasuta *et al.*, 1967), and animal reservoirs (Kruatrachue, 1964). In the Khmer Republic, Audebaud *et al.* (1968) reported the first case in that country from the provincial capital of Kratié. Subsequently, a number of epidemiological studies were published from the Calmette Health Center and the Pasteur Institute of Phnom Penh (Bazillio, 1969; Jolly *et al.*, 1970a; Tournier-Lasserve *et al.*, 1970). In Laos, the initial warnings of Vic-Dupont and his colleagues (1957) and of Barbier (1966) helped to secure the participation of the World Health Organization which sent a number of short-term expeditions to the single known focus of transmission at Khong Island (Ito and Jatanasen, 1961; Zeville and Santos, 1961; Iijima and Garcia, 1967; Iijima *et al.*, 1971; Lo *et al.*, 1971) to assess the severity of the problem and to search for transmitting snails. However, no transmitting snail was found.

Inception of the Study

Faced with this situation, additional sources of help in field research were sought by the Mekong Committee with a view to defining the scope of the schistosomiasis threat, and possibly the status of other snail-borne diseases, in the Lower Mekong Basin. At the request of the Mekong Committee, the Waterborne Diseases Project began as an activity of the Office of Environmental Sciences (now the Office of International and Environmental Programs) of the Smithsonian Institution with funds provided by a contract with the Agency for International Development. The project was planned in two phases. Exploratory work began in the field on 12 January 1970 and terminated 28 February 1970. During this period, site visits were made to Thailand (Bangkok and Khon Kaen Province), Laos (Vientiane and Khong Island) and Khmer Republic (Phnom Penh, Prek Thnot and Kratié) in order to survey existing capabilities for field support and to contact individuals and institutions interested in cooperating in the proposed field studies.

Initially it was recommended that field studies be pursued both in the vicinity of Kratié and Khong Island. The events of April 1970 forced the abandonment of plans to work at Kratié. Subsequent studies concentrated on Khong Island, Laos, as well as certain areas of northeast Thailand, on the Mun River.

Field research commenced in August 1970 with the arrival of permanent staff in Bangkok. Headquarters were initially established in the Center for Thai National Reference Collections, a part of the Applied Scientific Research Corporation of Thailand. Agreements were also reached with the Faculty of Tropical Medicine of Mahidol University, Bangkok, for the cooperation of trained personnel.

The field investigations reported herein thus represent a joint effort of the Smithsonian Institution, the Faculty of Tropical Medicine and the Applied Scientific Research Corporation of Thailand, with generous assistance from the Service National de Laboratoires de Santé Publique of Laos.

Purpose of the Study

The Waterborne Diseases Project was conceived as an investigation into the risk of spreading waterborne diseases as a result of water resource development projects in the Lower Mekong Basin. By agreement with the Mekong Committee, the activity would be limited to snail-borne diseases with emphasis on schistosomiasis. This involved assessment of the dimensions of such threats and evaluation of means for removing or reducing them to manageable proportions by drug treatment programs, snail or parasite control, or project modification. The first step was to mount a search for the intermediate snail host of human schistosomiasis in the Lower Mekong Basin.

The project was originally planned as a three-year activity with third-year support to depend upon the availability of funds. Emphasis was placed upon the establishment of the taxonomic identity of the transmitting snails, their ecology, and their seasonal and geographic distribution within the region (see Appendix I). The investigations included field searches to determine the environmental requirements of the snail vector as well as parallel efforts to cultivate it in the laboratory. The laboratory work was intended to identify physiochemical parameters (soil analyses, substrate preferences, water chemistry, and structure of the natural microhabitat) in addition to biological parameters (food sources, feeding habits, reproductive cycles, growth patterns) of the snail host.

In association with the above-mentioned studies, there was a need to characterize the Mekong schistosome (also known as human blood fluke). Its relation to the "classical" *Schistosoma japonicum* of China, Japan, Taiwan, Philippines, and Indonesia was unclear and in the literature it has been identified as a "*Schistosoma japonicum*-like parasite," as the Mekong strain of *S. japonicum*, or merely as the Mekong schistosome. It was planned to complete its life cycle under experimental, laboratory conditions, to develop proof of natural transmission, to identify reservoir hosts wherever possible, and to evaluate the pathobiological responses of humans to the parasites.

Third-year support enabled the team to proceed with the above and fourth-year funding was approved to expand the studies with a view to reviewing and recommending suitable methods for schistosomiasis control in the Lower Mekong Basin. While the principal focus of research remained at Khong Island, Laos, the expanded scope included provision to review schistosomiasis control programs associated with large water development projects elsewhere in the world and to evaluate the potential effectiveness of these programs within the limitation imposed by the special ecological situation at Khong Island. It was hoped that such data would permit prediction of any conditions of future mainstream impoundment that might contribute to the spread of schistosomiasis. In addition, it was proposed that the relative public health importance of other snail-borne diseases that may constitute present problems, or prove increasingly troublesome under conditions of future impoundment, be identified and discussed.

Findings

The field research activities of the Smithsonian Team between August 1970 and September 1974, in cooperation with the Faculty of Tropical Medicine in Bangkok and the Public Health Laboratory in Vientiane and supplemented by data from the available literature, support the following findings:

1. According to present understanding of patterns of snail and parasite distribution, *there is no valid reason for opposing the construction of the Pa Mong dam on the grounds that this might cause an increase in incidence of human schistosomiasis*. The disease is not known to

occur in this part of the region. The transmitting snails (*Lithoglyphopsis aperta*) have not been found in the Mekong River between the Burma border and the Khemarat rapids and suitable habitats for these snails have not been located in this part of the river. Given these conditions, there appears to be no reason to anticipate the introduction of human schistosomiasis into the Pa Mong area west of Vientiane.

2. The presence of an active focus of human schistosomiasis on Khong Island, Laos, presents a different situation and it is not possible to predict if and to what extent the disease may be spread by the construction of dams below this focus. The danger clearly exists. At the present time, there appear to be good reasons for making periodic observations of prevalence and for incorporating health considerations into feasibility studies for the dam proposed at Khone Falls as well as the Ban Khoum and Pak Mum dams farther north. The now limited populations at risk in these areas can be expected to expand to many times the present numbers during and following major dam construction.

3. There are two known foci of human schistosomiasis in the main Mekong River: one at Khong Island, Laos; the second at Kratié, Khmer Republic.

4. At Khong Island infection rates at all ages are believed to be about 15%. In the young, aged 4 to 15 years, the rate is about 30 to 40%.

5. The epidemiological picture at Kratié remains undetermined, as studies have been interrupted since March-April 1970 owing to security conditions.

6. At Khong, the transmitting agent is an aquatic hydrobiid snail, *Lithoglyphopsis aperta* Temcharoen, 1971.

7. This snail has been found in specific sites along the Mekong River from Khemarat south to Khone Falls, a stretch of about 200 miles. It is inferred that the snail also occurs in the Khmer Republic as far south as Sambor and Kratié. A distinct race of this species also occurs in the Mun River of Thailand near Phibun Mangsahan.

8. The snail, *L. aperta*, was found nowhere else. It was not found at the Pa Mong damsite or in the river west and north of Vientiane. Under *present* conditions it probably would not survive there, even if deliberately introduced. However, there is no way to predict changes that might result from impoundment. Equally, it is not possible now to predict that *L. aperta* could not be accidentally transferred by mechanical means to the region of the proposed Pa Mong impoundment west of Vientiane after a period of time.

9. At Khong Island, Ban Dan, and Khemarat, *L. aperta* is most abundant in the months of late March, April, May and early June. Variations in abundance occur from year to year in focal sites.

10. At Khong Island, the heaviest transmission of disease appears to be taking place in the main river adjacent to Khong Town. The risk is probably greatest from 10:00 a.m. to noon, tapering off thereafter, but there is a risk at all times of day and night during the afore-mentioned months.

11. The life cycle of the parasite is maintained because snails have access to infected human wastes. Dogs are also infected. It is not known whether the disease would persist in dogs if it were eliminated from humans. If the disease could persist, dogs would constitute a reservoir host.

12. Buffaloes on Khong Island have been surveyed and do not appear to carry the infection. The role of rats, pigs, and other vertebrate hosts in the epidemiology of the disease also appears to be negative but needs further exploration.

13. The size of the human population presently at risk on Khong is relatively small: about 10,000 people on Khong Island and in the surrounding area.

14. It is not yet known whether the Mekong schistosome is to be classified with the classical schistosomes of China, Japan, Philippines, and Indonesia. The response of this "strain" of parasite to therapeutic drugs such as trivalent antimonials or niridazole, which have been used against *Schistosoma japonicum*, has yet to be evaluated.

15. Reduction of transmission may be effected by a combination of methods, including snail control, engineering methods including sanitation, health education, and possibly the trial use of therapeutic drugs. Application of any of these methods will require studies of local conditions in order to adapt the measures to the peculiarities of the situation at Khong Island and, if eventually possible, at Kratié. It is desirable that certain additional basic scientific data be collected before initiation of pilot control studies.

16. The problem, at present, is relatively small in terms of human suffering. As such, control can be contemplated. But if the disease is not attacked under present conditions, it is believed that future conditions of water impoundment, such as planned at Stung Treng or at Khone Falls, may enable the transmitting snail to propagate on a year-round basis. The predictable consequence, if there is a concomitant influx of population of all ages to the shore of the new lake, would be year-round transmission of the disease. Assuming that the lake will be large and will have hundreds of miles of shoreline, it can be predicted that disease control would then no longer be feasible.

17. Extermination of the snail from the entire 300-mile stretch of main Mekong River in which it has been established apparently through geological time should not be attempted. It would be unlikely to produce measurable results, either in terms of snail populations over the years or in terms of disease reduction at the foci of infection. It is more probable that attempts at general snail extermination, which could be expected to rely heavily on chemicals, would introduce undesirable and possibly irreversible changes in the balance of nature. If snail control is attempted in the Mekong River, it should remain a focal activity.

Recommendations

The World Health Organization has recommended that ERADICATION of schistosomiasis be tried only in areas where technical problems are not great and resources are considerable. This does not describe the situation in the Lower Mekong Basin. On the other hand, the relatively small number of humans currently infected or at risk in Laos and Thailand probably does not exceed 10,000. This suggests that an attempt to REDUCE TRANSMISSION might have a fair chance of success and could have significant long-range implications for permanent control.

With this aim in mind, it is recommended that a Schistosomiasis Control project be initiated now in the Lower Mekong Basin. The project should consist of three phases: (I) Training; (II) Implementation; (III) Evaluation.

Phase I: Training

Training should be initiated as soon as possible. It is anticipated that the incidence of many infectious diseases transmitted by snails, mosquitoes, or other aquatic animals, will increase under the conditions of impoundment associated with water resources development schemes in the Lower Mekong Basin. In this connection, the recruitment of regional personnel trained to assume direction of research and control programs has high priority. At the same time, supervisory field workers and technicians are needed.

It is recommended that a Schistosomiasis Control project be initiated by beginning the training of personnel able to deal with all long-term aspects of schistosomiasis study and control in the riparian countries.

1. Funds should be sought to support long-term training programs for promising candidates who wish to improve themselves or seek advanced professional degrees in the fields of epidemiology, parasitology, aquatic ecology, malacology, and entomology. Such training could take advantage of such centers of excellence as now exist in Southeast Asia (*e.g.*, Mahidol University, Bangkok; Institute for Medical Research, Kuala Lumpur; University of Science of Malaysia, Penang) but should not ignore the possibilities offered by institutions of established reputation in Europe, Australia, the United States, and Japan. At the present time it is estimated that approximately US$10,000 is required to support one Asian student for one year at most American universities. The length of a PhD program will vary from three to five years, depending on the qualifications of the candidate.

2. With regard to schistosomiasis in particular, technicians should be trained in routine stool examination procedures. They should be able to recognize the eggs of the Mekong schistosome and be able to differentiate them from those of other locally acquired parasites. Such persons

are needed to work on Khong Island on a year-round basis in advance of the initiation of a control program as base-line data on prevalence will be needed. Supervisory personnel should be qulified Laboratory Technicians. Assistants do not need to be as advanced but might be recruited from the local school at Khong Island.

The activity should be centered in the laboratory of the PGNU Hospital on Khong Island.

A continuing parasitological surveillance will be needed, operating full-time in order to measure any degree of success or failure of the measures proposed in Phase II.

Phase II: Implementation

When technical personnel have been trained and are on duty, it is recommended that a Schistosomiasis Control project be instituted on Khong Island. The work should be coordinated with the staffs of the Faculties of Tropical Medicine and of Science, Mahidol University, Bangkok.

A multipronged approach should be adopted in beginning such a pilot Schistosomiasis Control project including (1) Snail Control, (2) Treatment, (3) Sanitation, and (4) Health Education.

In addition, when conditions in the Khmer Republic permit resumption of field investigations, research on transmission patterns of schistosomiasis at Kratié should be resumed in coordination with the Ministries of Health and of Education of the Government of the Khmer Republic and with the Pasteur Institute of Phnom Penh.

Control activities on Khong Island should be initially limited to the principal transmission site at Ban Xieng Wang, Khong Town. Since there is no previous control experience with the Mekong *Schistosoma*, attempts to interfere with transmission will necessarily fall into the category of field research. Thus the activity should be qualified with the title of "pilot project".

The target population should include the 4-15 year age group, the school children in the elementary schools and the intermediate school.

The goal of the Schistosomiasis Control project should be realistically limited to measurement of a reduction in incidence (percentage of new infections), a change in the intensity of infection (measured in terms of overall egg counts), or a decrease in severity of infection (measured in terms of the clinical gradient). The first of these measurements would provide the most convincing proof of success. A minimum of three years is envisaged as necessary for the collection of meaningful data. Base-line parasitological data on the children would be needed.

In the event of detectable success in reducing transmission during this period, it will be desirable to extend the project with a view toward achieving permanent control.

(1) *Snail Control*

This subject can be considered under three titles: (a) use of chemicals (molluscicides) to kill snails; (b) engineering improvements; and (c) biological control.

(a) *Molluscicides*

The sensitivity of the transmitting snails to available molluscicides is not known. Securing this information should constitute an important preliminary research activity. Since the supply of *Lithoglyphopsis* snails in nature is limited to the months of April, May and June, some means must be found to cultivate the snails in the laboratory. A serious approach to this problem has been begun in the present study but a final answer is not yet available. The laboratory and field testing of molluscicides must be preceded or accompanied by certain laboratory research efforts.

The life cycle of the transmitting snail, Lithoglyphopsis aperta, should be completed experimentally in the laboratory. Technology should be perfected to permit the constant availability of these snails for experimental purposes including chemical sensitivity studies as well as other ecological studies useful in planning snail control measures. The snails are also needed for maintenance of the parasite in culture. These studies should be based on the work already accomplished with a view to perfecting the credibility of the data and enhancing their preditive value. The work should be repeated in more than one laboratory. During the life of the present contract, studies have been carried out simultaneously in the Bangkok Snail Laboratory, at the University of Michigan Snail Laboratory (Ann Arbor), and at the Water Reed Army Institute of Research (WRAIR). Each of these facilities has made important contributions to the present report. Thus far the work at Ann Arbor and WRAIR has cost the Project nothing in terms of salaries or overhead: it is unlikely that such arrangements can continue as they are unrealistic in terms of long-range planning. In Bangkok, support should be secured to permit the snail studies currently underway to be continued after termination of the present contract.

The study of the population dynamics of Lithoglyphopsis aperta in its natural habitat should be continued. Endemic areas should be searched periodically since snail populations may vary according to year or season. Non-seasonal factors such as suitability of the microhabitat, snail breeding habits, and unknown factors affecting snail populations in the Mekong and Mun Rivers should be included in the study. The estimation of riverine snail population density will require skill and judgment and no single method of sampling is satisfactory for all habitats. New sampling techniques should be developed. At the same time, studies should be initiated of the biology of hydrobiid snail communities in the Mekong River in terms of microecology, niche specialization, and sympatric groupings under natural conditions. A lengthy study period may be needed, spanning several years, because the snails appear in sufficient numbers only during a short part of each year (chiefly the months between February and June). The natural life history of Mekong Hydrobiidae should be emphasized and efforts made to discover the fate of these snails during high-water periods. All of this information will be needed in order to evaluate the effect of any eventual molluscciciding activities. Such studies will require the services of a professional malacologist or aquatic ecologist in the field and will require back-up support from the Bangkok Snail Laboratory.

The potential role of other hydrobiid snails in the epidemiology of schistosomiasis should be determined. There are more than 70 described species of Hydrobiidae in the vicinity of Khong Island. Some of these may prove to be as beneficial in preventing the spread of human schistosomiasis as *L. aperta* is active in promoting it, *i.e.*, related snails may act as "decoys" able to remove infective parasitic stages (miracidia) from circulation even though the miracidia cannot complete their larval development in these snails.

If the Mekong *Schistosoma* can complete its life cycle in any species of snail other than *Lithoglyphopsis aperta*, the snail should be identified and its relative susceptibility should be determined experimentally.

The sensitivity of Lithoglyphopsis aperta and its sympatric relatives to available as well as novel molluscicides should be determined. Molluscicides that are commercially available at the present time include sodium pentachlorophenate, Bayluscide, Yurimin, Niclosamide, and N-tritylmorpholine. All were field tested elsewhere against pulmonate (aquatic) snails and found to be effective except Yurimin which is more effective against the amphibious hydrobiid, *Oncomelania hupensis*. Other candidates for trial should include the plant molluscicide, Endod, which is active against pulmonate snails, and the organotin compounds (triphenyltin chloride and triphenyltin acetate).

Snails should be exposed to the chemicals at various concentrations, temperatures, and time intervals under controlled laboratory conditions in order to determine the concentration that will kill 50% of the snails (LC_{50}). The LC_{50} should be determined separately for each molluscicide and for each species of snail.

However, identifying an effective molluscicide in the laboratory must be followed by trials of its effectiveness in the field. This may be restricted by two factors: (1) the enormous dilution factor in the Mekong River may prevent the establishment of lethal concentrations in the vicinity of the snails, and (2) the erratic seasonal appearance of the snails may not coincide with time of application. These restrictions could be diminished if experimental evidence should demonstrate the utility of novel methods of introducing chemicals into the molluscan environment (*e.g.*, from slow-release rubber compounds or from rubber-base paints).

In the event that an effective, relatively inexpensive molluscicide is experimentally identified which can be applied efficiently under field conditions, a field trial in the Mekong River should be undertaken. The site should be selected to correspond with the needs of the Schistosomiasis Control project, but the trial should not interfere with subsequent interpretation of the results of other control measures. There are populations of *Lithoglyphopsis aperta* elsewhere than at Khong Island; initial field trials of molluscicides in snail control could be done in such places (Ban Dan or Khemarat).

Molluscicide evaluation will require adequate supplies of the chemicals to be tested, sufficient quantities of target species of snails, and the full-time participation of a professional malacologist-parasitologist who is familiar with techniques of snail culture and molluscicide application. In addition, at least two laboratory technicians should be assigned to this project.

(b) *Engineering Improvements*

In addition to sanitation improvements (separately discussed), other engineering improvements may prove useful in aquatic snail control at Khong Island and could prove to be key elements in minimizing human-snail contacts. These may include any or all of the following trial activities: (1) physical alteration of the shoreline at the main transmission site in Khong Town in order to change river current velocities and to remove snail habitats adjacent to areas frequented by bathers; (2) removal of the rheophytic euphorbeaceous shrubs at the main transmission site which offer concealment and make the place popular as a defecation site; (3) physical removal of the peninsula at the main transmission site and reconstruction of the shoreline along Khong Town; (4) using earth, sand or concrete to fill the still-water and eddy areas near Khong Town where cercariae find optimal conditions for contacting their final hosts; (5) construction of a stone-and-concrete embankment along the shore of Khong Town with broad stairs at intervals (such as seen at Luang Prabang) to encourage and limit access to safe sections of the river; (6) development of popular bathing areas by putting down sand, removing stones and weeds, and constructing public latrines on the shore in convenient locations.

Some of these proposed changes would involve altered flow rates in the Mekong River adjacent to Khong Island; this, in turn, would affect underwater topography, sedimentation patterns, *etc*. Adverse effects from such changes, as viewed by the local residents, should be anticipated.

Since the Lao have a long tradition of engaging in enterprises of collective interest, they should be able and willing to participate actively in such improvements. Sanitary needs occupy a strong position in the Lao way of life. It is anticipated that the Khong Town community will consent to a significant degree of active participation in the recommended changes if they are consulted in advance and if they are provided with the raw materials (cement and cement-working equipment).

The detailed planning of the engineering improvements will require the services of civil/hydraulic engineering consultants skilled in this type of work. Based on assistance provided by a Thai consulting firm specializing in environmental engineering*, such an improvement program would involve three steps; (1) preliminary studies including hydraulic modeling and preliminary engineering design of the proposed alterations; (2) final design, including preparation of construction plans and specifications; (3) construction. The first and second steps may be completed within one year; construction may require as long as two years.

(c) *Biological Control*

At present, methodologies have not been developed to a stage where any form of biological control can be seriously recommended for field use. However, in the long term, it is probable that biological control will prove to be more environmentally acceptable than other methods of snail or disease control. *With this in mind, it is recommended that support be found to pursue basic research on biocontrol of snails and/or snail-borne diseases*.

One of the most promising of the methods thus far suggested in the literature is the use of protozoan hyperparasites of the order Microsporida. These organisms have been reported to enter snails and infect their trematode parasites, reducing the latter to simple sacs of spores. Their ability to destroy schistosome larvae under experimental field conditions has been reported. Such hyperparasites, if identified, cultivated *en masse*, incorporated into snail baits, and distributed seasonally in areas infested by transmitting snails, might prove effective in reducing parasite populations.

Investigations into the use of Microsporida in the biocontrol of schistosomiasis would be categorized as basic research. The services of a full-time professional protozoologist and competent technician would be required.

(2) *Treatment*

The role of chemotherapy in the control of schistosomiasis is, at present, of marginal value. Suitable drugs, which are both efficaceous and safe, are not available. Drugs in current use do not give 100% cure, are toxic, and permit a certain amount of relapse. In addition, none of the existing schistosomicidal drugs has been assayed against the Mekong *Schistosoma*.

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*Southeast Asia Technology Co., Ltd. (SEATEC).

On the other hand, the development of a drug suitable for mass chemotherapy would have notable advantages in a control program. Eliminating worms or their eggs would solve individual health problems and, at the same time, block transmission at one of the crucial stages in the parasite life cycle.

In addition, the importance of using chemotherapy in individual cases cannot be underestimated in terms of community cooperation. The victims of schistosomiasis are the children. Justification for treatment is self-evident. The search for safer and more effective drugs will, hopefully, strengthen the role of therapeusis in schistosomiasis and may eventually lead to the development of a prophylactic drug. A number of trial drugs are now available for testing.

The response of the Mekong Schistosoma to known or novel antischistosomal drugs should be determined.

At present, the response of the Mekong schistosome to any of the available antischistosomal drugs is unknown. With regard to "classic" Asian schistosomiasis (with which the Mekong form may or may not be allied), only the trivalent antimonial compounds (sodium or potassium antimony tartarate, Astiban, Fuadin) are currently recommended. All are highly toxic and can be administered only with medical supervision. Among non-antimonial schistosomicides, only niridazole (Ambilhar) has been used against *S. japonicum* elsewhere in Asia, with varying success.

The Mekong *Schistosoma* resembles the "classic" *Schistosoma japonicum* in adult morphology, but differs genetically. Its response to the drugs now in use must be determined experimentally before drug treatment can be incorporated into a Schistosomiasis Control project.

In addition to those listed above, other promising drugs should be included in such a protocol. They include drugs known to be active against one or both of the other human schistosomes (*S. mansoni* and *S. haematobium*) such as hycanthone, lucanthone, and metrifonate, as well as potentially useful new compounds for which adequate clinical evaluation is not yet available (including nitrofurans, nitrothiazolyls, and tetrahydroquinolines).

Such a study would require two to three years for completion. Approximately 4000-5000 mice, 500 hamsters, and 250 dogs would be used. The drug niridazole should be used as a reference drug. Criteria of success would include demonstrated death of worms, sterilization of worms, or death of eggs in tissues. The drug will have to be free of harmful side effects in humans. The work should be centered in the Schistosomiasis Research Unit of the Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok.

The comparative pathobiology of Mekong schistosomiasis in a number of laboratory animals such as mice, rats, hamsters, rabbits, guinea pigs, dogs, and monkeys should be studied. In view of the impression reported by some workers that the Mekong schistosome is less pathogenic than the "classic" Asian *Schistosoma japonicum*, the true impact of the disease in animals should be thoroughly assessed and the comparisons carried over to humans. A long-term study in humans of the morbid impact of Mekong schistosomiasis, considered alone as well as in constellation with other endemic diseases, such as tropical anemia, tuberculosis, malnutrition, and other helminthiases, should be planned. The area to be studied should include the known range of the transmitting snail, *i.e.*, the Mekong River from Khemarat to Kratié, and the Mun River as far west as Ubon Ratchathani. Such a study will require the participation of physicians trained to recognize all the local infectious diseases. Bed-space and financial support for patients brought to the Hospital for Tropical Diseases, Bangkok, will be required.

The nature and species of the Mekong Schistosoma needs to be further elucidated. A well-planned, long-term study of the role of reservoir vertebrate hosts in all transmission sites should be contemplated. The Mekong *Schistosoma* differs clearly in certain respects from *Schistosoma japonicum* of China, Japan, the Philippines, and Indonesia. With added evidence it may prove that the Mekong *Schistosoma* represents a parasite new to science. If true, this will draw attention to the need to identify other possible reservoir hosts than those known (dogs). Such information would have strong implications for the continual success of any short-term reduction in transmission that may be achieved.

Studies carried out during the present project do not support the hypothesis that rats, cattle, or pigs are involved as reservoir hosts of the Mekong *Schistosoma* on Khong Island. If further studies confirm these impressions, control will be limited to humans and dogs. Approaches to control of the infection in dogs that are mutually satisfactory to health authorities and to residents of Khong Island should be explored; it is recommended that the good offices of the Sithandone Association be solicited for help in this regard.

(3) *Health Education*

Health education should not be directed at changing the personal bathing, working and defecating habits of the people of Khong Island. Such an approach would meet with almost certain failure. Efforts should be made in the direction of providing the townspeople with information regarding the engineering measures that are planned and the reasons for them.

It is foreseen that certain changes in life style will be asked of the townspeople. If they do not arrive independently at the decision to make these changes, and if they do not come to feel a sense of community for the engineering projects that are planned, then traditional habits deeply rooted in the national character will probably cause the Schistosomiasis Control project to fail.

When acceptable alternatives to present-day sanitary practices have been provided on Khong Island, the message in health education will acquire meaning.

The citizens of Khong Island, in particular children in grade school and high school, should be informed of the nature of schistosomiasis, the life cycle of the parasite, the role of the snail, and methods of avoiding infection. Educational posters and permanent photographic displays using scenes and faces familiar to Khong Town residents, should be mounted at places where people gather (the ferry landing, the market, the school yards, *etc.*). An illustrated booklet (comic book) presenting the elements of the problem with positive suggestions about ways to combat it should be prepared for free distribution to intermediate school students.

Finally, the possible role of *mohlam ku* performers in developing support for sanitary innovations should be explored. These entertainers, including a pair of singers accompanied by a man playing the bamboo harmonica (*kaen*) are skilled in the improvisation of comic musical dialogues. Clever performers can keep an audience's attention for many hours. With proper instruction and advance notice, they could introduce the idea of improved sanitation at an almost subliminal level.

The interest of the Ministry of Public Health of the Lao Provisional Government of National Union should be directed toward the desirability of coordinating health education activities with other activities that may be implemented in association with schistosomiasis control.

(4) *Sanitation*

The life cycle of the schistosome can be broken by removing egg-bearing feces from the vicinity of snails as well as by removing snails from the vicinity of people. It is essential to discourage promiscuous defecation on Khong Island. At present, disposal of feces is primitive. Most people have no alternative to using the fields or forests. Many use the river. Less than one-fourth of the houses in Ban Xieng Wang have latrines. In addition, people should be provided with an alternative to the Mekong River as a source of water for domestic use. At present, few houses have wells. *The installation of a safe, piped water supply on Khong Island, beginning with Khong Town, is an essential beginning measure in enforcing disease reduction*. Such a step has always been most successful in controlling fecal-borne disease. It will not prevent people from bathing in the river but it will reduce man-water contacts. It will also give an incentive to the feasibility of installing water-seal privies in private houses. Promiscuous defecation, particularly as tolerated in the very young, should become no longer socially acceptable and the public danger resulting from defecating in or near the Mekong River should become a matter of community knowledge.

Phase III: Evaluation

A Schistosomiasis Control project in the Lower Mekong Basin will represent an ambitious undertaking. It will require the active participation of many authorities and agencies from different countries, both in the region and abroad. It will also attract the notice of scientists and public health workers not directly associated with the work who wish to keep informed about its progress.

At present, regular channels of communication between all interested parties do not exist. Such channels should be created. The coordination of all activities connected with the Schistosomiasis Control project is desirable and should constitute a third phase of the project.

It is recommended that a scientific Schistosomiasis Consultative Group (SCG) be foremd under the sponsorship of the Mekong Committee. The host organization should be the Regional Tropical Medicine and Public Health Project of the Southeast Asian Ministers of Education Organization (SEAMO-TROPMED), in Bangkok.

Each interested organization (provisionally listed below) could be invited to designate a representative to participate in sessions of the Group. The function of such meetings would be to prepare periodic reports to the Mekong Commitee describing the overall status of schistosomiasis research and control activities in the region, and to recommend new policies, research schemes, or guidelines as these become warranted.

Meetings of SCG could be held with a regularity governed by need, either in Vientiane, Bangkok, or Phnom Penh. Funds should be sought to support the administrative costs of SCG meetings, as well as the costs of editing and publishing proceedings of the meetings.

The following organizations, agencies and institutions should be invited to become members of a Schistosomiasis Consultative Group and nominate delegates to pariticpate in its sessions.

HOST: SEAMO-TROPMED

PARTICIPATING MEMBERS:

INTERNATIONAL ORGANIZATIONS

1. Committee for Coordination of Investigations of the Lower Mekong Basin (*Mekong Committee*)
2. United Nations Children's Fund (*UNICEF*)
3. World Health Organization (*WHO*)

NATIONAL AGENCIES

1. *Provisional Government of National Union of Laos*
   a. Ministry of Public Health; Public Health Laboratory
   b. Ministry of Public Works
2. *Royal Thai Government*
   a. Ministry of Health
   b. Bureau of State Universities
      Mahidol University
         Faculty of Tropical Medicine
         Faculty of Public Health
         Faculty of Science
      Chulalongkorn University
         Environmental Engineering Institute

3. *Government of the Khmer Republic*
   a. Ministry of Health
   b. Ministry of Education
   c. Pasteur Institute, Phnom Penh
4. *Government of the Republic of South Vietnam*
   a. Ministry of Health, Directorate of Public Health
   b. Faculty of Medicine, Department of Parasitology
   c. National Institutes of Public Health
5. *United States Government*
   a. Department of State
      Office of Regional Economic Development (RED) USAID/Laos, Public Health Department
   b. Department of Defense
      Walter Reed Army Institute of Research

OTHERS

1. Sithandone Association, Vientiane, Laos
2. Thomas A. Dooley Foundation, Vientiane, Laos
3. College of Pure and Applied Science, Lowell Technological Institute, Lowell, Massachusetts, USA
4. Office of International and Environmental Programs, Smithsonian Institution, Washington, D.C., USA
5. Mollusk Division, Museum of Zoology, University of Michigan, Ann Arbor, Michigan, USA
6. Department of Malacology, The Academy of Natural Sciences, Philadelphia, Pennsylvania, USA

PART I.

SCHISTOSOMIASIS: THE PARASITE

1. THE SCHISTOSOME LIFE-CYCLE

The sole causative agent of human schistosomiasis known in Asia has been a trematode worm, *Schistosoma japonicum*.

Like most trematodes, the schistosomes require snails as intermediate hosts. *Schistosoma japonicum* is a parasite of man as well as a number of other mammals including dogs, rats, goats, cattle, pigs and water buffalo.

In the vertebrate host, the adult worms inhabit the veins and venules of the small intestine (Fig. 1). Here they copulate and the females lay many eggs. Some of the eggs break through the thin wall of the vein and lodge in the tissue or fall into the lumen of the intestine where they are carried to the outside environment in the feces. However, many of the eggs remain within the veins and, when blood pressure is sufficient, are pushed toward the liver. Passage is blocked by the liver where most of the eggs become trapped. The body treats the eggs as foreign objects and attempts to wall them off with fibrotic deposits. In sufficient quantity such massive fibrosis can impair the function of the liver, producing disease. As far as the parasite is concerned, this is a blind alley in its life cycle for these eggs will eventually die.

Viable eggs that pass in the feces hatch quickly in fresh water. The eggs die in feces deposited in privies or on land. In fresh water the newly hatched larva, called a MIRACIDIUM, swims by means of hair-like cilia and is probably chemically attracted to the vicinity of a snail. The miracidium is equipped with histolytic glands to penetrate actively into the body of the snail. In general, a particular schistosome can develop in only one species of snail. If it is in a suitable host, the miracidium metamorphoses into a sac-like stage called a SPOROCYST. In the family of schistosomes there are typically two generations of these sporocysts, the second one giving rise to a fork-tailed larva termed a CERCARIA (Fig. 2).

The cercariae leave their sporocyst, bore through the snail's tissues, and emerge from its body into the surrounding water where they swim freely.

When a cercaria comes into contact with a suitable warm-blooded host, it is able to penetrate, using histolytic glands, directly through the skin. This may be accomplished in less than a minute, the parasite losing its tail in the process. It is then known as a SCHISTOSOMULE, or juvenile schistosome. The schistosomules enter the peripheral lymph and eventually gain access to the circulatory system. In time they reach the portal system of the liver, where maturation and copulation occur. In the case of *S. japonicum*, the paired worms then migrate through the portal veins to the mesenteric veins in order to lay their eggs, thus completing the cycle.

In summary, this cycle requires fresh water in which suitable snails are able to flourish, requires that the human host deposit feces in this water (to give the miracidia access to the snails), and requires that the human host come in sufficient contact with the same water to permit infection by the resulting cercariae.

So far as is known, the Mekong schistosome conforms to this broad outline. It has been called *Schistosoma japonicum*-like (Harinasuta *et al.*, 1972) and *Schistosoma japonicum*, Mekong strain (Sornmani *et al.*, 1973; Kitikoon *et al.*, 1973). However, in view of morphological differences in eggs and miracidia as well as the important difference in transmitting snail species (see below), the specific identity of the Mekong *Schistosoma* cannot now be stated with certainty. On the other hand, the existence of variations in the biological characteristics of schistosomes of the same species which may be endemic in different geographic regions has been noted (Sitrewalt, 1973).

To avoid misunderstanding, the blood fluke with which this report is primarily concerned will be designated "Mekong *Schistosoma*" or "Mekong schistosome".

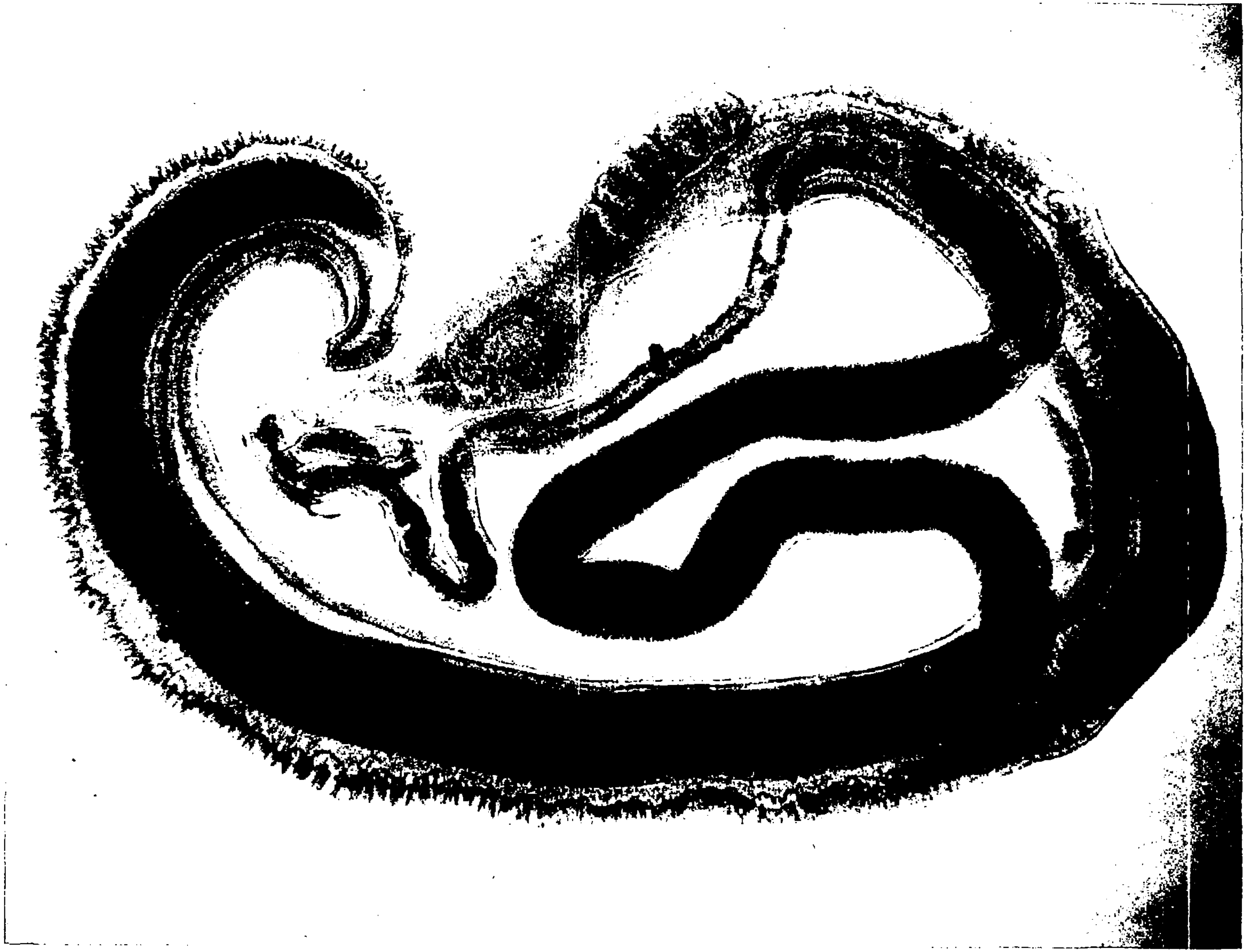


Fig. 1.--Male and female adult worms of the Mekong *Schistosoma* recovered from a mesenteric vein of a naturally infected dog.

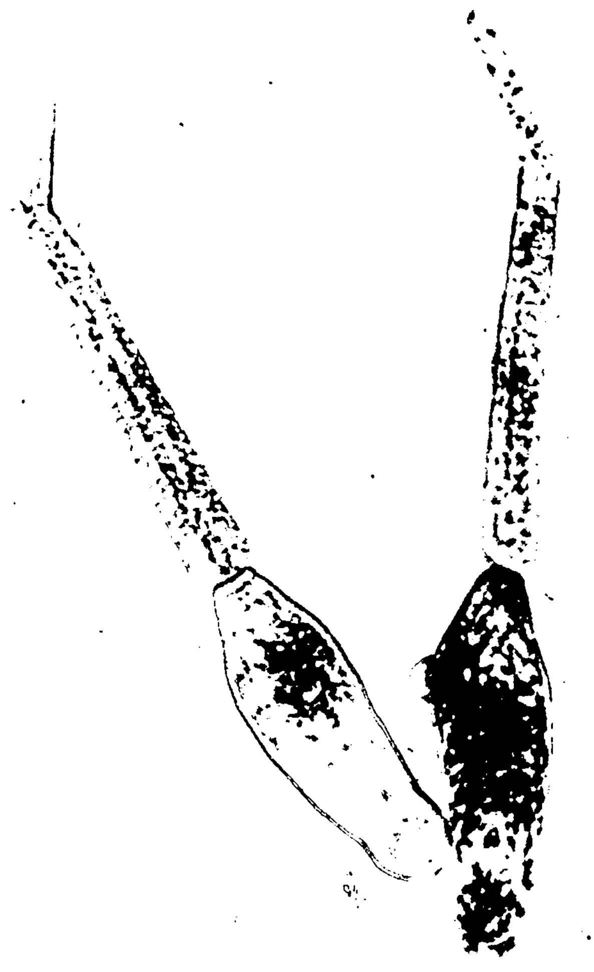


Fig. 2.--Cercariae of the Mekong *Schistosoma* from experimentally infected *L. aperta* snails.

2. EPIDEMIOLOGY OF SCHISTOSOMIASIS IN THE MEKONG BASIN

Two main foci of transmission of human schistosomiasis have been found in the Mekong Basin: Khong Island, Laos, and Kratié, Khmer Republic. A third site, near Ubon, Thailand, on the Mun River, has yielded only a few cases thus far and has not been sufficiently investigated clinically to warrant inclusion in this report.

Khong Island, Laos

The first cases of schistosomiasis, detected in Lao patients residing in Paris, originated on Khong Island.

Iijima and Garcia (1967) surveyed the villagers of Khong Town, the principal community of the island and the seat of government of Sithandone Province. They did skin testing, using as antigen a preparation of adult worms of *Schistosoma japonicum*. When a skin-test was positive or doubtful, a stool examination was done using the formalin-ether concentration technique with approximately one gram of feces. In this way, of 1012 subjects tested in various communities on Khong Island, there were 184 (18.2%) positive skin reactions, 319 (31.5%) doubtful reactions, and 509 (50.3%) negative reactions. Of 78 individuals with positive skin tests, 20 (25.6%) were found to be passing eggs in the stools, following a single stool examination. These workers reported essentially no difference in prevalence according to sex. The ages of the subjects were not recorded.

In 1970, Iijima (1970a) skin-tested primary school children in Vientiane, Paksé, and Khong Island. There were positive results from all three areas and it was assumed that many or most of them represented "non-specific" or "cross" reactions.

When stool examinations were performed on subjects with positive skin tests, no evidence of *Schistosoma* was found in children from Vientiane or Paksé, but 49 of 149 (32.9%) from Khong Island were positive. The technique used was to examine two to five direct smears from each child. This is a relatively inefficient method and it can be assumed that the figures reported represent minimal values.

The most complete epidemiological study of schistosomiasis on Khong Island was made by Sornmani *et al.* (1971). Their intradermal skin testing of 871 subjects in six villages on Khong Island elicited positive responses from 249 (28.6%) and doubtful responses from 277 (31.8%). Feces collected from 209 of the skin-test positive subjects were examined by the formalin-ether concentration technique and 30 (14.4%) were found positive.

The data from these three reports are summarized in Table 1, but the figures cannot be compared with precision as single-stool examinations for schistosome eggs always give minimal values for prevalence. This problem is probably related to the habit of the female schistosome worm of laying eggs in clutches which may collect in intestinal wall abscesses for varying periods of time before breaking out and passing with the feces.

TABLE 1

EXAMINATION OF KHONG ISLAND RESIDENTS OF ALL AGES FOR SCHISTOSOMIASIS: SUMMARY OF EXISTING DATA USING SKIN-TESTS AND STOOL EXAMINATIONS

	Skin Tests					Stool Exam.		
Authors	Exam.	Pos.	%	Doubtful	%	Exam.	Pos.	%
Iijima and Garcia, 1967	1012	184	18.8	319	31.5	547[a] 78[b]	47 20	8.6 25.6
Iijima, 1970a	223	178	79.8	4	1.8	149[a]	49	32.9
Sornmani *et al.* 1971	871	249	28.6	277	31.8	209[b]	30	14.4

[a]Direct smear.

[b]Formalin-ether concentration.

A rough estimate made from these studies, however, indicates that the prevalence of schistosomiasis on Khong Island is about 15% in all age groups and 30 to 40% in primary and secondary school children.

Age Distribution

The data of Sornmani *et al.* (1971) indicated that schistosomiasis on Khong Island was primarily a disease of children between the ages of 4 and 15. In individuals aged 30 or more who displayed signs or symptoms suggestive of schistosomiasis (splenomegaly, liver disease, ascites), the diagnosis could not be confirmed because eggs of the worm were not demonstrated in the feces. Proof that older persons have suffered from chronic schistosomiasis might be obtained by liver biopsy or at autopsy. Epidemiologically, it is of interest that persons beyond the age of 30 have ceased to participate in the transmission pattern if they have ceased contributing eggs in their feces.

Season of Transmission

Evidence suggests that transmission of schistosomiasis on Khong Island is limited to low-water periods when the intermediate hosts are present, abundant, and mature (Kitikoon *et al.*, 1973). This corresponds roughly with the three-month period of April through June during a year of average rainfall. During this period the water reaches its lowest level in the river. As the depth becomes shallower near shore, people must wade farther out into the river in order to bathe. There are larger numbers of shallow areas where current is reduced or absent and water is clear, conditions that probably permit cercariae to find and penetrate the skin of their final host with more efficiency.

Main Transmission Site

The main transmission site on Khong Island appears to be centered in Khong Town at a flat section of the shore of the Mekong River in the section known as Ban Xieng Wang. Here the slope of the bank permits easy access to the river in a place where there is a heavy growth of the rheophytic shrub, *Homonoia* sp. This cover makes the site popular as a defecation site. Also, youngsters frequently defecate directly into the water; their behavior in this regard seems to be uncontrollable.

Relative Efficiency of Transmission

The life cycle of the schistosome can be completed only if an unlikely juxtaposition of events occurs. The eggs must be passed with the feces in such a way that they reach the water safely and do not become desiccated. The larvae which hatch out must find receptive snails in which to complete this phase of the cycle within a few hours. The conditions must be good for maintaining the snails. The same conditions must prove attractive to the final hosts (humans and dogs) so that they will again come in contact with the infective larvae emitted by the snails.

Transmission of classical schistosomiasis, in China and Japan, is limited by the onset of winter. In the Philippines it may be perennial. The Mekong schistosome appears to have quite a different set of parameters governing its life cycle. It is limited by the behavior of the Mekong River to periods of low water. Transmission can occur only where populations of humans or dogs and populations of susceptible snails can come into contact. Such conditions are not found throughout the known geographic range of the host snail, *Lithoglyphopsis aperta*, but are known at present only from Khong Island. The snails may be presumed to occur at Kratié. It is possible that with further effort other localities of infection will be discovered in the Lower Mekong Basin.

On the basis of the above data, it appears that the pattern of transmission may differ at Khong and at Kratié, but the efficiency of transmission may be about equal in both places if prevalence in school children is used as a criterion.

Reservoir Hosts

In addition to humans, *Schistosoma japonicum* has been reported as a natural parasite of cattle, dogs, cats, pigs, goats, and rats. Schistosomiasis japonica in China, Japan, Taiwan, Philippines, and Sulawesi is considered a true zoonosis.

The role played by animals in the epidemiology of the Mekong schistosome appears to be more restricted. Only the dog has been demonstrated to be an important alternative host to man. Other animals have been investigated (see below) but with negative results.

(a) *Dogs:*

Several investigators have incriminated dogs as carriers of the Mekong schistosome. Iijima *et al.* (1971) examined 24 dogs at necropsy and found adult worms of *Schistosoma* in seven. Many of the worms were in the mesenteric veins of the large intestine and the portal vein of the liver. Smaller numbers of adult worms were found in the mesenteric veins of the small intestine. The worms resembled *S. japonicum* superficially, but slight differences were noted in the size of the ovaries (larger in the Mekong worms than in a Japanese strain), and a greater variation in the number of testes. However, the importance of these differences is not clear since the authors compared dog worms on Khong Island with rabbit worms in Japan; host-imposed differences are not unknown among the geographic strains of *Schistosoma* (Ito, 1955).

Sornmani *et al.* (1971) examined 46 dogs on Khong Island by taking feces directly from the rectum and making both simple smears and formalin-ether concentrations; five, or 10.9% were positive for eggs of *Schistosoma*.

Results of exposure of two dogs to cercariae of the Mekong schistosome derived from experimentally infected *L. aperta* were reported by Sornmani *et al.* (1973). A dog exposed to 124 cercariae by loop application began to show eggs in the feces 40 days later; a second dog, exposed to 214 cercariae, was positive 46 days after exposure.

(b) *Water Buffalo*

Domestic water buffalo (*Bubalus bubalis*) are found naturally infected with *S. japoniucm* in China and the Philippines. In China, the buffalo has been considered to figure significantly in the epidemiology of human disease (Wu, 1938), but in the Philippines, its role in transmission appears to be negligible (Pesigan *et al.*, 1958). During the course of the present work (Schneider *et al.*, 1975) 103 buffalo were examined parasitologically on Khong Island, using stool concentration and the hatching technique. The number examined represented virtually all of the buffalo living in Khong Town and about one-tenth of the buffalo population of the entire island. Only two animals, both calves, were found infected with schistosomes, one with *Orientobilharzia harinasutai* and one with *Schistosoma spindale*, but no samples were found containing eggs with the Mekong *Schistosoma*. It was concluded that water buffalo were not, in all likelihood, involved as reservoirs of human schistosomiasis on Khong Island (Table 2).

(c) *Wild Rodents*

Unpublished data produced during the present study showed that albino laboratory rats, *Rattus rattus*, were capable of developing experimental infections with the Mekong schistosome when suitably exposed to infectious cercariae. Eggs were deposited in the liver but were not passed in the feces of five infected rats.

Rats and related rodents occur on Khong Island and neighboring islands. The numbers fluctuate but appear to be relatively low. *Rattus exulans* was found principally in and around houses. *R. rattus molliculus* and *Bandicota savilei* were found in fields, in paddies, and by stream beds and the shore of the river.

Iijima and Garcia (1967) caught and dissected 45 "wild mice" (probably a misidentification of the small, slender *Rattus exulans*) and examined intestines and liver, but found all specimens negative for *Schistosoma*. Sornmani *et al.* (1971) trapped eight *Rattus rattus* at the water's edge within the boundaries of Khong Town. The livers and intestines were examined microscopically by crushing them between thick glass plates. No eggs or adults of *Schistosoma* could be demonstrated.

In the course of the present study, 103 rodents, including 10 *Rattus exulans*, 83 *R. rattus molliculus* and 10 *Bandicota savilei* were trapped in and near Khong Town and on islands in the Mekong River (Table 3). About a third of these were infected with various unidentified nematodes and cestodes, but careful examination of mesenteries and livers by perfusion failed to reveal any *Schistosoma* (Figs. 3, 4).

In view of the demonstrated experimental susceptibility of *Rattus rattus* to infection, these results were unexpected. It is remembered that "classical" *S. japonicum* is a natural parasite of rats elsewhere in Asia. The results from this survey may be explained in four ways: (1) The habits of the

TABLE 2

RESULTS OF A STOOL EXAMINATION OF WATER BUFFALO (*Bubalus bubalis*) ON KHONG ISLAND, USING THE M.I.F.C. TECHNIQUE

Sites	No.	Neg.	Positive									
			Trematodes[a]						Nematodes[b]			
			S.j.	S.s.	O.h.	Para.	F.g.	E.p.	B.sp.	M.sp.	N.v.	T.sp.
Ban Na Lao	13	0	0	0	0	13	0	1	0	3	0	0
Khong Town												
Xieng Wang	25	1	0	1	1	23	2	2	3	1	0	2
Wat Klang	7	0	0	0	0	7	0	0	2	1	0	2
Dooley H.	9	0	0	0	0	9	1	0	2	0	1	0
None Ka Sang	7	0	0	0	0	6	0	0	0	1	0	0
Ban Na	11	2	0	0	0	8	1	0	0	0	0	0
Sene Hat	31	1	0	0	0	26	2	0	3	4	4	1
Total	103	4	0	1	1	92 (89%)	6	0	10	10	5	5

[a]Legend: S.j. = *Schistosoma japonicum*; S.s. = *Schistosoma spindale*; O.h. = *Orientobilharzia harinasutai*; Para. = Rumen flukes, family Paramphistomatidae; F.g. = *Fasciola gigantica*; E.p. = *Eurytrema pancreaticum*.

[b]Legend: B.sp. = *Bunostomum* species; M.sp. = *Mecistocirrus* species; N.v. = *Neoscaris vitulorum*; T.sp. = *Trichuris* species.

TABLE 3

RESULTS OF A PARASITOLOGICAL EXAMINATION OF RODENTS ON KHONG ISLAND, USING PERFUSION TECHNIQUE AND GUT DISSECTION

Species	Site	No. Exam.	Positive		
			Trematodes[a]	Nematodes[b]	Cestodes[b]
Rattus exulans (in houses)	Dooley Hospital	8	0	0	0
	Done Khiao[c]	1	0	0	1
	Done Yang	1	0	0	0
Rattus rattus molliculus (edge of water)	Military camp	1	0	1	1
	Ban Xieng Wang[c]	2	0	1	1
	Done Yang	8	0	2	3
	Done Sam Rahn	25	0	7	15
	Small islands	4	0	2	1
	Dooley Hospital	8	0	5	3
	Ban Na	5	0	2	2
	Done Khiao	27	0	7	9
	Done Vern Khao	3	0	0	0
Bandicota savilei (edge of water)	Ban Nok Nok	5	0	4	0
	Done Vern Khao	5	0	0	0
Total		103	0	31 (27.9%)	36 (32.4%)

[a]Examined by perfusion or directly in the gut.

[b]Examined directly in the gut.

[c]Ban = village; Done = island.

Khong Island *R. rattus*, and other rodents, do not bring them into contact with water containing cercariae long enough for cercariae to penetrate. (2) An occasional rat may become naturally infected but the prevalence is so low that it was not detected in the relatively small number of animals examined. (3) The survey was conducted too early in the year to detect infections. (4) There is a real resistance on the part of wild rats to infection with the Mekong schistosome.

The first proposal seems the most likely and the last least likely. There is still work to be done on the role of rats as reservoirs on Khong Island. On the basis of work done thus far, however, it appears that rats do not participate in the epidemiology of the *Schistosoma* on Khong Island.

(d) *Swine*

Domestic swine are kept on Khong Island but they are not numerous. They seldom are allowed to live beyond an age of eight to 10 months before slaughter. Older pigs are kept for breeding purposes and are rarely allowed out of their pens (Fig. 5), although an occasional pig may be seen loose in a yard. It is thought that these domestic swine do not visit the river because the bank is usually too steep.

Iijima (1970a) examined an unspecified number of pigs on Khong Island but found none infected with *Schistosoma*. In the present study, stools from 15 pigs in and around Khong Town were examined by the MIFC technique but eggs of *Schistosoma* were not demonstrated, although other helminth parasites are harbored (Table 4).

(e) *Cattle*

Cattle have appeared on Khong Island only recently. A market for veal has been established in Paksé. Most of the cattle are short-term residents on Khong Island. In April 1974 a total of 43 cows living in the vicinity of Khong Island were examined parasitologically. Rumen flukés and certain nematodes were demonstrated in a small number, but no *Schistosoma* were found (Table 5).

TABLE 4

RESULTS OF A STOOL EXAMINATION OF DOMESTIC SWINE (*Sus Scrofa*) ON KHONG ISLAND, USING THE M.I.F.C. TECHNIQUE

Site	No. Exam.	No. Neg.	Positive: Trematodes[a] S.j.	O.h.	O.v.[d]	Nematodes[b] A.s.	A.sp.	O.sp.	Acanthocephala[c] M.h.
Khong Town									
Market Square	1	1	0	0	0	0	0	0	0
Chom Tong	2	0	0	0	0	2	1	0	0
Pho Chai	2	0	0	0	2	1	0	1	1
Ban Na	9	7	0	1	0	2	0	0	0
Done Yang	1	0	0	0	0	1	0	0	0
Total	15	8	0	1	2	6	1	1	1

[a]S.j. = *Schistosoma japonicum;* O.h. = *Orientobhilharzia harinasutai;* O.v. = *Opisthorchis viverrini.*

[b]A.s. = *Ascaris suum;* A.sp. = *Ascarops* species; O.sp. = *Oesophogostomum* species.

[c]M.h. = *Macracanthorhynchus hirudinaceus.*

[d]Ova of *Opisthorchis viverrini,* which is not a natural parasite of swine, may have come from human feces containing the eggs which were eaten by the swine. *O. viverrini* is not uncommon in humans on Khong Island.

Fig. 3.--Technician preparing to examine a wild rat for the presence of schistosome worms using the perfusion technique (Field team working on Khong Island, March 1974).

Fig. 4.--Scientist making a microscopic examination of rat intestines for the presence of parasites (Field team working on Khong Island, March 1974).

Fig. 5.--Technician collecting feces beside a pigsty on Khong Island, March 1974.

TABLE 5

RESULTS OF A STOOL EXAMINATION OF DOMESTIC CATTLE IN KHONG TOWN, KHONG ISLAND, LAOS, USING THE M.I.F.C. TECHNIQUE

Site	No. Exam.	No. Neg.	Positive: Trematodes[a]		Nematodes[b]			
			S.j.	Para.	N.v.	O.sp.	Th.p.	Tr.sp.
Xieng Wang	11	5	0	3	0	3	0	1
None Ka Sang	32	22	0	6	2	2	1	1
Total	43	27	0	9	2	5	1	2

[a]S.j. = *Schistosoma japonicum;* Para. = paramphistomatids (rumen flukes).

[b]N.v. = *Neoascaris vitulorum;* O.sp. = *Oesophagostomum* species; Th.sp. = *Thelazia* species; Tr.sp. = *Trichuris* species.

(f) *Cats*

Cats can be infected experimentally with the Mekong schistosome and the eggs which result from such infections are viable. However, it is not surprising that natural infections have not been found on Khong Island, considering the reluctance of domestic cats to venture near the water's edge. Iijima (1970a) examined an unspecified number of domestic cats in Khong Town but found no *Schistosoma*.

In summary, it appears that man and dogs are the principal, if not the sole hosts for the Mekong *Schistosoma*. Rats, pigs, cattle, and cats, all of which have been demonstrated to be good natural hosts in other Asian countries, have not been shown to be infected at Khong Island. Thus far, animal studies have not been done in the Khmer Republic.

Kratié, Khmer Republic

In June and July 1968, a team of French researchers from the Pasteur Institute and the Calmette Hospital of Phnom Penh surveyed children who lived on the floating houses or raft-houses that line the Mekong River at Kratié (Jolly *et al.*, 1970b). Stool examinations were done on children aged four to 13 years who presented any or all of the following signs and symptoms: general hypotrophy, digestive difficulties, hepatomegaly, or splenomegaly. *Schistosoma* eggs were found in 51 of 68 subjects examined within the boundaries of Kratié (Table 6). Smaller numbers of children were examined up-river (two of 53 positive) and down-river (three of 14 positive). Evidently, the greatest prevalence was in Kratié and seemed to be limited to the inhabitants of the raft-houses who were all fishermen having Vietnamese ethnic origins.

TABLE 6

COMBINED DATA FROM TWO SURVEYS AT KRATIÉ AND SURROUNDING AREAS, JUNE-JULY 1968[a]

Location	Stool Examined	Positive for *Schistosoma*	Per cent
Kratié, at the center of town, about 100 raft-houses	68	51	75.0
Up-river (four groups comprising 78 houses)	53	2	3.8
Down-river (five areas, with about 40 houses)	14	3	21.4
Total	135	56	41.5

[a]Adapted from Jolly *et al.*, 1970b. Stool examinations were done only on subjects with clinical signs and/or symptoms of schistosomiasis.

A concomitant examination of 164 children in a school in Kratié yielded only 11 clinical suspects; examination of stools from eight of these revealed no evidence of *Schistosoma*. The stool examinations were done by either direct smear or the formalin-ether concentration.

Iijima (1970b) skin-tested 2095 primary school children in Kratié, Stung Treng, Kompong Cham, and the villages along the Bassac River. There were few positives except at Kratié, where he found 202 of 563 (35.9%) positive. Subsequent stool examinations of 95 children inhabiting the floating villages yielded 40 positives (42.1%), results which are in good agreement with those of Jolly and his colleagues.

Iijima (1970b) found two children in Stung Treng and one child in Chhoung Leap (Bassac River) positive for *Schistosoma*. It is not known if these subjects had lived at Kratié.

The study of the epidemiology of schistosomiasis in the Khmer Republic has been impeded by military hostilities. As yet, published clinical studies raise more questions than they answer. The search for reservoir hosts needs to be done. The provenience of the few human cases occurring far from Kratié must be ascertained. Most importantly, the snail, *Lithoglyphopsis aperta*, has not been reported from Kratié or anywhere in the Khmer Republic.

When conditions permit, a thorough study of the conditions of transmission must be done at Kratié. It appears that such conditions may vary considerably from those reported from Khong Island, Laos.

3. PATHOBIOLOGY OF SCHISTOSOMIASIS IN THE MEKONG BASIN

Pathology of "Classical" Schistosomiasis Japonica

The following account is based on Hunter *et al.* (1961).

The initial symptom of attack by schistosomes may be itching due to cercarial penetration which often goes unnoticed except in highly sensitized persons, and is almost sure to be unreported by children.

After arriving in the mesenteric veins, the worms mature and lay eggs. The eggs are the main source of disease in schistosomiasis. The Asian schistosome, *Schistosoma japonicum,* produces the most severe form of disease among the three known human species; the female worm produces about ten times as many eggs a day as do *S. mansoni* or *S. haematobium.* The eggs are often laid in masses that form abscesses in the wall of the intestine. If the abscess is near the inner surface, it typically ruptures, releasing the eggs into the lumen so that they pass with the feces. The dysentery and fever that accompany this stage constitute an *acute attack* which is apt to go unrecognized, as dysentery and fever are characteristic more often of other diseases in the tropics, such as amebiasis or malaria.

Eggs which collect in abscesses deeper in the intestinal wall remain there and their presence stimulates a thickening of the intestinal wall. Many eggs are passively carried by the hepatic portal vein to the liver; here, they are treated as "foreign bodies" and each egg becomes the center of a deposit of scar tissue. When a very large number of such eggs are present, even though their individual size is small, the liver may become so heavily invaded by fibrin deposits that its function is impaired and blood flow is markedly slowed. This produces, initially, a degree of liver enlargement, which may later regress. Portal obstruction also leads progressively to enlargement of the spleen. As the blood must find new pathways to the liver, a collateral circulation in the form of prominent varicose veins develops on the abdomen. The esophageal vein may also develop varicosities subject to sudden rupture, which may cause sudden death. Ascites may develop. In a small proportion of cases, worms may migrate from the mesenteric to the spinal veins and eventually reach the brain where abscesses, due to egg production, may result in Jacksonian epilepsy.

Liver fibrosis, enlargement of the spleen, esophageal varices and ascites constitute *chronic* signs and symptoms and may develop gradually over several years. The typical symptoms of chronic schistosomiasis include the discomfort of splenomegaly, malaise, poor appetite, and an increased susceptibility to other infections. Schistosomiasis is mainly a wasting disease although death is by no means an uncommon result.

Treatment of Schistosomiasis Japonica

Newer drugs that have been effective in treatment of other forms of schistosomiasis caused by *S. mansoni* and *S. haematobium* have not proved useful in schistosomiasis japonica. At the present time, the trivalent antimonials are the most potent against this species. These compounds are highly toxic, and must be administered by a physician under controlled conditions, and are not suitable for mass therapy or prophylaxis. There is a high incidence of moderate to severe side effects, particularly when the dosage is increased in order to achieve increased efficiency.

There is a continuing need for the development of an easily tolerated, efficient schistosomicide that may be used either therapeutically or prophylactically (Santos, 1972).

Schistosomiasis on Khong Island, Laos

Barbier (1966) made a careful clinical study of five cases of schistosomiasis in Lao patients residing in Paris who had spent a large part of their lives on Khong Island. The five subjects all came to medical attention for different clinical reasons, none was suspected initially of harboring schistosomes, and the discovery that they were infected was incidental to their diagnosis. At the time it was considered that human schistosomiasis in southeast Asia was confined to certain sections of southern Thailand.

The cases cited by Barbier did not represent schistosomal disease (three of the five also had other parasites, including either *Opisthorchis viverrini* or *Clonorchis sinensis*). All were adults and all had experienced exposure in varying degrees to the waters of the Mekong River at Khong Island during their youth; yet clinical changes in liver and spleen were slight, although detectable. Eggs were rare in the feces but the results of rectal biopsy were positive in all cases. Liver function tests were normal or only slightly modified.

Iijima (1970a), studying the epidemiology of schistosomiasis at Khong Island as a WHO consultant, found numerous patients with enlarged livers and spleens but noted that, in general, the symptoms of schistosomiasis in persons with positive skin tests seemed mild in comparison with schistosomiasis japonica elsewhere in Asia. He found a high degree of correlation between positive skin tests and the presence of eggs in the feces.

Sornmani (1973) reported that 25 of 45 infected individuals (55.5%) on Khong Island had slightly enlarged livers and spleens. All were in the 8- to 19-year age group. These subjects usually were infected with numerous other parasites, including *Opisthorchis, Ascaris* and hookworm.

Schistosome egg output, as evaluated by Stoll's technique, did not correlate with the size of liver and spleen. Frequently, positive individuals were passing too few eggs to be counted by the Stoll method.

Two children had ascites, one severely, but these represented the exception rather than the rule. One of these children, in addition to schistosomiasis, was suffering from hookworm infection, opisthorchiasis, tuberculosis, anemia, and malnutrition. Under the circumstances, it was not possible to assign the severity of the child's condition to schistosomiasis alone.

The relationship of the human schistosome of the Mekong to that reported from the south of Thailand is not clear. However, eggs of the Thai schistosome resemble those of the Mekong schistosome more closely than they do eggs of "classical"

Schistosoma japonicum (Barbier, 1966). Clinical features of some cases from south Thailand have been reported (Harinasuta *et al.*, 1967). Symptoms were mild and included slight abdominal pain, flatulence, loss of appetite and weakness. Nearly all subjects had a past history of dysenteric symptoms and occasional fever. One of these patients died of liver cancer but the others showed eventual improvement without the use of drugs and with only symptomatic treatment.

Schistosomiasis at Kratié, Khmer Republic

Jolly *et al.* (1970a) made a clinical study of children of the "floating villages" of Kratié (houses built on bamboo logs, inhabited by fishermen who were all ethnic Vietnamese). A group of 70 children, from 4 to 13 years old, were examined and 51 (73%) were found to be passing eggs of *Schistosoma*. These 70 children were selected for examination because they had one or more of the following signs or symptoms: poor appearance (thinness or slight fever), digestive upsets, enlarged liver, enlarged spleen.

Tournier-Lasserve *et al.* (1970) reported clinical findings in three children who were admitted to hospital in Phnom Penh. All were from Kratié and lived on floating houses. A 12-year-old girl was seriously ill with dysentery, hepatomegaly, splenomegaly, collateral circulation and ascites. She was passing 600 to 1200 eggs per gram of feces (indicating a very heavy worm burden). She was treated with niridazole which proved efficaceous and well tolerated, but suffered a relapse four months later and had to be treated again. Her two brothers, aged 8 and 6, were also infected with schistosomes and passing eggs, but showed no signs or symptoms of disease.

Iijima (1970b) observed 78 school children in Kratié. All were residents of the "floating villages". Of the 70, 40 (51%) were positive for *Schistosoma*. He noted that "the symptoms of schistosomiasis japonica seemed mild in comparison with those in Japan and the Philippines. Nevertheless, patients with enlarged livers and spleens were observed in all the endemic areas."

Evaluation of the Seriousness of Mekong Schistosomiasis

To date, clinicians who have made observations at Khong and at Kratié have acquired the general impression that cases of schistosomiasis in the Mekong Basin are not as serious as elsewhere in Asia. This may not result from the intrinsic nature of the parasite but may reflect the manner and rate of infection. In other forms of schistosomiasis it is thought that frequent exposure to small numbers of cercariae causes milder disease than does an initial exposure to many cercariae; that is to say, the presence of a few mature worms in the body induces a certain degree of immunity which seems to prevent later invading worms from maturing to the adult stage (Hunter *et al.*, 1961).

Except for the work of Sornmani (1973), no effort has been made to relate disease to worm load in a general clinical study. On the other hand, it is possible that the low pathogenicity is indeed a true characteristic of the Mekong schistosome, setting it specifically apart from human schistosomes elsewhere in Asia.

The "mildness" of Mekong schistosomiasis is a matter that must be resolved as soon as possible. It would be difficult to recommend a large expenditure of time and money (which is what will be required) on the control of an infection which may prove to be only exceptionally serious and which, at present, puts a population of less than 10,000 people at risk in the Lower Mekong Basin. It is also of primary importance to ascertain the efficacy of known as well as novel antischistosomal drugs against the Mekong schistosome.

4. ATTITUDES AND BELIEFS REGARDING SCHISTOSOMIASIS AMONG THE INHABITANTS OF KHONG TOWN, KHONG ISLAND, LAOS

In the four riparian countries of the lower Mekong Basin (Khmer Republic, Laos, South Vietnam, and Thailand), cultural environment and social attitudes are expected to play a role in determining the success or failure of disease control measures, whether or not they are novel, since such measures will require social participation.

With regard to the control of a waterborne disease associated with fecal pollution of the Mekong River, such as schistosomiasis, reaction to and understanding of the disease must be ascertained and any beliefs or practices that might interfere with the establishment of a control program must be anticipated.

In order to develop an idea of the importance attached by local people to needs of a sanitary nature, a preliminary questionnaire survey was carried out in Khong Town, Khong Island, Laos, in cooperation with the medico-sociology section of the World Health Organization office of Vientiane. The preliminary questionnaire was designed to assess local practices which affect transmission, local understanding of the schistosomiasis problem in its various manifestations, and attitudes toward possible control measures (see Appendix II). The survey was limited to one section of Khong Town, namely, that part closest to the main transmission site. It is called Ban (=village) Xieng Wang. A map of the Ban Xieng Wang is shown in Figure 6.

Selected heads of households were requested to consider a list of activities aimed at social improvement and to rank them by priority. The list included nine items, five of which had no direct bearing on sanitation and four of which were intimately involved with sanitation.

The first five included:

1. School improvements
2. Road improvements
3. Improvement of irrigation canals
4. Rehabilitation and improvement of *wats* (temple compounds)
5. Installation of electricity

The others included:

1. Building and staffing a dispensary
2. Installation of running water
3. Construction of latrines
4. Management and sanitation of bathing areas

(With regard to the last item, the intent was explained as follows: bathing areas along the edge of Khong Town would be limited to places that could be regularly controlled from a standpoint of sanitation; they would be situated away from infected zones, *i.e.*, from sites near the rocky islands supporting seasonal populations of transmitting snails around their edges; they would be made attractive by constructing stairways for easier access, laying down white sand in the river, and installing public latrines nearby.)

A brief summary of the results, without tabulation, is presented here. Of a total of 108 ethnic Lao households in the Ban Xieng Wang sector, the heads of 54, or 50%, were questioned.

Knowledge of Early and Late Stages of Schistosomiasis

The early symptoms of acute schistosomiasis may include fatigue, shortness of breath, fever and/or diarrhea. Most of the people contacted considered that these symptoms were so common in everyday life that they were discounted as illness. Treatment was seldom sought since the symptoms were known to be self-limiting.

However, blood in the stools was a cause for reflection. Although commonplace, it was recognized as sickness, especially in the very young, and there were many remedies available from the local pharmacy.

The development of an enlarged spleen (*pen pang* in the southern idiom) or ascites ("big belly") were, on the other hand, causes for alarm. People were uniformly frightened of these conditions which were known to be not self-limiting and which sometimes anticipated death. Each family had a memory of one or more members who had been crippled or had died with these signs and symptoms.

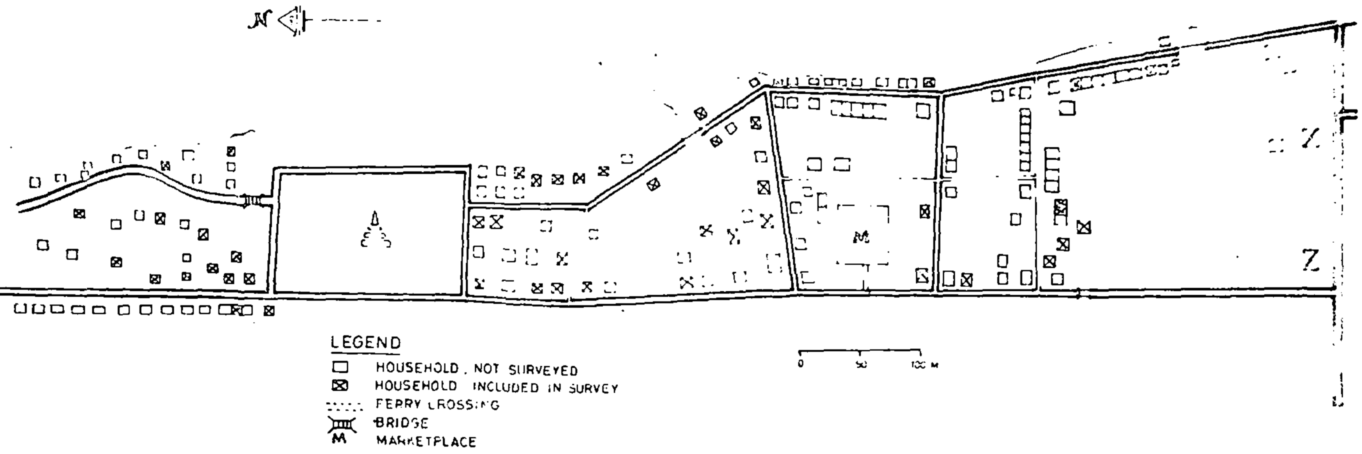


Fig. 6.--Map of the Ban Xieng Wang section of Khong Town, Khong Island, Laos, showing the peninsula (between the *wat* and the market) which is the principal transmission site for schistosomiasis on Khong.

In the past, the cause of chronic schistosomiasis has not been understood. Supernatural causes were usually blamed, including vague concepts of "bad" air, water, or odors. "Big belly" might also be a retribution for breaking a promise made before a monk. It was found that some people were able to attribute "big belly" both to bad air and broken oaths, without a sense of contradiction. The most common cause, however, was bad *phi* (evil spirits).

It was of great interest that a small number of adults now attribute splenomegaly and ascites to something coming from the tiny snails in the river. The investigators acquired the impression that this more accurate knowledge had developed only within recent years following visits of the Trop Med-Smithsonian Team to the community. During this time there had been opportunity for many conversations between scientists and local residents. The observation is of particular interest in view of the need to provide health education on Khong Island. Within the cultural environment of the Lao village, the residents of Khong Town are not unsophisticated and if some of them laugh at the "snail" idea, others do not.

Attitudes Toward Disease in General and Schistosomiasis in Particular

In Laos, animism attributes much illness to *phi*. Buddhist monks are frequently asked to exorcise evil spirits or to appease them. Almost uniformly, the people contacted during the present study indicated that their first recourse in the case of splenomegaly or ascites was to go to the *wat* for religious ceremonies. As a double precaution, many at the same time would visit a "magician" of some kind (there are several kinds).

Recourse might also be had to medicines, either traditional or modern, if the patient thought it might help. The people of Laos are much preoccupied with disease as indicated by the large number of pharmacies in every community of any size. If modern (read, Western) drugs, even patent nostrums, are suggested by the pharmacist they will be accepted readily and used. But the people consider the "traditional" medicines of the herbalists and magicians to have more durability than modern drugs, although the latter are thought to act more quickly.

When all other recourses fail, people may consent to travel to Vientiane, or even to Bangkok, for surgery (to relieve portal hypertension by splenectomy) or to hospital-supervised administration of dangerous drugs such as the trivalent antimonials. But the Lao are reluctant to stay away from home and if they develop any notion that they are going to die, they insist on returning home.

Practices Affecting Transmission of Schistosomiasis on Khong Island

On Khong Island, life is centralized around the Mekong River. Everyday practices such as fishing, bathing, drawing water for domestic uses, even defecation, place people in direct contact with the river. This way of life is solidly entrenched both by necessity (there are few alternative sources of water) and the traditional values of a rural Lao town. The river constitutes the prime source of revenue. As a source of water it is interesting that less than one-fourth of the households included in the present survey had the use of a well. Most people must go to the water's edge and carry water for every kind of domestic use back to the house.

The practice of promiscuous defecation is admitted. People say they use the forests and the fields. Admissions that the Mekong River is used as a place of defecation were rare; it was considered that such a practice was socially reprehensible. Most answers to questions about defecation habits were in response to questions about children. The Lao child enjoys a traditional freedom in its daily behavior more in accord with that granted to the domestic dog or cat. It is hardly to be expected that parents of children who may be old enough to become infected with schistosomiasis will be able to exercise much control over their children's activities.

On the other hand, there was much interest in the subject of latrines. In those households possessing indoor latrines (32 of the 54 questioned), the possibility of controlling children's sanitary behavior was considered positive in half. In households without latrines, 13 of 22 thought control was a possibility. The difference is small. It must be assumed that a transition from current practice to general use of latrines by all concerned is a long-term possibility only.

Everyone except the wealthiest individuals goes fishing on Khong. The equipment includes set lines, gill nets, circular nets, or bamboo fish traps weighted with stones. Fishing is done in the main in the main current or near shore but in all cases the techniques require getting the feet wet.

Most important, everyone questioned admitted to bathing in the river. It is a source of pleasure, one which people anticipate with lively pleasure. Not all areas of the populated section are equally desirable as swimming places. In some, the bank is steep and can be reached only by a narrow and treacherous path over exposed tree roots down an incline of as much as 60 degrees. In other places, houses built out over the water make the site unpleasant. In the Ban Xieng Wang sector there is a large peninsula which, at low water, can be reached by a gradual slope. It is the most popular bathing site in town (see Fig. 6) and is also the location of most schistosomiasis transmission on Khong Island.

When questioned, the villagers of Ban Xieng Wang expressed *no* hesitation in agreeing that their favorite bathing site should be altered, when this was posed in the context of controlling schistosomiasis. Using dynamite to remove some of the larger rocks seemed to pose no problem to a people who have learned, after decades of war, how to fish with grenades.

Conclusions

Although the sanitary habits of the villagers of Khong are such as to promote the spread of schistosomiasis, it would appear unrealistic to expect any rapid changes in this behavior, particularly on the part of the very young, in response to simple requests.

The remedy will have to be based on engineering measures such as the installation of running water, increase in the number of latrines, and physical alteration of the present bathing site at Ban Xieng Wang accompanied by the destruction of some of the small neighboring islands.

If this is done in the name of schistosomiasis control, it is expected that local cooperation will be forthcoming.

The role of sanitary education should not be based on an attempt to force new sanitary patterns on old traditions, but on making information available to the villagers that will insure their acceptance of and support for the envisaged engineering measures. Passive acceptance is never sufficient. If the *active* cooperation of the villagers can be obtained, so that they will feel a sense of personal responsbility for the engineering measures proposed, the inhibiting effect of uninformed indifference will not cause this part of the project to fail. But popular participation must start at the *conceptual* level; it should not be suddenly demanded only at the time of execution of the projects.

Cleanliness is already entrenched in the Lao way of life. Another Lao tradition is that of cooperative effort at the village level in order to achieve a purpose of collective utility to the community (Taillard, 1973). If, in the past, such efforts have been more or less limited to the raising of *wats* and construction of schools, the present climate on Khong Island would indicate that the time may be ripe for the inauguration of community health efforts.

The social structure of the Lao village is well developed. Khong Town is not an exception. Given a supply of raw materials and a way to transport them to Khong, the people would be capable of getting the work done, whether it consists of simply removing the brush at the Xieng Wang peninsula, or destroying the peninsula, or destroying some of the other small islands that harbor transmitting snails, or building a concrete embankment at the water's edge to run the length of the town.

In this connection, it will be important to include the community leaders of Khong Island in the initial planning measures for a control project. Their active participation could be mediated through the Sithandone Association, a Vientiane-based group of businessmen and government officials whose roots are in Sithandone Province in general and Khong Island in particular, and who think of Khong when they think of home.

PART II.

SCHISTOSOMIASIS: THE SNAIL

1. IDENTIFICATION OF A SNAIL INTERMEDIATE HOST

Snails of the genus *Oncomelania*, which are the intermediate hosts of human schistosomes in China, Japan, and the Philippines, are not found in continental Southeast Asia (Davis, 1969). The search for an alternative intermediate host for the Mekong schistosome has been undertaken by a number of investigators (Ito and Jatanasen, 1961; Iijima and Garcia, 1967; Brandt, 1968). Initial success in this endeavor was achieved when Harinasuta *et al.* (1972) demonstrated that a hydrobiid snail, *Lithoglyphopsis aperta* Temcharoen, 1971, was experimentally capable of transmitting the disease.

Materials and Methods

One particular focus of infection on Khong Island, Laos, has received concentrated attention in recent years because of the relatively high infection rates in humans and dogs that have been recorded there, and also because of the accessibility of this site within a region where security for visiting scientists has been, for many years, highly problematical.

In April 1971 a joint team representing the Faculty of Tropical Medicine, Mahidol University, Bangkok, and the Office of Environmental Sciences of the Smithsonian Institution, Washington, D.C., spent more than a month on Khong Island searching for a transmitting snail for the local human schistosome. In that year the dry season in southern Laos was at its height during the months of April and May and there was no water in the paddies or small rivers in the interior of the island. Thus the search for vector snails in areas away from the river was precluded.

A previous study had indicated a riverine source for the infection. Most of the human cases were reported from Khong Town. The largest number of these cases had a history of regular bathing either at the shore in the Ban Xieng Wang section or in front of the Royal Lao Government hospital. Thus the search for transmitting snails was concentrated in and between these areas.

Snails

The mollusk fauna in the Mekong River at Khong Island was very rich. Collections during the months of January through April 1971 had contained examples of many families of gastropods, including Viviparidae, Ampullariidae, Stenothyridae, Hydrobiidae, and Buccinidae. The hydrobiids and stenothyrids were dominant. In April, a single large collection of hydrobiids was made in the shallow water adjacent to the market at the Ban Xieng Wang area. The snails were brought by air directly to Bangkok for examination and identification.

In the laboratory, snails were transferred to rain water in brick-clay bowls of approximately 18" diameter and 6" center depth. They were provided with constant aeration.

They were examined once a week for three or four weeks but no evidence of natural infection with any trematode parasite was observed. In the absence of cercariae after this interval it was assumed that the snails were negative and were used in the experiment described below.

Miracidia

Three dogs, collected on Khong Island, had been found to harbor naturally-acquired schistosome infections. They were transported by air to Bangkok. Eggs were obtained from the feces by sedimenting in 0.85% NaCl solution, resuspending until clean, transferring the material to fresh water and allowing the miracidia to hatch by exposing them to strong light.

Exposure of Snails to Miracidia

Snails were separated according to species into 50- or 100-ml beackers, depending on the number to be exposed. Freshly hatched miracidia were then introduced and allowed to remain with the snails for one hour. Although exposure was thus *en masse*, it was estimated that five to seven miracidia were available for each snail. Table 7 identifies the snails by genus or species.

TABLE 7

RESULTS OF EXPOSING SNAILS FROM KHONG ISLAND *EN MASSE* TO MIRACIDIA OF THE MEKONG SCHISTOSOME DERIVED FROM DOGS (APRIL 1971)

Species of Snails	No. of Snails	Pre-Patent Period (days)	No.+ve Snails Recovered	Remarks
Manningiella sp.	220	-	-	Only 86 snails left after three months, when crushed all negative.
Hubendickia sp.	226	-	-	All died after one month; no cercariae were found.
Lithoglyphopsis aperta	97	45-53	20	29 died, 48 were negative. Only 19 of the negatives left after three months and when crushed all were negative.
Pachydrobia crooki[a]	200	-	-	Only 165 snails alive after three months; when crushed all were negative.

[a]Collected from Ban Dan between Thai and Laos border.

After exposure, snails were kept in clay bowls and were provided with constant but slow aeration. Artificial fish food was initially added as a food supplement.

Starting three weeks after exposure, snails were examined twice weekly for cercarial emergence. The work was done during regular working hours, mostly in the morning between 0900 and 1200 hours.

Exposure of Experimental
Animals to Cercariae

Pools of cercariae from all positive snails were used to infect hamsters (by dipping) and mice, dogs and rabbits (by loop application to the shaved skin of the anaesthetized animal). Beginning on or about the fifth week after exposure, stools were examined for the presence of eggs by means of the formalin-ether concentration technique. The approximate numbers of cercariae used in each exposure are shown in Table 8. Adult worms for morphological examination were recovered by perfusion.

TABLE 8

RESULTS OF THE EXPERIMENTAL INFECTION OF LABORATORY ANIMALS WITH CERCARIAE OF THE MEKONG SCHISTOSOME

Animals	No.	No. of Cercariae Inoculated/Animal (Via Skin by Loop)	No. of Pre-Patent Period (Days)	Worm Recovery
Dog #1	1	124	49	not done
#2	1	214	46	not done
Mice	2	40	46	not done
Hamsters	5	est. 100/cer/hamster (dipping tech.)	43	One hamster was sacrificed on 36th day of infection, 24 males and 13 females were recovered.
Rabbits	2	64	-	One killed after 79 days; the other sacrified after 143 days; few eggs were found in the liver of the latter.

Results

Of the snails listed in Table 7, only *Lithoglyphopsis aperta* (Figs. 7, 8) eventually shed cercariae. Infections in these snails became patent as early as 45 days after exposure to miracidia.

The cercariae were brevifurcate and nonocellate (Fig. 2). After swimming actively for a few seconds they came to rest in flexed posture at the surface film. They could be induced to emerge at almost any time of the day. They resembled cercariae of *S. japonicum* in obvious respects but because of scarcity, all of those that emerged were retained for animal infections and no attempt was made to study cercarial morphology in detail.

Mice and dogs developed diarrhea during approximately the seventh week of infection. One hamster was killed 36 days after infection and 24 male and 13 female worms were recovered by perfusion. Only four pairs were recovered *in copula*. Eggs had not been seen in the feces of this animal up to and at the time of necropsy, but infertile eggs were observed in its liver.

After this time, the remaining animals were checked daily for the presence of eggs in the feces. The dogs began to pass eggs on the 46th and 49th days. Two mice were patent on the 46th day. The four remaining hamsters were all positive on the 43rd day. Two rabbits never developed patency; they were killed 79 and 143 days after exposure and adult schistosomes were recovered from both by perfusion of the portal vein. No eggs were found in the first rabbit although a few eggs were found in the liver but not in the feces of the second. These data are summarized in Table 8.

The eggs of this parasite were the short, round eggs that have been reported by other investigators (Kitikoon, 1970; Taylor and Moose, 1971) to be morphologically distinct from those of "classical" *S. japonicum*.

Adult worms, recovered by perfusion, resembled *S. japonicum* in all obvious respects. Male worms had typically seven testes (Fig. 1).

Discussion

The snail, *L. aperta,* is entirely aquatic. It was collected on twigs, dead leaves, rocks, or tin cans, usually under 6 inches to a foot of water. It was found in the presence of a detectable current, high oxygen tension, high pH, and sunlight. Its aquatic ecology, which is discussed below, appeared to be associated with the small islets of rock-bearing woody rheophytes during low water that are numerous near Khong Town. It is particular interest that this snail has no known amphibious habits, unlike its distant relative, *Oncomelania*.

L. aperta from Khong Island is distinguished in the field by four prominent patches of black pigment on its mantle which are easily seen through the translucent shell of the live animal (Fig. 8). These markings are absent in dried specimens (Fig. 9). Adult *L. aperta* measured 2 to 3 mm in length and 1 to 2 mm in width. A discussion of the taxonomy of this snail is given in Part II, Chapter 4.

Although *L. aperta* was the only one of the four species of snails tested that proved capable of completing the life cycle of the parasite, preliminary evidence indicates that related, sympatric snails can also accept miracidia of this schistosome (Brandt and Temcharoen, 1971; Lo *et al.*, 1971). Thus it is too early to state that *L. aperta* is the only transmitting snail in nature. It should be pointed out, however, that *L. aperta* was a very efficient intermediate host under experimental conditions (of 97 snails exposed, 68 survived of which 20 eventually shed cercariae).

The strongest evidence for a genetically distinct group of schistosomes probably can be based on the differences between snail hosts. Subspecies of *Oncomelania hupensis* are taxonomically distant from *Lithoglyphopsis aperta*. Although both belong to the family Hydrobiidae, the former are ranked in the subfamily Pomatiopsinae and the latter in the subfamily Triculinae (Davis, 1969). Preliminary cross-infectivity studies indicated the inability of the Mekong parasite to infect five geographic subspecies of *O. hupensis* (Lo *et al.*, 1971).

Fig. 7.--Living "*Lithoglyphopsis*" *aperta*, gamma race.

Fig. 8.--Mature "*Lithoglyphopsis*" *aperta*, gamma race, showing apical erosion.

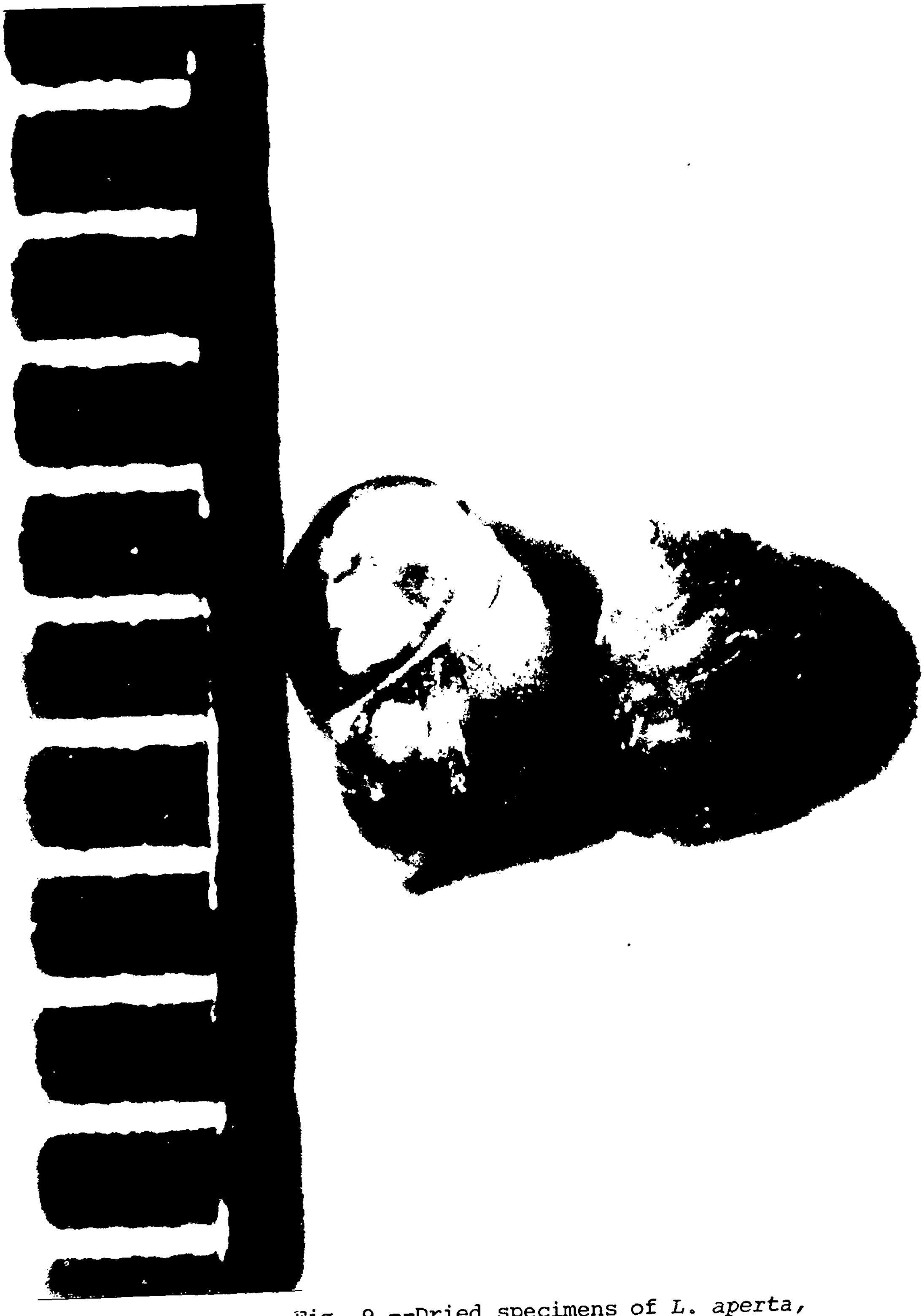


Fig. 9.--Dried specimens of *L. aperta*, gamma race; pigment on the mantle is no longer visible. Specimens were photographed beside a millimeter rule.

2. NATURAL TRANSMISSION OF SCHISTOSOMIASIS ON KHONG ISLAND

Previous reports (Sornmani *et al.*, 1971) suggested that the principal source of infection with the Mekong strain of *Schistosoma* at Khong Island was the river itself. The evidence, however, was indirect: the heaviest worm burdens were reported from residents of Khong Town and its immediate vicinity beside the river.

Direct evidence of transmission was sought, using two methods: (1) sentinel mice were exposed in cages on the river in or near areas suspected of heavy contamination, and subsequently examined for the presence of schistosome infections; and (2) efforts were increased to collect naturally infected snails (*Lithoglyphopsis aperta*) at Khong Island.

Materials and Methods

Description of Exposure Cages

The literature contains several descriptions of cages devised for exposing mice to schistosome-contaminated waters (Dazo, 1965; Pitchford and Visser, 1962; Radke *et al.*, 1961). In the present work, a cage was developed by a carpenter following verbal descriptions of what was wanted and the use to which the contraption would be put. The somewhat unwieldy result (Fig. 10) proved to be essentially serviceable. The cages were equipped with ¼-inch galvanized screen on five sides, the screen protruding about ½-inch below the level of the bottom frame. Teak was used for the frame, with a plywood lid made with an overhang to protect mice from the sun. Outside measurements, not including the lid, were 9 by 6.5 by 6.5 inches. The inside volume was approximately 300 cubic inches. Because the screen did not extend inside beyond the top horizontal frame, mice were unable to crawl into the top of the cage. Adjustable clamps on two sides supported styrofoam floats and the depth of immersion of the floating cage could be controlled by raising or lowering these floats. When the floats were removed, the heavy cage could only float totally submerged.

Four such cages were constructed in Bangkok and were flown directly to Khong Island where the following experiments were performed.

Exposure of Mice on the
Mekong River: 1971

Three sites were selected near the Ban Xieng Wang section of Khong Town, where transmission was thought to be active. The cages were anchored in position on short lines in one or two feet of water. Ten laboratory-raised white mice were placed in each cage and exposed at sites A, B and C for three hours a day (0900 to 1200 h) during five consecutive days (April 14-18). Approximate locations are shown in Figure 11. The floats were adjusted to allow the bottom half-inch of the cage to remain submerged. The current at site A was judged to be somewhat slower than at sites B and C, but there was a gentle movement of water at all three sites. True velocity was not measured. Most of the mice got thoroughly wet at the beginning of the exposure but subsequently climbed up the screening, leaving only their tails in the water. A fourth cage of 10 mice, placed too close to shore, was attacked by a dog which broke it open and killed or released the mice; these animals are not considered in this report. In June and July 1971, another 30 mice were exposed in the water adjacent to Ban Xieng Wang but owing to unavoidable technical difficulties, none survived long enough to permit inclusion in this report.

Exposure of Mice on the
Mekong River: 1972

When the team members returned to Khong in April 1972, they found that in addition to the cage destroyed by the dog, two others had disappeared. Moreover, the styrofoam floats on the remaining cage had fragmented to such a dgree that they were unable to support the cage in the water. Accordingly, this cage was propped in shallow water on rocks for

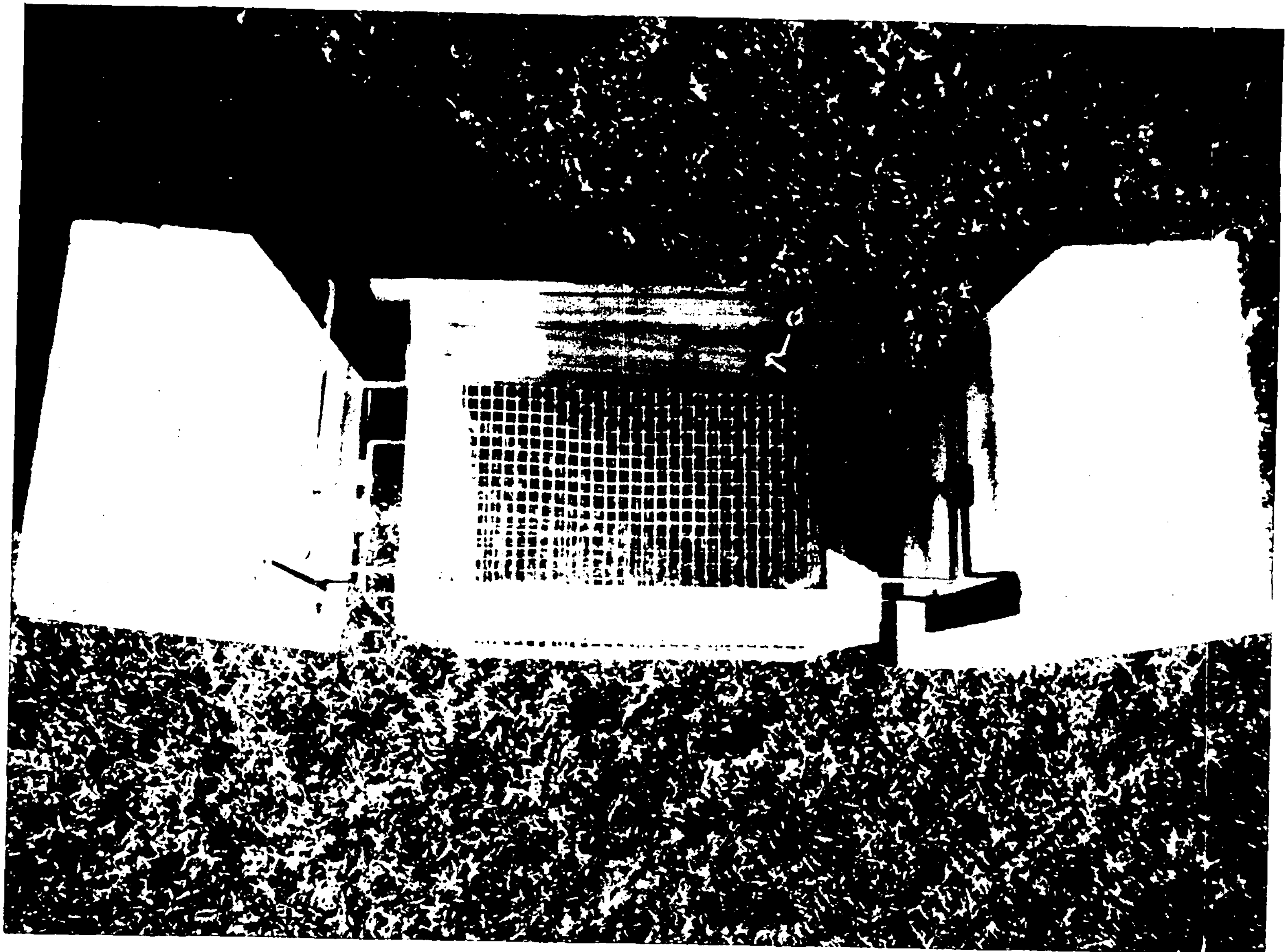


Fig. 10.--Floating cage for exposing sentinel mice to possible infection with the Mekong *Schistosoma*.

the following experiments. Two groups of 10 mice each were exposed for a total of 40 hours on alternate days at sites D and E (Fig. 11). Exposures at site D were made on April 22, 24, 26 and 28 while exposures at site E were made on April 23, 25, 27 and 29. The two sites were in about a foot of water, separated by a low spit of sand. Water currents were dissimilar at these sites. Site E had a detectable, although gentle, current whereas site D was protected from the main current and water movements were by means of eddies. The cage was submerged in about 1½ inches of water; the mice stood up on their hind legs at first, getting completely wet, but later crawled up on the screen letting their tails hang in the water.

Snail Collections

In previous work, a snail assigned to *Lithoglyphopsis aperta* Temcharoen, 1971, was found experimentally capable of serving as an intermediate host of the Mekong schistosome (Harinasuta *et al.*, 1972). A search for this snail was made during a two-month period (April-May) in 1972 at sites around the perimeter of Khong Island, with emphasis on inhabited localities. Snails were collected by removing submerged branches, twigs and rocks and shaking them over a bucket, or they were taken individually from submerged rocks, twigs, leaves, beer cans, and other solid objects to which they adhered.

In April 1971, small numbers of *L. aperta* were found in collections consisting predominantly of *Manningiella* species on the east side of Khong Island in the vicinity of Khong Town (Sornmani *et al.*, 1973). However, in April and May 1972, there was a virtual population explosion of *L. aperta* near Khong Town, particularly in the neighborhood of Ban Xieng Wang, the same site collected in 1971. Relatively large numbers of *L. aperta* were taken here, as well as at most suitable sites along the southeast edge of Khong Island. Smaller numbers of this species were collected on the western shore of the island. *L. aperta* were not found on the sandy shores of the north end of the island.

Crushing was not used in examining snails for schistosome infections because large numbers of live snails were required for subsequent life-cycle studies planned for the laboratory in Bangkok. Instead, snails were isolated in plastic shell vials in groups of 20, left for three hours under a lamp or in the sunlight, and then examined under a dissecting microscope for the presence of naturally shed cercariae. Vials containing cercariae were set aside and the snails in them re-isolated into individual vials.

Positive snails shed eyeless, brevifurcocercous cercariae which resembled those of *Schistosoma japonicum* in appearance and behavior. A pool of cercariae from all of these snails was used to infect three mice and a weanling puppy on Khong Island. The number of cercariae available at a given time was small. Infected snails shed from two to three to 20 cercariae per day. There were never more than 20 snails shedding at one time. Each mouse was anaesthetized and exposed to 40 cercariae by looping. The puppy was anaesthetized with Nembutal and 550 cercariae were applied with a loop to a shaved portion of the abdomen.

Animals exposed in the field were brought directly to the laboratory in Bangkok for further investigation.

Results

Mouse Exposures

Of the mice exposed on the river in April 1971, pre-patent survival was relatively high (26 survivors out of 30, or 87%). No eggs appeared in the feces of any of these mice, however, and no worms were recovered when mice were killed and perfused 60 days after the initial exposure on April 14 (Table 9).

In Groups D and E, exposed April 1972, a total of 17 mice out of 20 (85%) survived the 45-day pre-patent period which had previously been observed in mice exposed to the Mekong schistosome (Sornmani *et al.*, 1973).

In Group D, one mouse was found dead on June 9, 48 days after its initial exposure on April 22; when tissues were examined microscopically, eggs were found in the liver and in the mucosa of the small intestine, but none were seen in simple smears of the feces. The tissues were too macerated to recover or count adult worms. Three other mice in this group were found dead on June 16, 55 days after initial exposure; eggs were found in liver and in feces but again the tissues were macerated and the adult worm load could not be determined.

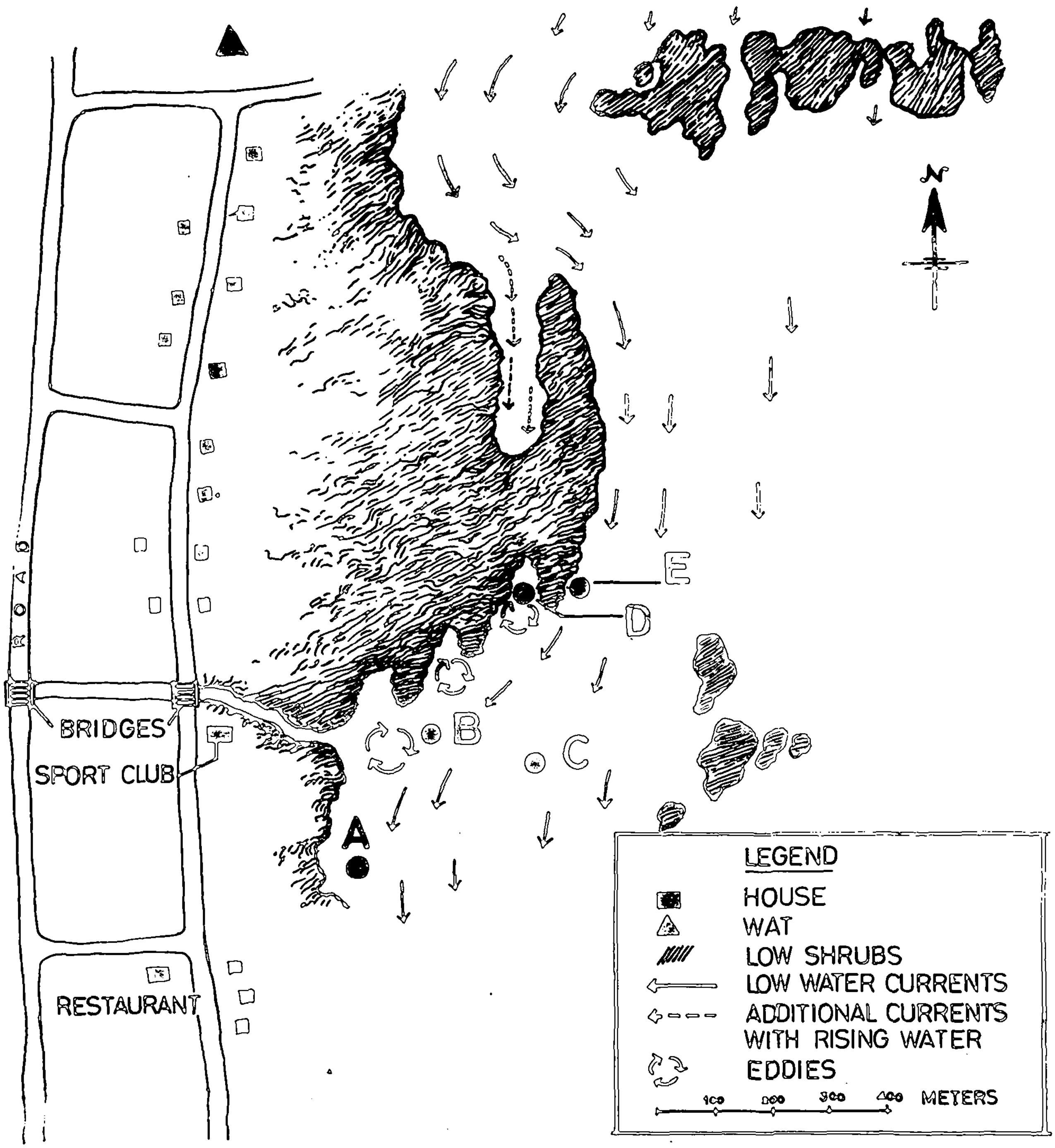


Fig. 11.--Rough sketch of the shoreline of Khong Town in the Ban Xieng Wang section, showing sites where sentinel mice were exposed to detect schistosomes in 1971 (sites A, B, and C) and 1972 (sites D and E). NOTE: Figure 6 represents a more accurate map of Ban Xieng Wang.

The six remaining mice in Group D were killed and perfused 55 days after initial exposure. Five proved to contain male and female worms which appeared morphologically indistinguishable from *Schistosoma japonicum*. However, of eight mice surviving in Group E, only two proved to be infected when killed on June 22, 60 days after initial exposure on April 23, and these had only male worms (Tables 9, 10).

TABLE 9

NATURAL INFECTIONS WITH MEKONG *Schistosoma* IN SENTINEL MICE EXPOSED ON THE MEKONG RIVER AT KHONG ISLAND IN AREAS SUSPECTED OF AFFORDING HEAVY HUMAN TRANSMISSION

Group[a]	Date of Exposure	No. of Mice	Time of Exposure (Hours)	No. of Mice Alive 45 Days After Initial Exposure	No. of Mice Positive for Adult Worms
A	April 14-18 1971	10	15[b]	10	0
B	April 14-18 1971	10	15[b]	7	0
C	April 14-18 1971	10	15[b]	9	0
D	April 22,24, 26,28 1972	10	40[c]	10	9/10
E	April 23,25, 27,29 1972	10	40[c]	8	2/8

[a]For locations of group sites, see Figure 11.

[b]Exposed three hours per day for five consecutive days.

[c]Exposed 10 hours per day on four alternate days.

TABLE 10

RESULTS OF EXPOSING SENTINEL MICE TO POSSIBLE SCHISTOSOME INFECTIONS ON THE MEKONG RIVER AT KHONG ISLAND, APRIL 1972

Group	Mouse	Infection	Worms Recovered 55 Days After Initial Exposure: Single Males	Single Females	Pairs *in copula*	Total Worms
D[a]	1	+	2	1	2	7
	2	+	7	3	6	22
	3	+	5	1	3	12
	4	+	9	8	3	23
	5	+	4	4	1	10
	6	+[c]				
	7	+[d]				
	8	+[d]				
	9	+[d]				
	10	-				
E[b]	1	+[e]	2	-	-	2
	2	+[e]	5	-	-	5
	3	-				
	4	-				
	5	-				
	6	-				
	7	-				
	8	-				

[a]Exposed April 22, 24, 26, and 28, 1972. Cf. Fig. 11.

[b]Exposed April 23, 25, 27, 29, 1972. Cf. Fig. 11.

[c]Found dead on June 9 (48 days after initial exposure). Mature eggs in liver, immature eggs in mucosa of intestine; no eggs in feces. Tissues too macerated to recover adults.

[d]Found dead on June 16 (55 days after initial exposure). Mature eggs in liver and feces. Tissues too macerated to recover adults or count worms.

[e]Worms recovered 60 days after initial exposure.

Snail Collections

Forty-eight snails were found to be positive during the 1972 field work on Khong Island, and 18 more positives were detected when surviving collections of *L. aperta* previously recorded as negative were re-examined by shedding later in the Bangkok laboratory.

The largest population of *L. aperta* was found adjacent to the Ban Xieng Wang area, which yielded 15,790 specimens. The Military Camp produced 1750, whereas immediately downstream, at the Dooley Hospital, 4132 specimens were collected. The village of Ban Na yielded 6342 specimens. Elsewhere around the island, populations of *L. aperta* appeared to be much smaller and fewer individuals were collected (Table 11, Fig. 12).

Natural infections detected by shedding were found only in snails collected near Ban Xieng Wang, the prevalence being 0.30% (48 of 15,790).

Multiple collections were made at the Khong Town sites and at Ban Na but the other localities around the island were visited only once, in May. For this reason the numbers of snails collected and reported in Table 11 do not represent accurate differences in population density. However, the collecting team, comprising the same personnel throughout the work and utilizing the same methods throughout, were aware of the relative abundance and ease of collecting from place to place and acquired the strong impression that snail population densities at Ban Veun Thong and on the western shore of the island were indeed considerably lower than those at Khong Town and Ban Na.

Because shedding instead of crushing was used to detect infections, many immature infections may have escaped attention. The infection rate of 0.3% (Table 11) in the Ban Xieng Wang area must be considered to represent a most conservative figure. Indeed, when the snails were transferred to Bangkok and re-examined a week later, 18 more positives were detected among those previously recorded as negative, so that a total of 66 positive snails were obtained on this expedition. Nevertheless, since exact death records of these snails were not available, it is not possible to include these 18 in the overall natural infection rate.

It was demonstrated by animal infection that the cercariae from the wild-caught *L. aperta* represented the Mekong strain of *S. japonicum*. Three mice exposed to 40 cercariae each developed bisexual infections in 43 to 46 days. Upon perfusion, the adults appeared indistinguishable from *S. japonicum*. The puppy exposed to 550 cercariae began to produce mature eggs in the feces after the 43rd day. The eggs resembled the short, round eggs of the Mekong schistosome.

TABLE 11

SITES WHERE *Lithoglyphopsis aperta* WERE COLLECTED AT KHONG ISLAND IN APRIL AND MAY 1972, AND EXAMINED FOR CERCARIAE OF THE MEKONG *Schistosoma*

Site	No. of Snails Collected	No. of Snails Positive	Per cent Positive
East Shore			
Ban Veun Thong	185	-	-
Khong Town:			
Military Camp	1,750	-	-
Ban Xieng Wang	15,790	48[a]	0.30
Dooley Hospital	4,132	-	-
Ban Na	6,342	-	-
West Shore			
Ban Hang Khong	150	-	-
Ban Hin Siou	185	-	-
Moung Sene	375	-	-
Ban Sen Hat	750	-	-
Total	29,659	48[a]	0.16

[a]When surviving *L. aperta* were returned to Bangkok they were re-examined for cercarial infections by shedding; 18 more positives were found among snails previously recorded as negative. However, in the absence of exact records of snail deaths, it is not possible to include these positives in the natural infection rate detected in the field.

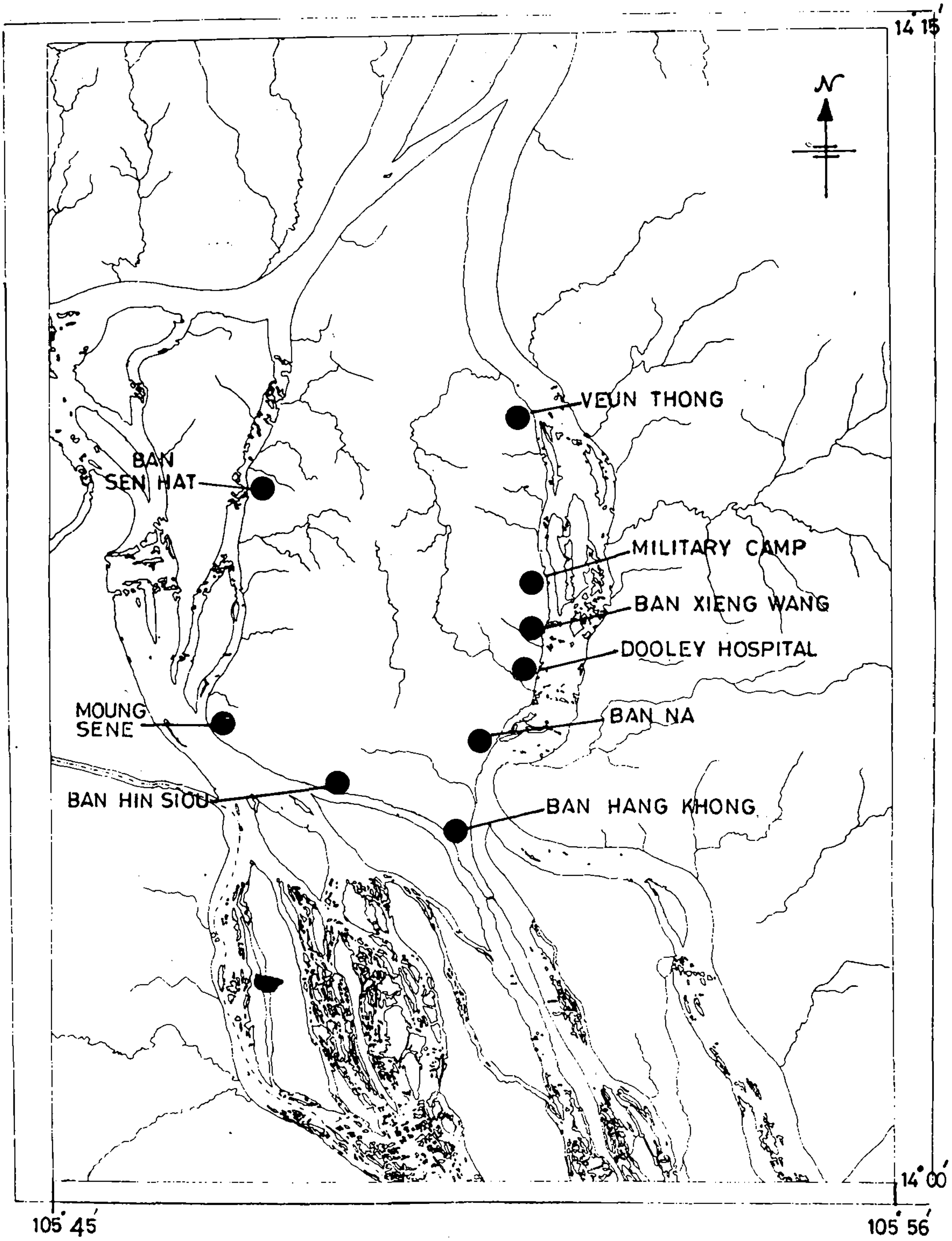


Fig. 12.--Map of Khong Island, showing populated sites around the shore where *L. aperta* were collected in 1972.

Discussion

With regard to the sentinel mice exposed in April 1972, it is of interest that nine of 10 animals in Group D became infected but only two of eight in Group E did so, the latter being unisexual infections with few worms. Although sites D and E were close, water movement was different in the two places; at site E there was a detectable, if gentle, current, whereas at site D there was little or no current and the water movement was due to eddying (Fig. 11). We must conclude either (a) that the infections in Group D resulted from a "lucky strike," or more probably, (b) that the acquisition of infection was influenced by the difference in water movement, since cages exposed to areas of detectable current (A,B,C, and E) produced few or no infections. That is to say, the presence of current may have reduced the chance of infection.

These experiments provided evidence that the Mekong schistosome was being transmitted in the body of the main river. Yet, during the dry season many quiet, pond-like pockets of still water can be found along the shore of the Mekong River at Khong Island (Figs. 13, 14). Water currents in such places may be locally reduced or absent. The pattern of transmission at Khong Island, therefore, seems to differ from that of classical schistosomiasis japonica in China, Japan, and the Philippines, where infection is picked up in seepage areas, wet-grass areas, and paddy fields. On the other hand, transmission at Khong Island does not quite approach the situation reported for schistosomiasis mansoni in certain regions of Puerto Rico (Radke *et al.*, 1961), where infections were picked up by sentinel mice from rather rapid water in small streams.

The failure to obtain infections in mice exposed in April 1971, might be explained in several ways. (1) The water velocity may have been too great; although not measured, it was considered "detectable" close to shore and "substantial" farther away from shore. To avoid misunderstanding, it should be mentioned that the main stream of the Mekong River, with very fast current even at the height of the dry season, occurs close to the left bank and several hundred meters away from the vicinity of Khong Town. Still, the current is very fast in some areas close to Khong Town. (2) The mice may have been exposed for too short a time. (3) Cercariae may have been absent. Very few *L. aperta* were collected here by team members in 1971 (Sornmani *et al.*, 1973). (4) Cercariae may have been present but may have come from so far upstream that they had lost vitality and/or infective ability by the time they reached the mice in the exposure cages. Radke *et al.* (1961), working with *S. mansoni* in streams of Puerto Rico, reported two factors that limited their mouse infections: the velocity of the water and the distance the cercariae were carried downstream. In the latter case, the number of worms maturing in exposed animals decreased markedly with increased distance from the infected snails.

We have no doubt that our success in recovering infections from sentinel mice in 1972 was associated with the large numbers of *L. aperta* that appeared in the water adjacent to Khong Town in that year from April onward. In previous years, their numbers at Khong Island appear to have been small, but most collections seem to have been made earlier than April or May. In the early dry seasons of 1968 and 1969, Brandt and Temcharoen (1971) found small numbers of *L. aperta* at Khong Town and Ban Na but did not examine them for the presence of cercariae or try to infect them. Lo *et al.* (1971) made extensive mollusk collections in December and January 1968-69, but did not report *L. aperta* (at that time still an undescribed species) among the snails they examined for cercariae. *L. aperta* were present in small but easily detectable numbers in April 1971 (Sornmani *et al.*, 1973) and in very large numbers in April and May, 1972 (present work).

The shore of the Ban Xieng Wang area of Khong Town is flat during the low-water period and there is a heavy growth of shrubby rheophytes (predominantly a euphorb, *Homonoia* sp.) which offer concealment and make the area popular as a defecation site (Fig. 15). The recovery of naturally positive snails in this area may be related to this situation.

More must be known of the natural life cycle of *L. aperta* and its relatives before predictions can be made about when and where the snails will appear in abundance. Thus far, the evidence suggests that this species occurs in the waters near Khong Island in large numbers only toward the last months of the dry season. In this part of Southeast Asia, the dry season begins in November or December and ends usually in mid-June. Thus, in April, May and June, river contact at Khong Island, especially in the Ban Xieng Wang section of Khong Town, must be considered extremely risky.

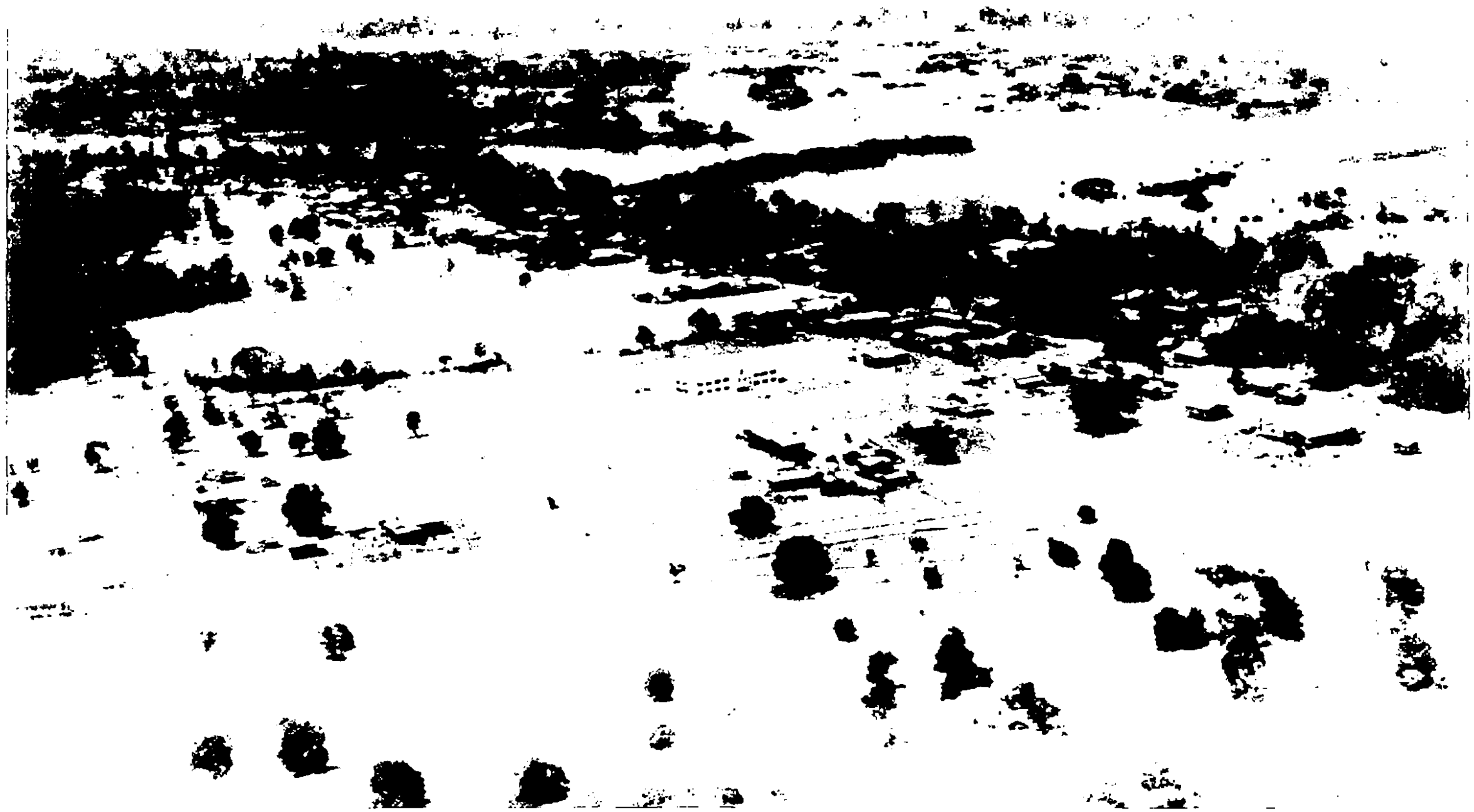


Fig. 13.--Aerial view of Khong Town and the adjacent Mekong River, March 1974, showing many small islands that appear during low-water.

Fig. 14.--Principal transmission site at Ban Xieng Wang, showing a heavy growth of filamentous green algae in the clear, slow water.

Fig. 15.--Luxurious growth of the euphorb shrub, *Homonoia* sp., at the Ban Xieng Wang transmission site, March 1974.

It may be of interest to report here an isolated fact regarding an "accidental" sentinel animal detected on Khong Island. In May 1971, a five-month-old puppy was found shedding schistosome eggs. The puppy had been born and weaned in the Ban Xieng Wang area. Assuming that it was born in January, that the pre-patent period was approximately six to seven weeks, and that it managed to get infected as soon as it was able to leave its mother (probably at two months of age), we may conservatively guess that it became infected in late March or early April. If so, this puppy became infected at the time sentinel mice *failed* to pick up the infection in the Ban Xieng Wang area.

It is precarious to attempt to extract too many facts from the few data at hand. Nevertheless, it appears that the risk of becoming infected with the Mekong schistosome at Khong Island is intermittent in time and place and may be limited to the latter part of March, the months of April and May, and possibly mid-June when the rains usually begin.

3. ROLE OF SYMPATRIC SNAILS IN TRANSMISSION

Some 94 species of hydrobiid snails have been described from the Mekong River. Although all of these species are probably not taxonomically valid, speciation within the family Hydrobiidae clearly has progressed in the Mekong River. This family seems to be much more diversified within a relatively small geographic region than are most other mollusk groups.

Within the family, *Lithoglyphopsis aperta* was identified experimentally as an efficient transmitter of the Mekong *Schistosoma*. Three distinct races of this species were observed in the Mekong River and its tributary, the Mun River. The "tiger-striped" snail which was first incriminated has been identified as a member of the *gamma* race, as described in Part II, Chapter 4. Unpublished preliminary data collected during the course of the present study indicated that the *alpha* and *beta* races were also capable of transmitting the infection under experimental conditions, but no information is yet available on the comparative efficiency of these races as hosts for *Schistosoma*. The *beta* race has been found in the Mun River, east of Phibun Mangsahan, in northeast Thailand, in an area where human cases of schistosomiasis have been reported (Desowitz *et al.*, 1967).

Other genera apparently related to *Lithoglyphopsis* have been suspected of involvement in transmission on the basis of the behavior of miracidia toward them. Brandt (1968) found that *Hydrorissoia hospitalis* (=*H. munensis*) accepted miracidia derived from a human infection at Khong Island. Sporocysts were reported as developing but all the snails died before maturation to cercariae could occur.

Lo *et al.* (1971) exposed *Pachydrobia bavayi* which had been wild-caught in the Mekong River at Khong Island to miracidia of the Mekong *Schistosoma*, eggs of which were obtained from infected school children. The miracidia penetrated the snails but no infection resulted.

Lo (unpublished data) attempted to infect *Pachydrobia crooki* and unidentified species of *Manningiella*, *Hydrorissoia*, *Hubendickia*, and *Stenothyra*, all wild-caught in the Mekong River at Khong Island in the month of January 1972. The miracidia used were derived from infected children. He noted that the miracidia were attracted to and successfuly penetrated all snails but *Stenothyra*, in which they showed no interest. It is worhty of note that *Stenothyra* does not belong to the family Hydrobiidae but to the family Stenothyridae.

When snails which had accepted miracidia were fixed and sectioned at intervals of 15 hours, and one to five days after exposure, it was found that development of the miracidia into sporocysts had not progressed beyond 15 hours in *P. crooki* before they died and began to degenerate, losing their characteristic cell types. Sporocysts were found in *Manningiella* sp. and *Hubendickia* sp. through the third day of infection, and in *Hydrorissoia* sp. through the fourth day. Thereafter, the sporocysts degenerated and could not be recognized (Table 12).

Clearly, additional work is needed on this subject. Given the large number of hydrobiid species in the Mekong, those in which schistosome development can be initiated but not completed may act as decoys for miracidia, taking them out of circulation and interferring with transmission.

On the other hand, one or some of the non-transmitting species may be transmitters with a very low rate of efficiency, *e.g.*, one in a hundred thousand. Many years of work, collecting snails during the few months of low water and attempting experimental infections, will be required to answer these questions.

TABLE 12

PENETRATION OF MIRACIDIA AND SURVIVAL OF SPOROCYSTS IN SNAILS FROM THE MEKONG RIVER AT KHONG ISLAND, JANUARY 1972

Snail	Number of Sporocysts Counted in 3 Specimens of Each Species					
	15 hrs	1 day	2 days	3 days	4 days	5 days
Pachydrobia crooki	9	0	0	0	0	0
Hubendickia	?[a]	1[a]	4[a]	1[a]	0	0
Hydrorissoia	2	5	2	1	3	0
Manningiella	11	1	4	1	0	0
Stenothyra	0	0	0	0	0	0

[a]Poorly fixed specimens, difficult to read.

4. THE TAXONOMY OF *Lithoglyphopsis aperta*

Morphology

Shell

Alpha Race (Table 13). Shells of mature animals are 6.0 to 6.5 whorls and 4.20 ± 0.11 mm long. Other shell dimensions are given in Table 13. The whorls are shouldered and increase regularly in size. Some young have a concave outline for the first three or four whorls. There is no spiral microsculpture. Sculpture consists of five axial growth lines.

The body whorl is inflated; the aperture is pyriform, produced adapically like a beak. A distinct crease or groove runs from the adapical limit of the peristome into the shell following the contour of the body whorl. The peristome is complete and edged with brown periostracum. The inner lip is expanded over the base of the shell; it is often wide, sharp at the edge and keel-like. The outer margin of the aperture, 0.63 ± 0.06 mm in width, is translucent and glistening. Internal to that margin the aperture is chalky white.

Young shells are translucent and yellowish; adult shells are opaque and white. The periostracum is olivaceous. Males and females do not differ significantly in shell length or width. Shells with 6.0 whorls dominate the adult population. In this size class males and females differ significantly in length of body whorl, aperture length and width.

Beta Race (Table 14). Shells of mature animals have 6.0 to 6.5 whorls and are significantly smaller than those of the alpha race. The greatest length attained by an uneroded shell with 6.5 whorls was 3.81 mm. The shells also differ from those of the alpha race in having flat-sided whorls or a smoothly concave spire outline. The suture is very narrow. Raised spiral microsculpture is pronounced in 58 percent of the shells, faint in 25 percent and absent in 17 percent (40 specimens).

TABLE 13

ANALYSES OF SHELLS FROM LIVING ANIMALS OF THE ALPHA RACE OF *Lithoglyphopsis aperta* (MEASUREMENTS IN MM)[a]

Shell Trait	Females		Males		S.D.	p
	$\bar{x}$	Sd	$\bar{x}$	Sd		
6.0 whorls (n = 9)						
Length	4.20	0.11	4.03	0.10	N	.05
Width	2.79	0.08	2.72	0.16	N	.05
Length of body whorl	3.15	0.11	2.81	0.30	S	.05
Aperture length	2.62	0.09	2.49	0.10	S	.01
Aperture width	2.13	0.09	1.96	0.10	S	.01
6.5 whorls (n = 8)[b]						
Length	4.22	--	4.18	.06	N	--
Width	2.81	--	2.90	.27	N	--
Length of body whorl	3.26	--	2.90	.22	S	--
Aperture length	2.69	--	2.60	.08	N	--
Aperture width	2.19	--	2.03	.08	S	--

[a]Legend: n = number of shells measured; N = not significantly different; S = significantly different; Sd = standard deviation; S.D. = significant difference; $\bar{x}$ = mean.

[b]Probably only 2-3 shells of 6.5 whorl females were found.

Gamma Race (Table 14). The animals mature with a shell of predominantly 5.5 whorls; few reach 6.0 whorls. Females from Khong Island maintained in the laboratory several months matured with an average shell length of 2.93 mm (standard deviation, 0.16 mm; standard error of the mean, 0.06 mm). An insufficient number of males was available for comparison.

Living mature animals thus far have not been collected and preserved in the field so that accurate shell measurements are lacking. Several collections of dead shells were made in the Mekong River near Khemarat. The shells were collected from pockets in the river where currents had deposited them. Their position with regard to living immature snails suggested that they were of the same population but of a previous generation.

TABLE 14

SHELL DIMENSIONS OF BETA AND GAMMA RACE *Lithoglyphopsis aperta*
(MIXED MALES AND FEMALES)

Race	Whorls	Length	Width	Lbw[a]	Lap[a]	Wap[a]
Beta, Complete Shells	6.0	3.44	2.00	2.63	1.88	1.31
ANSP 332316[b]	6.5	3.81	2.56	2.94	2.31	1.81
Beta, Eroded Shells ANSP 332316	6.0 est. (n = 8)[c]	--[d]	2.23±.13[e]	2.68±.10	2.06±.13	1.52±.12
	6.5 est. (n = 11)	--	2.46±.11	2.95±.04	2.34±.07	1.73±.06
Gamma, ANSP 330925	5.5 to 6.0 (n = 7)	--	2.29±.17	2.68±.0138	2.11±.22	1.66±.19
Gamma, ANSP 327506	5.0 est. (n = 7)		1.91±.20	2.32±.12	1.76±.17	1.44±.14

[a]Lbw = length of body whorl; Lap = length of aperture; Wap = width of aperture.

[b]Academy of Natural Sciences of Philadelphia catalog number.

[c]Number of shells measured.

[d]Eroded, measurement useless.

[e]± = Standard deviation.

Data from two of these populations are given in Table 14. The shell apices were so badly eroded that shell lengths could not be measured. Mature shells were readily recognized because of the fully developed aperture with the pronounced parietal shelf and keel-like appearance of the edge of the expanded inner lip. Estimates of whorl number were derived from the one or two nearly entire shells.

As seen in Table 14, as well as Figures 7, 8, and 9, and from the data on laboratory-reared specimens, shell size of mature gamma race snails may vary greatly. This variation has been observed both within and between populations. As seen in Table 14, the length of body whorl and width of gamma race snails (ANSP 330925) equalled those of eroded beta race snails of the same estimated whorl size. Most populations of beta race snails examined, however, have been significantly smaller. The influence of sex on shell size in the beta and gamma races has yet to be determined.

The whorls are slightly convex and thus there is only a shallow suture. The base of the inner lip meets the base of the shell in a sharp keel. The translucent outer margin of the aperture is 0.25 to 0.30 mm wide; the area within the aperture is the usual chalky white. This race tends to have a less developed beak-like adapical projection of the aperture and a less developed parietal shelf than alpha race snails. The shells have no spiral microsculpture.

The Generic Status of "*Lithoglyphopsis*" *aperta*

The type-species of *Lithoglyphopsis* is *modesta* Gredler from mainland China. Thiele (1928) created the genus and assigned the type species. He illustrated the radula, in which the anterior edge of the central tooth has one large, smooth-edged triangular cusp; the lateral tooth has 3 or 4 large cusps, and the marginals have 4 to 6 cusps. This type of central tooth is seen in the Mekong hydrobiid genera *Wykoffia*, *Lacunopsis*, *Pachydrobiella*, and *Hydrorissoia*.

The shell of "*L.*" *aperta* Temcharoen (1971) somewhat resembles shells of Chinese "*Lithoglyphus*" and *Lithoglyphopsis*. Temcharoen (1971) illustrated a radula supposedly from "*L.*" *aperta* which appears similar to Thiele's drawing of *modesta*. However, the radula shown by Temcharoen is not that of *aperta* which we have thoroughly studied from the alpha and gamma races. It appears that Brandt made the radular illustration for Temcharoen and on the basis of shell and drawing determined that *aperta* belonged within the genus *Lithoglyphopsis* (Temcharoen, personal communication).

The radula of *aperta* has a central tooth with an anterior central cusp flanked by 4 or 5 minor cusps. The marginals have from 12 to 24 cusps. On the basis of radula, *aperta* does not belong in *Lithoglyphopsis*. The radula suggests a relationship with *Tricula*, not with *Pachydrobiella* or *Lithoglyphopsis*. In initial studies of *Pachydrobiella brevis* we noted that *brevis* has a female reproductive system structured in a way radically different from that of *aperta*. The radula of *brevis* is similar to that of *Lithoglyphopsis modesta*. This further supports the contention that *aperta* and *modesta* belong to different genera.

A new monospecific genus should not be created at this time for *aperta*. Too few data are available for the numerous species of the Mekong and Yangtze Rivers, which are in the genera listed in Table 15. Thus, *aperta* is temporarily left in "*Lithoglyphopsis*."

Subfamily Status of "*Lithoglyphopsis*" *aperta* (Gastropoda: Hydrobiidae)

Several authors, but most notably Thiele (1928, 1929) placed *Lithoglyphopsis, Lacunopsis, Pachydrobia, Pachydrobiella, Tricula, Paraprososthenia* and other allied genera in the tribe Lithoglypheae or subfamily Lithoglyphinae. The subfamily name "Lithoglyphi" based on the genus *Lithoglyphus*, was created by Troschel (1859), who included in it *Lithoglyphus, Assiminea,* and *Tomichia*. The type-species of *Lithoglyphus* Hartmann 1821 is *L. naticoides* (C. Pfeiffer) from Europe. The superficial similarity between the shells of *L. naticoides* and certain Asian taxa caused the latter to be included in the genus *Lithoglyphus*. Thiele (1928) considered that some of these Asian species assigned to *Lithoglyphus* deserved new generic status. Accordingly, as mentioned above, he erected the genus *Lithoglyphopsis*.

TABLE 15

HYDROBIID GENERA OF THE MEKONG AND YANGTSE RIVERS PLACED IN THE SUBFAMILY TRICULINAE

Delavaya[a]	Heude, 1889
Fenouilia[a]	Heude, 1889
Hubendickia	Brandt, 1968
Hydrorissoia	Bavay, 1895
Jullienia	Crosse and Fischer, 1876
Lacunopsis	Deshayes, 1876
Lithoglyphopsis	Thiele, 1928
Manningiella	Brandt, 1970
Pachydrobia	Crosse and Fischer, 1876
Pachydrobiella	Thiele, 1928
Paraprososthenia	Annandale, 1919
Parapyrgula[a]	Annandale and Prashad, 1919
Saduniella[a]	Brandt, 1970
Tricula	Benson, 1843
Wykoffia	Brandt, 1968

[a]Placement uncertain.

Detailed studies of the anatomy of *Tricula* and "*Lithoglyphopsis*" coupled with general observations of *Hubendickia, Hydrorissoia, Jullienia, Manningiella, Pachydrobia* and *Pachydrobiella* show that these genera belong to a phylogeny apart from *Lithoglyphus*. The anatomy of *Lithoglyphus naticoides* was published by Krull (1935) and Krause (1949). The similarities to some of the Asian genera here placed in the Triculinae (Table 15) are shell shape, the simple penis, three basal cusps on either side of the central tooth, and an elongate ospharadium.

There are pronounced differences in the Hydrobiinae and Pomatiopsinae: (1) The most important is seen in the female reproductive system, which has the ground plan of the Hydrobiinae where sperm traverse a ventral channel of the pallial oviduct to reach the bursa copulatrix, *i.e.*, there is no spermathecal duct as in the Triculinae. (2) Some Hydrobiinae and Pomatiopsinae have a pronounced gonopericardial duct not seen in any Triculinae studied to date. (3) In the nervous system of *L. naticoides* there is near fusion of the right pleural ganglion and the supraesophageal ganglion; in the Triculinae there is a pronounced pleuro-supraesophageal connective. (4) The pedal commisure of "*L.*" *aperta* is long while it is very reduced in *Lithoglyphus naticoides*. (5) The ciliated region of the ctenidial filament is 20 to 22% of the length of the gill filament in *L. naticoides*; it is 54 to 50% the length in "*L.*" *aperta*. (6) The pedal glands of *L. naticoides* are relatively small (Krause, 1949, fig. 3a) but comparatively huge in "*L.*" *aperta*. The esophagus of *L. naticoides* apparently has no pronounced dorsal folds. The pronounced left dorsal fold persists along the length of the esophagus in "*L.*" *aperta*.

The Triculinae to which "*L.*" *aperta* is assigned have the following traits:

(1) Sperm enters the female reproductive system via a conduit which opens at the posterior end of the mantle cavity. (2) The penis is "simple," *i.e.*, without appendages and with only one duct. (3) The snails are aquatic and progression is by ciliary glide. (4) The eyes are in slight swellings at the outer bases of the tentacles. (5) The prostate overlies the posterior end of the mantle cavity. (6) The basal cusps of the central tooth exceed one on either side. (7) Eggs are laid singly; all taxa thus far studied from the Mekong coat the egg capsule with minute sand grains. (8) The operculum is corneous and paucispiral.

Geographic Distribution and Habitats of *Lithoglyphopsis aperta*

Localities

Snails collected for this study came from five localities. Collections were made for the most part in March and April 1973.

1. Thailand, Ubon Ratchathani Province, 2.5 to 3.0 miles below Khemarat Town, Ban Khee Lek (= Ban Khi Lek); 16°2'30"N, 105°17'30"E. Mekong River.

2. As above, except several miles above Khemarat. Mekong River.

3. Thailand, Ubon Ratchathani Province, Ban Dan Village, isles at mouth of the Mun River; 15°19'15"N, 105°30'45"E. Mekong River.

4. Thailand, Ubon Ratchathani Province, Amphoe Phibun Mangsahan, last large island E of Phibun Mangsahan (= Ban Sai Mun); 15°14'45"N, 105°17'30"E. Mun River.

5. Laos, Sithandone Province, Khong Island; 14°7'30"N, 105°51'45"E. Mekong River.

Type Locality

The type locality of *"Lithoglyphopsis" aperta* Temcharoen (1971) is Ban Na on Khong Island, Laos. Temcharoen (1971) gave the distribution as being between Champassak three-quarters of the distance between Khong Island and Paksé and Sompamit Falls near Khone, Laos (at the Khmer border).

The holotype and paratypes by original designation are in the collections of the Senkenberg Institute, Frankfurt am Main, Germany. The holotype conforms to the alpha race snails discussed here. Part of the paratype series (SMRL 16282) is housed in the Academy of Natural Sciences of Philadelphia (ANSP 320061). Some of these paratypes correspond to the alpha race, some to the gamma race. The paratypes are badly worn. Temcharoen (1971) collected only shells.

Distribution

Collections to date show that "*L.*" *aperta* is distributed in the Mekong River from a point several miles upstream from Khemarat to the Khmer border, *i.e.*, Sompamit Falls near Khone. This is a distance of 154 direct miles, 200 river miles. It is suspected that the species lives as far south as Kratié, Cambodia, because schistosomiasis has been reported from this region and because the hydrobiid fauna extends at least to Kratié (Fig. 16). If this occurrence is verified, the species will be distributed over 253 direct miles, 300 river miles.

The alpha and gamma races are sympatric at localities 1 and 2 combined, 3 and 5. The beta race is known only from the Mun River at locality 4.

Habitats and Life Cycle

Rainy and dry seasons are pronounced in the Mekong basin. Heavy rains begin in early June plus or minus one or two weeks. The river rapidly rises 40 to 90 feet and becomes a raging torrent. With cessation of the rains in November to December, water levels decline. A marked decline is usual in late November. Lowest water is found in April (Fig. 17).

Young "*L.*" *aperta* are found in late March. Snails having 3.0 to 3.5 whorls and a shell 1.3 mm long have been found on 22 March. By late April the majority of the snails have 4.0 to 4.5 whorls and are about 1.9 mm long. A few reach 5.0 to 5.5 whorls and a length of 2.8 mm. Full maturity occurs in late May to early June, thus coinciding with the beginning of the rainy season.

At locality 1, Ban Khee Lek, rock outcroppings and islands cause the river to narrow. Gamma race snails were found on 14 April 1973 massed on sticks and rocks at depths of 0.5 to 1.5 m in moderate to swift current near the Thailand-shore side of islands at Ban Khee Lek. Later, on 5 June, the water had risen and the current was swifter. Alpha race snails were found underneath the rocks at about 1.5 to 2.0 m. Only one gamma race snail was found in this collection.

A pure population of gamma race snails was collected upstream from Khemarat on 5 June (locality 1). Snails were under rocks in about 2 m of water. They could only be collected by diving for rocks. The snails were only half-grown.

The channel running between the two principal islands at locality 3 was 1 m deep at low water. There was little current. Collections on 18 April 1973 yielded thousands of half-grown gamma race snails. These were massed on sticks, clam shells and rocks. A few alpha race snails were found with 3.5 to 5.5 whorls. Mud-coated egg capsules of "*L.*" *aperta* were fixed to the shells of some of the snails.

Both alpha and gamma races have been collected from Khong Island. The gamma race predominates along the eastern side of the island. The snails are found in shallow water on rocks, leaves and sticks which rest on the mud substrate. The water may be only 40 to 50 mm deep, with little or no current, and hot, *i.e.*, 26° to 27°C. This shallow environment is quickly changed with the onset of heavy rains. Then there is a strong current, and the snails are found beneath rocks. The habitats are totally inaccessible at the height of the rainy season.

Several collection sites are shown on Khong Island in Figure 12: (1) the shore line at the Lao Army Installation, (2) that section of Khong Town called Ban Xieng Wang, (3) Dooley Hospital, (4) Ban Na, the type-locality of "*L.*" *aperta*. "*L.*" *aperta* can be found at all four sites. Human infections with the Mekong schistosome appear to be derived from site 2 where people frequent the shallows to bathe, wash clothes, and defecate. The center of Khong Town is less than a quarter mile south of site 2.

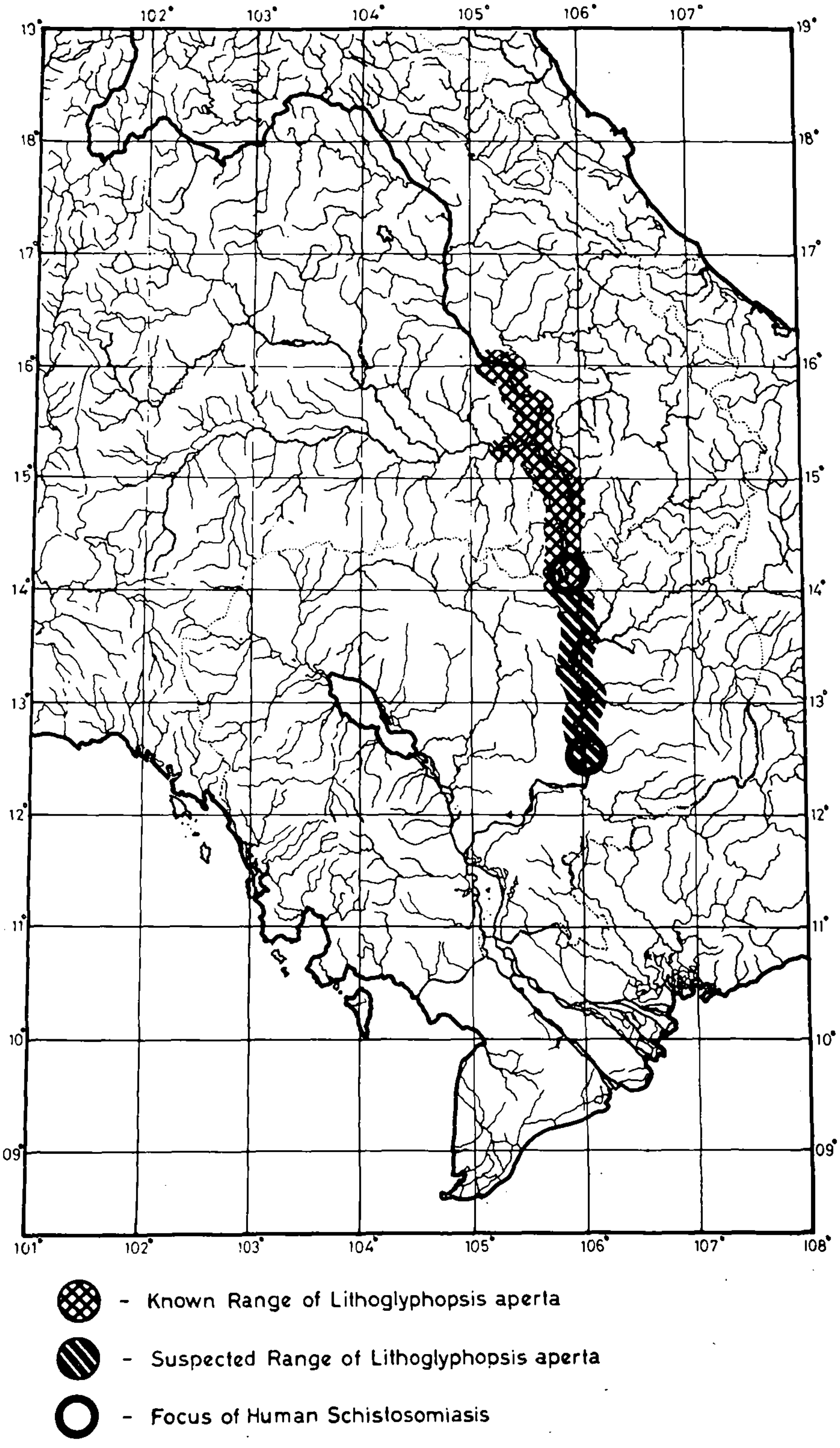


Fig. 16.--Known and suspected geographic range of *L. aperta* in the Lower Mekong River.

MEKONG-PAKSE
1967

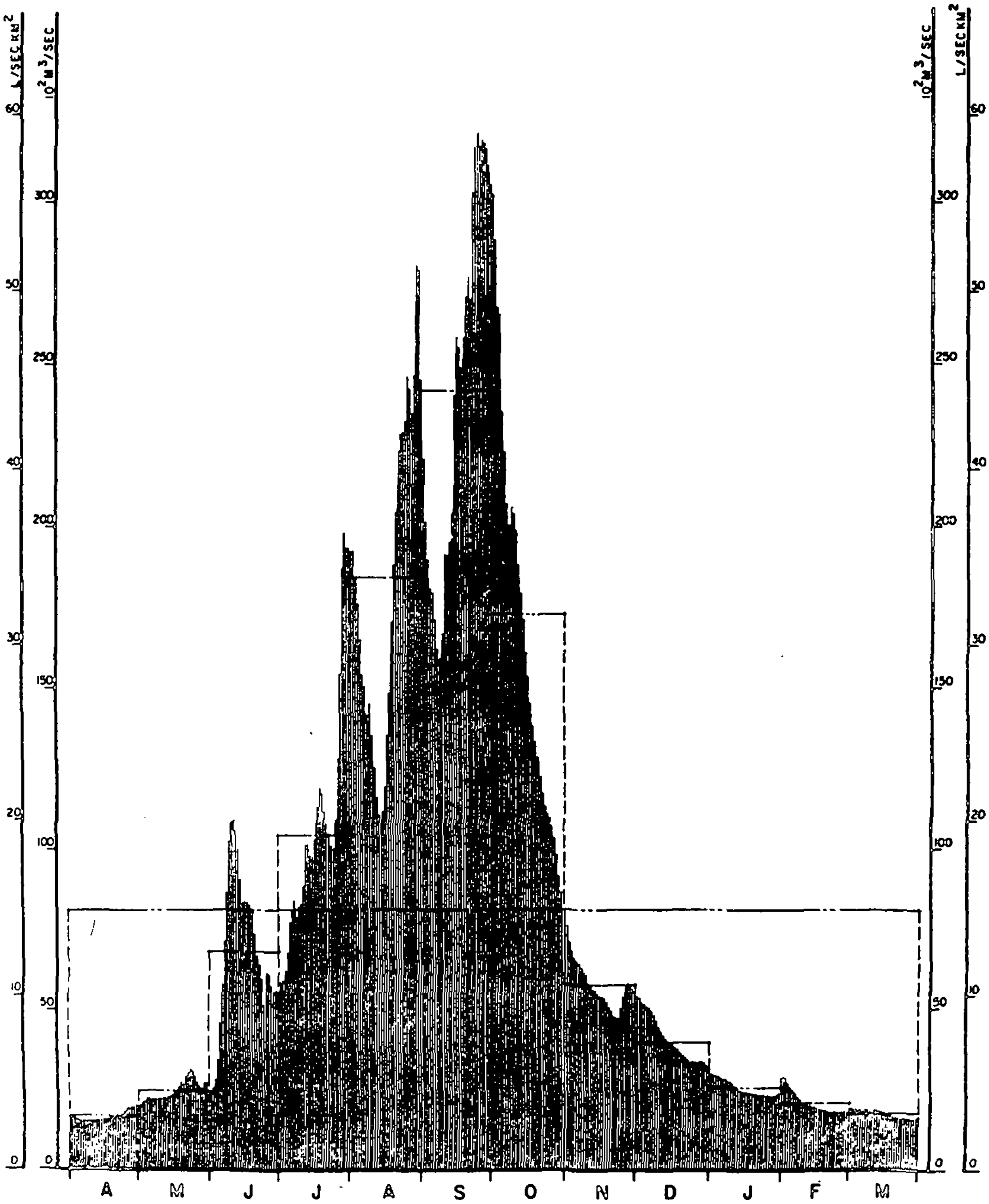


Fig. 17.--Typical water-level profile of the Mekong River at Paksé, 100 kilometers north of Khong Island.

5. COMPARISON OF THE PA MONG DAM SITE AND THE KHONE FALLS DAM SITE AS FOCI OF SCHISTOSOMIASIS

Biotic studies on schistosomiasis vectors have drawn attention to irregularities in the general richness and abundance of molluscan fauna in the Lower Mekong River.

The River

From Chiang Saen to Pak Tha the riverbed is dominated by granitic boulders and pebbles with fine and coarse sand collecting in discontinuous stretches. At periods of low water, much of the riverbed is exposed and supports heavy growths of grasses and sedges. Snails are difficult to find in the region, even at the period of lowest flow. In May 1973, when the water had reached its lowest level of the year, a few *Stenothyra hybocystoides* were located in the axils of sedges newly submerged by rising water but such snails (parasitologically uninteresting) could not be found in more exposed sites. A small number of an undescribed species resembling *Lithoglyphopsis* (not, however, *L. aperta*) were taken on stones at Chiang Khong, along with *S. hybocystoides* and a few *Jullienia munensis*.

From Pak Tha to Luang Prabang, the river flows east and is obstructed by a series of rapids. At Luang Prabang, the river turns in a southward direction. The stretch between Luang Prabang and the Pa Mong dam site flows between mountain ranges of diminishing size. Collections of mollusks were not made on this segment of the river.

The Pa Mong dam site is located at a narrowing of the Mekong River approximately 15 kilometers west of Vientiane. Collections were made in January 1970 and February 1971, and in May 1973 and 1974. The area proved to be relatively sterile with regard to mollusk fauna. The river bottom was dominated by dense, micaceous sand. Filamentous green algae were rare or absent. At the first narrows at Wat Hin Mahk Peng, outcroppings granite, limestone, and conglomerate rock were found extending in shelves into the river. Under water, their surface was covered with fine silt. These rocks represented part of the river bed.

At Wat Hin Mahk Peng, there were few snails. The most numerous were the ubiquitous *Stenothyra hybocystoides* and *S. basisculpta*. Less common and found clinging to the underside of rock shelves in areas of moving water were *Lacunopsis coronata*. A few *Clea helena* were recovered alive after persistent sifting of the silt and sand. Dead shells of the Asiatic clam, *Corbicula* sp., were sometimes found in the sand strainers. None of these mollusks was of biomedical interest.

Further west, a small rocky island lies athwart the Pa Mong dam site. It was densely covered with micaceous sand and rimmed with rheophytes. At low water it supported a heavy growth of grasses and sedges. Intensive sifting of the sand and investigation of submerged plant roots revealed only a few *Stenothyra hybocystoides*.

At the police camp at Ban Pha Thang, still further west (and beyond which point it was considered best not to travel) the river bottom was exposed at low water. In the shallows, the rocky shelves were overlaid with silt and the snails collected were identical with those found to the east at Wat Hin Mahk Peng.

From Vientiane to Savannakhet the river flows through open country over a sandy gravel bed. Many feeder streams and larger tributaries enter along this stretch. The sand is densely packed near shore and contains grains of mica which tends to become concentrated at the water's edge. Collectors have learned to associate the presence of mica grains with poor conditions for snails.

From Savannakhet south to Khone Falls the river changes its character drastically when viewed as a habitat for snails. This section of the Mekong contains many rapids, numerous divided channels and islands varying from small outcroppings of rock to inhabited islands several square kilometers in area. The narrows and rapids form good niches for mollusks. As the water level drops with the advent of the dry season, the emergence of many rocks and islets reduces the rate of flow in localized sections. At this time, shelves of rock in shallow water may support heavy growths of dense, filamentous green algae which, in turn, conceal substantial snail populations. Such foci are discontinuous but, where they occur, the number of snail species is apt to be relatively large. More than 80 species of the family Hydrobiidae alone have been reported variously from different segments of the Mekong between Khemarat and the Lao-Khmer border. During the present work, about half of the named species of Hydrobiidae were collected at one time or another in the Mekong, and 12 were found in the Mun River. Three species proved to be undescribed (see Appendix III).

It was common to find that one or two species of Hydrobiidae or Stenothyridae predominated in numbers at a given location and time. This predominance changed seasonally.

Water Chemistry

The Mekong River in the area around and above Vientiane is generally poor snail habitat, whereas the area around and below the confluence of the Mun River with the Mekong River is highly favorable habitat. The question arises whether the water in these two sections of the river differs chemically to an extent sufficient to explain the differences in snail fauna.

Some limited water chemistry data were taken to provide initial background information on the snail habitats (see Appendix IV). The resulting data were more indicative than comprehensive, and cover only a portion of the year at each of the two regions. However, the data were free of gross error and were internally inconsistent only to the degree expected considering the survey nature of the work.

Analysis of the Data at Hand

The monthly data on areas of favorable snail habitat (Mun and Mekong Rivers near the Mun confluence) (see Appendix IV) showed that the ionic content of these waters varied seasonally by a factor of approximately 2. During the period of low flow (dry season), evaporative concentration of the water was thus substantial. It is possible that seasonal changes in ionic content in the Pa Mong area were different, but seasonal data were not available.

During the period of low flow, the water of the Mekong around and above Vientiane was only about half as rich in ions as that of the favorable snail habitats at the Mun River confluence. This was largely due to the very high sodium content of the Mun River, which was also reported in the Mekong around and below its confluence with the Mun. The high sodium content of the Mun is explained by the presence of highly soluble salt deposits in the watershed. Presumably some of the salt also reaches the Mekong above the Mun by means of small feeder streams, and this explains the extension of high sodium concentrations somewhat north of the Mun-Mekong confluence.

It would appear that the Pa Mong and Mun Confluence habitats contain more than sufficient divalent cations and carbonate to support mollusk growth. Temperate-zone snails are found doing well in habitats far more extreme than those considered here (Harman and Berg, 1971).

The favorable Mun Confluence habitat differs from the Pa Mong site with respect to algal growth, which would suggest a difference in basic fertility that cannot be deduced from the data at hand. Algal growth, which is likely to be related to snail production, is probably limited by either nitrogen or phosphorus in these waters. It is conceivable that the favorable snail sites receive increased nitrogen or phosphorus input from runoff. Different rice culture practices in the Pa Mong and Mun Confluence areas might lead to differences in nitrogen input, since vigorous nitrogen fixation by blue-green algae occurs in rice fields.

The high pH values recorded in the favorable snail habitats were probably due to intense photosynthesis, which scrubs the water free of CO_2 and even of bicarbonate. This may result in a short-term CO_2 deficit during midday or afternoon, which, in turn, could account for high pH. Concomitantly, the presumed high photosynthesis should imply sources of nitrogen phosphorus, or both: these elements are ordinarily limiting factors in photosynthesis.

Conclusions

The density and diversity of snail populations in the Mekong River may be controlled by the direct influence of dissolved constituents on the populations or by indirect influence of water chemistry on snail food supply. At present the latter explanation seems most likely. (It is of course possible that the true explanation for the dearth of snails at the Pa Mong site and their seasonal abundance at and around the Mun Confluence is entirely hidden from the present perspective, but in view of the information discussed here this does not seem likely.)

6. THE SITUATION AT SAMBOR AND AT KRATIÉ, KHMER REPUBLIC

The snail, *Lithoglyphopsis aperta*, has not been reported south of Khone Falls, Laos. It is inferred that it exists as far south as Kratié (Fig. 16) because of the presence of human cases there. There appear to be suitable habitats for *L. aperta* in discontinuous foci among the islands north of Sambor. Field searches for this snail are indicated as soon as the situation permits, and emphasis in the search should be upon the entire stretch of water between Khone Falls and Kratié.

The pattern of transmission at Kratié, Khmer Republic, was studied by staff members of the Pasteur Institute, and the Calmette and Preah Ket Mealea Hospitals in Phnom Penh (Audebaud *et al.*, 1968; Jolly *et al.*, 1970a).

During the present study, a visit was made to Kratié during Phase I, just before the outbreak of hostilities. In the absence of further data, inferences must be drawn from the reports already published.

These indicate that transmission of schistosomiasis at Kratié is associated with the floating houses that line the shore. The houses are constructed on rafts made of large bamboo poles. They usually remain in place, however, rising and falling with the water-level of the river. Between each house and the shore there is a relatively small space of still water, protected from the river currents by the presence of the house. Children are apt to be found playing and swimming in these protected areas.

Each floating house is equipped with an enclosed toilet, usually located on the southwest corner of the house. Waste is discharged directly into the river.

Jolly *et al.* (1970a) made an examination of these houses and their riverine vicinity, searching for snails that could be transmitting schistosomiasis. They found specimens of the following:

Filopaludina speciosa Deshayes
Mekongia moreleti Deshayes
Pachydrobia pellucida Bavay (=*P. bavayi* Brandt)
Pachydrobia spinosa Poirier
Clea (Anentome) bocourti Brot (*C. helena* [Philippi])

Of these species, *Pachydrobia bavayi* was a prime suspect as transmitter. It was experimentally infected with miracidia derived from Lao patients with schistosomiasis (Brandt, 1970), but snails died before development of parasites was complete. The snails, which lived beneath the houses, were indeed in a good position to take miracidia out of circulation, but there is not yet any experimental proof that they contribute to the transmission cycle directly.

More likely, the tiny *Lithoglyphopsis aperta* will eventually be found clinging to the underside of the floating houses. If this proves to be the case, transmission at Kratié will differ substantially from that at Khong Island. Since the houses rise and fall with the river, the snails may not be subject to the annual high-water cycle as they are at Khong, and may have adapted to an uninterrupted reproduction cycle. Infected snails may therefore be present at most or all times of the year. This possibility will have to be determined by direct investigation. But if this hypothesis proves true, the implications for increased incidence of schistosomiasis following impoundment would be much weaker. In effect, transmission from floating rafts would presumably not be affected by a cessation in rise and fall of the water, although the results of any change in water chemistry cannot now be predicted.

It is noted that the focus of schistosomiasis at Kratié is well downstream of proposed major impoundments at Sambor, Stung Treng, or Khone Falls.

7. LABORATORY CULTIVATION OF *Lithoglyphopsis aperta* AND RELATED FORMS

Preliminary Investigations

After a transmitting snail (*L. aperta*) had been experimentally identified, attempts were begun in three different laboratories to establish parameters essential to the cultivation of this snail, a necessary prior condition for definitive laboratory studies. The laboratories involved in this work (with their abbreviations indicated in parentheses) were the Schistosomiasis Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok (Trop Med); the Mollusk Division, Museum of Zoology, University of Michigan, Ann Arbor (Ann Arbor); and the Department of Medical Zoology, Walter Reed Army Institute of Research, Washington, D.C. (WRAIR). Each of these laboratories has made various contributions to the work and their findings to date are included as a single unit in this report.

Field Observations

Initial attempts at cultivation were based on preliminary field studies of the Mekong River habitats where the snails were collected. A general ecological "profile" of the natural habitat, during the period March through June, when hydrobiid snail populations appeared to be at their peak, was as follows:

Water

The snails were entirely aquatic. They were taken most often in shallow inlets and marginal pockets along the shore where the water depth ranged from only a few to about 50 cm. They were also sometimes taken at greater depths, such as 2 to 3 m, but this was not typical. The water was subject to gentle mixing as a result of both air movements and direct continuity with stronger offshore currents.

The water appeared clear. Turbidity was 0 to 15 Jackson Units.

Water temperature varied according to depth but was variously measured at 27° to 33° Celsius (air temperature 25° to 35°).

Substrates

Snails were found crawling on submerged stones, boulders, rock outcrops, water-logged branches and twigs, glass bottles, and leaves. They were rarely collected on aquatic macrophytes and never on filamentous green algae (where other hydrobiids were sometimes found). A few were taken on soil (which proved to have high percentages of fine sand, clay, and silt). They were not found on the dense, micaceous sand which characterizes much of the Mekong River shoreline.

Aquatic Plants

The available aquatic plants included a sparse community of macrophytes and a varying but moderate community of algae, including species of Chlorophyta, Chrysophyta and Cyanophyta.

Shore Plants

The predominant riverine plants along this stretch of the Mekong River are shrubby euphorbeaceous rheophytes, *Homonoia* sp. During high water these shrubs are completely covered by water, but as the level drops, they begin to put out new leaves. Water-logged twigs and leaves of these shrubs were often found with snails crawling on them.

Water Chemistry

In April 1972 analysis of the water near the Ban Xieng Wang district of Khong Village gave the following values:

1. Total dissolved solids: 140 to 170 parts per million (ppm).

2. Conductivity (=EC x 10^{-6} @ 25°C): 240 to 250 ppm.

3. Hydrogen ion concentration (pH): 7.8 to 8.4.

4. Dissolved oxygen: 6.2 to 7.6 ppm. The percentage of saturation was from 78 to 103% (=supersaturation).

5. Alkalinity: 70 to 80 ppm $CaCO_3$.

6. Total hardness: 70 to 75 ppm $CaCO_3$.

7. Carbon dioxide: 4 to 10 ppm.

Egg Deposition Sites

Eggs apparently laid by hydrobiid snails were found in the river on rocks, twigs, and the shells of other snails. In the latter case it was assumed (perhaps unjustly) that such eggs had been laid by the same species as the one carrying them on its shell. To date, specific field identification of the eggs of *L. aperta* has been made only on rocks taken from the river near Khemarat; these eggs hatched in the laboratory (see below).

Laboratory Investigations

Based on these findings, investigations were initiated at Trop Med, Ann Arbor, and WRAIR aimed at establishing laboratory parameters for the artificial cultivation as well as maintenance of *L. aperta* and its relatives. It seemed logical to begin by trying to provide the snails with the elements that could be identified in their natural habitat in the Mekong River. Attention was paid to temperature, pH, day-night cycles, food preference, aquarium water levels, aeration and substrate.

The cultures were both static and dynamic.

Static Cultures

Static cultures as utilized at Trop Med included aquaria of several sizes (2.5-, 5-, 10-, and 13-liter tanks), filled with conditioned water (see below) to a depth of 18 cm, with or without bottom sand filters and various substrates (stones, leaves, wood), and maintained at a pH of 8.0 to 8.4, and a temperature of 26.0° to 28.0°C (Fig. 18). These (and all other cultures) received weekly additions of culture diatoms and unicellular green algae as food whether or not a "natural" algal growth existed or developed in them. "Conditioned" water consisted of tap water that had been allowed to stand in large (65-liter) earthenware or plastic containers for at least 72 hours prior to use, with a chalk supplement. The chalk used was an inexpensive, non-dust-free type which did not contain harmful metal salts as binders. It proved useful in keeping the pH high.

Clay bowls of various sizes were also suitable as holding vessels for short periods of time but snail mortality in them was 100% within a few months.

The aquaria employing bottom sand filters were found to be unsuitable over prolonged periods. The size of the tanks limited observation of mortality and snail activity. Also, after four or five months there was a heavy growth of aquatic fungus (Phycomycetes) despite the fact that the sand, rock, and water had been autoclaved prior to use. Finally, it was difficult to recover dead shells from among the sand grains. This type of aquarium was abandoned after seven months.

Similar tanks, containing wood or rock substrates, but without sand or filtration have proved to be more useful. One culture of this type, to which a potted plant of *Vallisneria* had been added, still contained live snails after 15 months.

Dynamic Cultures

Dynamic cultures were not successful. Two types were attempted. The first was a "continuous flow" apparatus using non-recirculated water, in 3.5-liter volumes to a depth of 8 cm. It was stocked with representative substrates (see above) and received continuous replacement of conditioned water at varying flow rates. This was intended to imitate the physicochemical characteristics of the natural river habitat. The pH ranged between 7.8 and 8.1 and the temperature varied from 27.0° to 29.0°C. This apparatus was unsuitable for long-term maintenance of *L. aperta* and was abandoned after seven months. It proved difficult to control pH. The tanks became plagued with ostrocods which could not be removed, given the complexity of the apparatus. Snail mortality was very high in this system.

A second "dynamic" culture was established in a 13-liter aquarium with conditioned water, equipped with an external water-recirculation unit operated by an aeration pump. This tank contained wood and rock substrates but no sand. Such a tank was found capable of sustaining *L. aperta* for about four months. Owing to a laboratory accident, it was abandoned before a final decision about its usefulness was made. It was not started up again because interest in "dynamic" cultures had taken on a lower priority than static cultures.

Laboratory Parameters

During a seven-month period the following variable laboratory parameters were established for maintaining *L. aperta* and some of its relatives:

1. *Aquarium type*. Glass aquaria without sand but containing conditioned water, rocks with rough surfaces, or pieces of wood taken from the Mekong River, proved most suitable. Static water systems were preferable to dynamic ones. Clay bowls were suitable for short periods, only a few months.

2. *Food*. Several types of food substance were offered to the snails and found by trial and error to be inappropriate. These included dried fish food, raw or boiled lettuce, finely ground powder including dry milk, wheat germ, fish food, and cerophyl, balanced rat chow pellets in alginate bars, dry and powdered lettuce, and various types of plants from the shore of the Mekong River at Khong Island. Cultures of diatoms containing more or less pure suspensions of the diatom, *Navicula*, as well as unidentified unicellular green algae were found to be preferred by *L. aperta*. This preference was substantiated by two additional findings: (1) *Navicula* frustules were seen in histological sections of snail intestines, using freshly collected snails; (2) *Navicula* frustules were also detected in fecal pellets of newly caught *L. aperta*.

Fig. 18.--Technician attending to the maintenance of static cultures of *L. aperta*.

3. *pH*. A hydrogen concentration of 7.0 or lower caused *L. aperta* to crawl out of the tank and die. With an established pH of 8.2 to 8.5, *L. aperta* remained healthy and apparently contented. The higher range resembled that recorded in the natural river habitat.

4. *Temperature*. When water temperature ranged between 23° and 26°C, *L. aperta* were inactive. At a range of 28° to 30°C, snails became very active. On occasions when the temperature of the water was allowed to rise to 33° or 34°C, snails began to leave the tank and some died.

5. *Aeration*. Aquaria containing relatively large quantities of water in relation to the size of the snail (such as the 13-liter size) appeared to need aeration. Without air, the tanks became cloudy and overgrown with water fungus. Moderate aeration was found to be best; strong aeration disturbed the snails, which often could not adhere to the substrate surface. In this connection, it is interesting to recall that in nature the snails are found in waters containing about 100% saturation with oxygen, but where the current is strong they tend to collect on the undersides of rocks where they are protected.

6. *Substrate*. *L. aperta* appear to require some type of solid substrate. In nature they were never found on sandy bottoms. The addition to aquaria of green leaves and pieces of wood which had not been soaked completely free of their natural acids pushed the pH of the water toward the acidic side (7.9 or lower) and were thus not suitable as substrates in enclosed waters. On the other hand, pieces of wood which had been soaked for a long time in the Mekong River and were waterlogged were found to be attractive for *L. aperta*. Rocks with irregularities and crevices (the best were pebble conglomerates collected at Khong Island) were also suitable.

7. *Conditioned water*. White blackboard chalk free of impurities appeared to aid in keeping the water alkaline. Water was "conditioned" by allowing tap water to sit in 65-liter plastic or earthenware jars for several weeks prior to use. Several pieces of chalk were added to each container and the water was aerated. The pH in these containers was checked twice weekly and the water was considered ready to use when the pH reached 8.2 to 8.4. Several pieces of chalk had to be added each week to achieve this balance. However, it was also necessary to exercise care in adding chalk to the aquaria themselves since too high concentrations were harmful to snails.

8. *Photoperiod*. A day-night cycle of 8 to 10 hours of light and 15 to 16 hours of darkness was used. Further investigation of this requirement is indicated.

Studies on the Life Cycle of *L. aperta* in the Laboratory

With the above parameters as guidelines, attempts to complete the life cycle of *L. aperta* in the laboratory were carried on for 17 months. The following account is derived primarily from reports of work done in Ann Arbor but includes some data from Trop Med and WRAIR.

Egg Laying

Three types of cultures were employed in Ann Arbor in an attempt to induce egg laying. (1) Shallow plastic trays (12" x 8½" x 2¼") appeared to give excellent results; the largest number of eggs were laid in them and they maintained the highest adult survival rate. The shallow depth allowed easy observation of all stages when the trays were scanned under a binocular microscope provided with a swing-arm. Young snails could be picked up without damage by using an extra-fine artist's brush. The volume of water was sufficient to maintain stable chemical conditions and also retard the growth of undesirable mats of algae (see below). (2) Glass dishes (3½" diameter by 2") were useful only for microscopic studies over short periods (not more than three weeks). A serious disadvantage was that diatoms and green algae grew too rapidly in such dishes, forming mats within a short time. These mats easily trapped young *L. aperta* and discouraged them from feeding. Another disadvantage was that the pH fell rapidly in these dishes. Such cultures had to be changed every two or three weeks. (3) Cylindrical battery jars (6" diameter and 8" tall) and glass aquaria (10" x 4½" x 8") were adequate for keeping adult *L. aperta* alive and for the production of eggs. But the weight of the walls (8") made picking young snails off with fine brushes impossible. It was necessary to recover these young snails by siphoning them through a rubber tube into a shallow tray. The cultures were bulky and cumbersome. The small *L. aperta* adults were hard to pick up with long forceps since the shell surface was slippery. Also, removing adults with forceps may have accounted for much of the mortality that was experienced by cracking shells inadvertently. Battery jars were easier to maintain than aquaria and the round shape provided a wider surface area for collecting snails.

Problems in Rearing Young
L. aperta to Maturity

a) Water temperature. Room temperature (23°-25°C) was found to be too cold for hatching eggs. When the largest number of eggs were laid by *L. aperta* in one tray kept at room temperature, many mature embryos were found dead inside their capsules. Increasing the temperature to 26°-30°C, on the other hand, produced an excellent hatch and more than 200 young were recovered in one month. Because the number of surviving adults was low, temperatures over 30°C were not attempted and, in consequence, the critical temperatures for this species are still unknown. But a range between 26° and 30°C was recommended by the Ann Arbor Laboratory.

At Trop Med, an interesting although accidental observation of hatching temperature was made. In March 1973, a stone bearing eggs of a hydrobiid snail was collected at Khemarat, Mekong River, and brought to Bangkok. Here it was placed in a holding tank containing *Pachydrobia spinosa*, *Hubendickia* sp. and two different species of *Lancunopsis*. This tank was then ignored for several months, during which time the water remained clear, the pH averaged 7.84, and the water temperature averaged 25.4°C. Mortality was high: of 906 snails placed in the tank in March, only 41 were found in October. But of these, 11 were mature *L. aperta* which had not been present among the original 906 snails. Since they had not been introduced, it was assumed that they had hatched and grown up under the conditions provided in the tank.

b) Food. Based on the finding that *L. aperta* is algophorous (see above), a number of cultures of Chrysophyta (diatoms) and Chlorophyta (green algae) were cultured and tested on the snails for suitability as food (Fig. 19).

All algal cultures were maintained in liquid medium in screw-capped test tubes or Erlenmeyer flasks (Ann Arbor), or, in addition, Petri dishes (Trop Med). In Ann Arbor, the medium was a basic soil-water solution prepared by autoclaving a mixture of 5 or 6 tablespoonfuls of dried mud from the river bank (or garden soil) with approximately 1300 ml water. At first, distilled water was used but tap water was later found to be as effective. After autoclaving, the flasks were placed in a cold chamber or refrigerator for at least two weeks until all of the fine particles had settled out. The supernatant was then siphoned into another flask (or culture dishes or test tubes) and re-autoclaved for 15 minutes. After cooling, algae were introduced, using sterile procedure. The culture dishes were placed under fluorescent lighting at room temperature and left alone until growth was evident.

Cyanophyta (blue-green algae) have also been found in the culture dishes, probably introduced with the snails. They appear to be harmful, particularly the filamentous types; the long trichomes grow over the snails, trapping them and preventing them from foraging.

Unsuccessful diatoms included a large benthic form, unidentified but similar in size and shape to *Suriella,* and a centric form, also benthic, also unidentified. Both types seemed too large for young snails to grasp. They were not adhesive to the glass surface and therefore were shoved in front of the snails as they attempted to feed.

Two algae which proved successful as food were *Navicula pelliculosa* and *Chlorococcum macrostigmatum.* Both were considerably smaller than the above two.

Young snails readily ate *N. pelliculosa* which grew adherent to the glass in very thin mats. It was necessary, however, to remove excess diatoms almost daily from older cultures. In excess, the diatom mats pulled away from the glass surface and balled up, making feeding impossible for the snails.

C. macrostigmatum showed growth habits similar to *Navicula.* The algae were small enough for the young snails to eat, but formed mats in older cultures. Snails refused to eat older preparations of *C. macrostigmatum.*

A collection of wild algae scraped from the shells of *L. aperta* was successfully cultivated but could not be identified. Divisions represented were Cyanophyta, Chlorophyta, and Cryptophyceae. The dominant forms were Chlorococcales, probably *Chlorococcum* sp., and a lunate unicellular form that resembles *Closteridium.* These mixtures were fed to the snails and proved satisfactory. At present, cultures of *Navicula, Chlorococcum,* and the native Mekong algae are being maintained in the Ann Arbor laboratory as snail food.

Fig. 19.--Technician inspecting pure cultures of diatoms intended as snail food.

Fig. 20.--Female *L. aperta* laying eggs on an air sparger in a static aquarium.

Life History of *L. aperta*

A complete life history of *L. aperta* cannot yet be described.

Adults were observed to lay eggs on clean spots on objects such as rocks, walls, and air spargers in the culture dishes (Fig. 20). The eggs were 0.5 mm long, naked, but sometimes covered with microscopic sand-like particles. Within the egg a one-celled embryo, bright yellow in color and about one-tenth the diameter of the egg, is suspended. It undergoes cell division until it can be recognized as a shelled infant snail. At this stage the yellow color disappears. The now colorless embryo is about half the size of the egg. In about a week, numerous black pigment spots appear in the mantle. At this time, the embryo approaches the size of the egg.

The egg hatches and the young snail emerges in 3 to 5 weeks. The newly hatched snail is 0.5 to 0.75 mm in length. The shell has 1½ to 2 whorls. Growth, when compared with *Oncomelania*, is slow. The whorls increased to 2 or 2½ at the age of 2 weeks, and 3 to 3½ at 8 weeks. At this time the shell length was about 1 mm.

Thus far, in Ann Arbor, only two snails survived for 21-22 weeks (see below). At WRAIR, young were successfully reared to the age of 13 weeks but then died. At Trop Med, *L. aperta* that had been collected as eggs on a rock hatched and grew to adulthood but time intervals or measurements were not recorded.

The sexes of *L. aperta* are separate. Sex usually cannot be differentiated in the young snails until they develop reflexed lips or varices on their shells. The varices have been used to indicate that such snails are mature, as in *Oncomelania*. Adult female *L. aperta* were between 2.9 and 3.7 mm in length. Adult males measured 2.7 to 3.3 mm.

One pair of snails was observed mating. A male climbed onto the right side of a female's shell and extended its verge in the female's cavity. The pair remained *in copula* for two hours, while the female browsed around the culture dish.

The time interval required for this species to go from egg to egg in nature remains unknown.

Algal Cultures

Algae were cultured by the method described by Liang (1974). Briefly, about 40 g of wet autoclaved mud was used to make a round mud mound in a Petri dish (10 x 2 cm), to which 60 ml of autoclaved glass-distilled water was added as overlay. *Nostoc muscorum,* a blue-green alga, was inoculated and the dish was incubated under a continuous fluorescent light at a temperature of 28° ± 1°C for two weeks.

Petri Dish Preparations for Culturing Snails

Two preparations were tried for culturing snails. In both, the pH of the water was between 7.4 and 7.8. After the introduction of snails, these preparations were maintained as described above.

1) Since *L. aperta* tend to dig into mud, only a small amount of the mud with algae growing on it (about 1 cc in volume) and 40 ml of the water taken from the algal culture dish were placed into a clean Petri dish (10 x 2 cm). This kind of preparation was used for parent snails. For newly hatched young snails, a smaller Petri dish (6 x 1.5 cm) was used. To this dish only 1/8 cc of mud and algae and 15 ml of water were added. In both cases the dishes were renewed weekly.

2) Most of the mud and algae were removed from an algal culture dish (10 x 2 cm), leaving only a trace of mud and algae on the bottom of the dish. This kind of preparation was used for accommodating both young and parent snails in the later part of snail cultivation.

Cultivation of Snails

Parent Snails

Ten half-grown parent snails (4 males and 6 females) were placed into the first kind of Petri dish preparation (size 10 x 2 cm) on May 17. The dish was renewed weekly. Snails began to lay eggs on August 9.

Eggs

These were laid on the wall and the bottom of the Petri dish. They were hemispherical, their flat sides being attached to either the wall or the bottom of the dish. Their diameters were between 0.37 and 0.43 mm. The surfaces of the eggs were coated with mud, but were not as thick as those observed in *Oncomelania* snails.

Incubation of Eggs

The Petri dish containing eggs was filled with about 40 ml of aerated tap water. The mud and algae left around the eggs and other open spaces of the dish were wiped off with the aid of a small cotton ball grasped by a pair of forceps. The muddy water was then replaced with fresh. The dish was then incubated under the conditions previously mentioned. Water was renewed weekly until eggs hatched. Usually this happened between 4 and 5 weeks after they were laid.

Young Snails

Altogether, 11 eggs hatched, 5 on September 25, 1 on October 4, 2 on October 8 and 3 on October 9. The body length of the newly hatched snails was about 0.24 mm. The whole appearance of the snails was very much the same as that of *Oncomelania* snail except that they were semi-transparent.

With the aid of a Pasteur capillary pipette and a dissecting microscope, snails were introduced into the first kind of Petri dish preparation (with dish size of 6 x 1.5 cm). Altogether 4 dishes were used to accommodate these 11 snails: snails 1 through 5 in dish 1; snail 6 in dish 2; snails 7 and 8 in dish 3; and snail 9 through 11 in dish 4. The dishes were again renewed weekly.

However, this method of cultivation appeared unsatisfactory for the growth of young snails as all but two (snails 5 and 6) died by December 6. Meanwhile, the cultivation of the parent snails was also not going well, only 2 snails (one male and one female) surviving then. The 2 young snails (snail 5 being 10-weeks old and snail 6 being 9-weeks old), together with the surviving parent snails were then placed in the second kind of Petri-dish preparation (with dish size of 10 x 2 cm). The dish was renewed weekly. Snail 5 reached maturity at the end of the 20th week and snail 6 at the end of the 17th week. Both were found to be female.

Table 16 indicates the survival time and body length of the 11 young snails. Figure 21 indicates the growth curves of snail 5 and snail 6.

TABLE 16

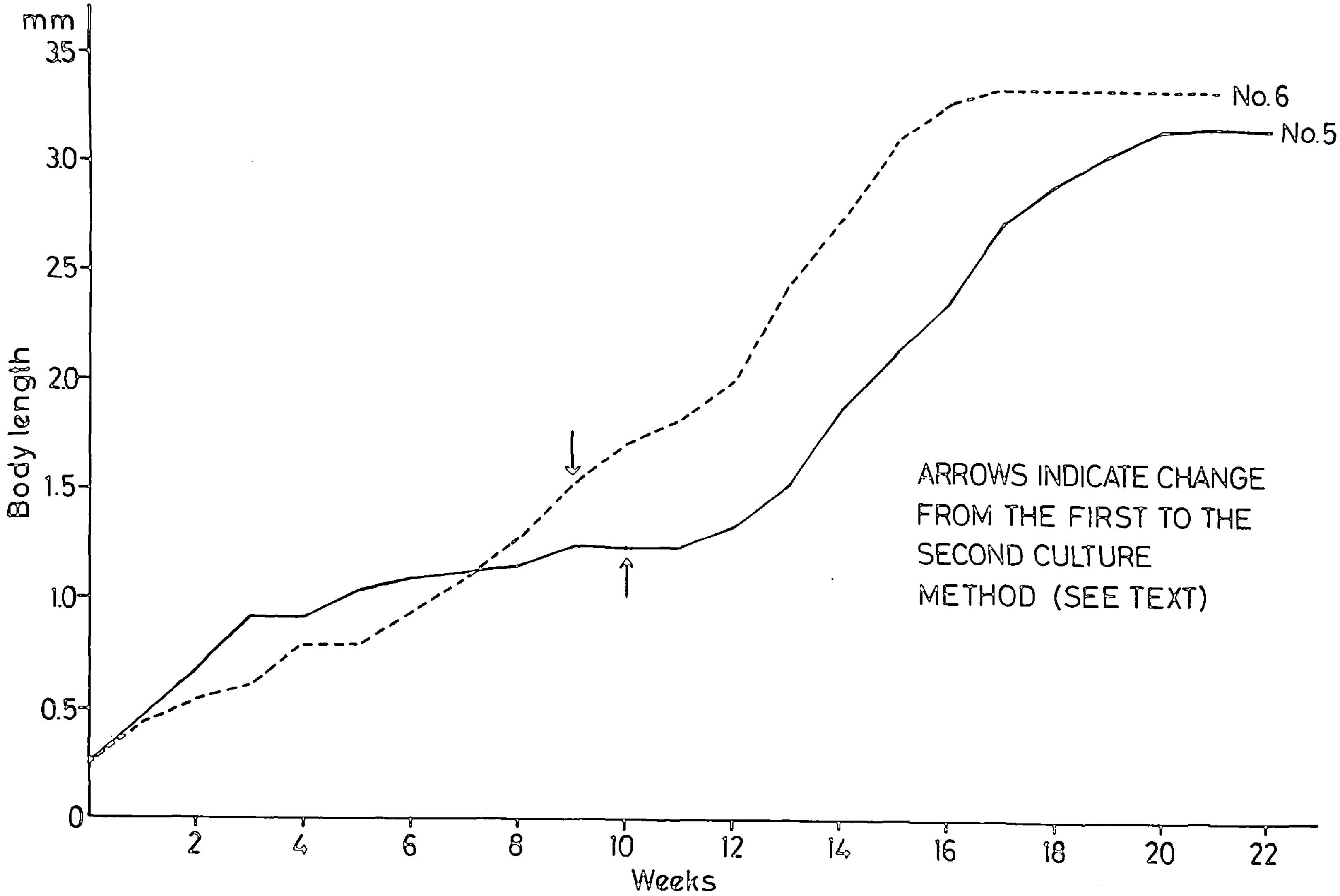
MEASUREMENTS OF THE BODY LENGTH OF *Lithoglyphopsis aperta* SNAILS MAINTAINED IN CULTURE (SEPTEMBER 25, 1973 - MARCH 8, 1974)

W[a]	Serial Number of Snails[b]										
	1	2	3	4	5	6	7	8	9	10	11
0	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
1	-	-	-	-	-	0.43	0.31	0.43	0.37	0.37	0.37
2	0.49	0.55	0.55	0.55	0.67	0.55	0.49	0.61	0.43	0.43	0.49
3	0.49	0.61	0.61	0.67	0.92	0.61	0.49	0.67	0.43	0.43	0.49
4	0.55	0.73	0.73	0.73	0.92	0.79	0.55	0.67	Died	Died	0.49
5	0.61	0.73	0.73	0.79	1.04	0.79	0.55	0.73			Died
6	0.61	0.79	0.79	0.79	1.10	-	0.67	1.04			
7	-	-	-	-	-	1.10	0.73	1.10			
8	Died	0.79	0.79	0.79	1.16	1.28	0.73	1.10			
9		0.79	0.79	0.79	1.22	1.53	Died	Died			
10		0.79	0.79	0.79	1.22	1.71[c]					
11		Died	Died	Died	1.22[c]	1.83					
12					1.34	2.14					
13					1.53	2.44					
14					1.89	2.75					
15					2.14	3.11					
16					2.38	3.29					
17					2.75	3.36					
18					2.93	3.36					
19					3.05	3.36					
20					3.17	3.36					
21					3.17	3.36					
22					3.17						

[a]W = Weeks.

[b]1-5 hatched on September 25; 6 hatched on October 4; 7-8 hatched on October 8; and 9-11 hatched on October 9.

[c]Snails were cultured with the second method.



Growth in body length of <u>Lithoglyphopsis aperta</u> (No. 5 and 6).

Fig. 21.--Growth in body length of *L. aperta*.

PART III

SCHISTOSOMIASIS: CONTROL OF MEKONG SCHISTOSOMIASIS

1. REVIEW OF THE LITERATURE

The voluminous literature on the control of schistosomiasis throughout the world has been summarized in certain outstanding publications.

In the field of schistosomiasis, one turns first to the bibliography of Warren and Newill (1967), which spans the period 1852 to 1962. Titles only are listed. No critiques are offered. The work has the minor disadvantage that all entries must be double-checked for accuracy and for original phrasing when this was not in English.

In 1964, a symposium on schistosomiasis was sponsored by the Fifth International Congress of the International Academy of Pathology, meeting in London. The proceedings, updated, were published in 1967 (Mostofi, 1967). They present a valuable source of modern information on the pathology of schistosomiasis, with additional chapters on epidemiology, ecology, and diagnosis. The references are voluminous. Asian schistosomiasis receives less attention than other forms.

A *Symposium on Schistosomiasis* was sponsored by the Tropical Disease Center, St. Clare's Hospital, New York, N.Y., and held on 27 May 1967. The papers presented were published originally in the *Bulletin of the New York Academy of Medicine* and then reprinted in *Clinical Tropical Medicine*, Volume 1 (Cahill, 1968). Although limited to a discussion of the American form of the disease, with the exception of the chapter on schistosomiasis as a world problem, the reference sections are thorough and the discussions useful in evaluating modern concepts.

The book of Jordan and Webbe, *Human Schistosomiasis* (1969), contains outstanding chapters on chemotherapy, epidemiology, and control of all three known species of human *Schistosoma*.

A report on *Engineering Measures for Control of Schistosomiasis* was prepared by McJunkin (1970) for the Office of Health, Bureau of Technical Assistance, Agency for International Development. This report was supported by a contract with AID. It discusses the design and operation of drainage systems and storage reservoirs, the effectiveness of sewage treatment and water treatment in control, and the place of various agricultural practices and naturalistic control methods.

In 1972, with the support of the Agency for International Development, a group of schistosomiasis experts was convened in New Orleans by the Tulane University Department of Tropical Medicine and Parasitology. They were concerned with the future of schistosomiasis control in relation to international development. The proceedings of these meetings have been published (Miller, 1972). They include working papers, discussions, workshop group reports, and reviews of major control programs throughout the world.

A monograph on *Snail Control in the Prevention of Bilharziasis* was published by the World Health Organization in 1965 (WHO, 1965). Recent technical reports of WHO Expert Committees on schistostomiasis in 1967 and 1973 have summarized advances in the development of specific schistosomicidal drugs, the status of molluscicide research, and current thinking on other control procedures (WHO, 1967, 1973). These three publications have been cited in the present report and proved particularly valuable in planning this work.

A recent, long treatise of 752 printed pages entitled *Epidemiology and Control of Schistosomiasis (Bilharziasis)* (Ansari, 1973) prepared under the auspices of the World Health Organization will probably stand for a long time as the definitive work on this subject. It contains factual material on the geographic distribution of schistosomiasis, guidance on the development of surveys and control programs, and discusses the place of chemotherapy, molluscicides, engineering of all kinds, and technical training in control. Stress is laid on gaps in present knowledge that need to be filled.

In the chapters that follow it will be apparent that the technology thus far developed to control American and African schistosomiasis (transmitted by pulmonate pond and canal snails) or "classical" Asian schistosomiasis (transmitted by amphibious snails) is not exactly suited to the situation that has been described in the Lower Mekong Basin. This epidemiological situation combines features of the transmission patterns of all other human schistosomes but is equivalent to none of them. New technology will have to be developed for the Mekong schistosome.

2. THE GOALS OF SCHISTOSOMIASIS CONTROL ON KHONG ISLAND

The following paragraphs are taken from a recent report of a WHO Expert Committee (1973):

> *The same aim for schistosomiasis control cannot be proposed for all countries. The objective of any programme will be dictated by the epidemiology and severity of the infection, the resources available for control, and the priority accorded to schistosomiasis in the relation to other prevalent diseases. For instance, a country might lack the resources to stop transmission in the endemic areas but be well able to reduce greatly the morbidity due to schistosomal infection.*
>
> *A most important aspect of control in the widest sense is the prevention of the spread of schistosomiasis to areas where it does not yet occur; in several countries this should have high priority in the schistosomiasis control programme. The effects of water resources development projects, particularly irrigation schemes and the construction of dams, are likely to increase schistosomiasis unless preventive measures are taken.*
>
> *Eradication of the infection may be attainable in certain very limited areas where the technical problems are not great and the resources are considerable. In most other situations a lesser goal is more realistic. Interruption--i.e., cessation--of transmission is another possibility that can be envisaged only in highly favourable circumstances. Reduction in the rate of transmission by a specified amount or some reduction in morbidity might also be chosen as a reasonable goal. The decision on a reasonable goal can only be made on the basis of technical considerations, the resources available, and the type of methods and techniques to be applied. In any case, progress must be measurable.*

The options for control of schistosomiasis on Khong Island at the present time appear to demand the "lesser goal," *i.e.*, a measurable change in some aspect of transmission, whether it be simply a reduction in prevalence, in worm burden, or, over the long term, a detectable reduction in incidence.

In any case, at the present time, there are limited facilities for measuring any changes in transmission patterns on Khong Island. The Thomas A. Dooley Foundation Hospital is not at present equipped or staffed to do this work, and no other health agency is available in the area.

However, as mentioned above, "progress must be measurable." Thus, in advance of control measures, there will have to exist a cadre of trained technicians capable of recognizing the eggs of the Mekong schistosome and of differentiating them from other parasites. Such technicians, ideally, will be drawn for future training from among the residents of Khong Island, since it is expected that they would function on a year-round basis for many years to come.

Only when such technicians have been trained and are working will it be possible to "measure progress" of any control program.

None of the methods that have been tried in the control of schistosomiasis throughout the world exactly fits the situation on Khong Island (see Appendix V). But they can be adapted. The options for control remain identical with those of schistosomiasis everywhere and are dictated by the life cycle of the parasite. They include drug treatment, health education, sanitation, and snail control.

An attempt to forecast the possible costs of an overall control program is given in Appendix VI. Costs relating to snail control studies, clinical studies, and training are considered. The cost of health education in endemic areas, under the charge of the Laos Ministry of Pubic Health, will be governed by decisions relating to national policy. Capital expenditures, such as may be associated with improved sanitation, are also included.

3. DRUG TREATMENT

As mentioned earlier, the Mekong schistosome will probably prove to be a species distinct from *Schistosoma japonicum*. Its response to drugs currently in use against known types of schistosomiasis has yet to be determined.

If the Mekong parasite proves to be similar to "classical" *Schistosoma japonicum*, there will still remain a need to assay its response to certain new drugs which have not yet been tested against known *S. japonicum*. Trivalent antimonials (antimony sodium tartrate and stibocaptate) and the nonantimonial niridazole have been used with some success against *S. japonicum*, but none of these can be considered for mass chemotherapy. Cure rates are not high and side effects can be severe (WHO, 1973).

The antimonial drugs require a lengthy treatment. They must be given intravenously (antimony sodium tartrate) or intramuscularly (stibocaptate); thus continuous medical supervision is essential.

Niridazole has certain advantages over the antimonials, but cure rates are not high with moderate doses, and increased doses are accompanied by central nervous system disturbances. Tolerance is said to be greater in children than in adults and greater in earlier stages than in the advanced hepatosplenic form of the disease. Nevertheless, it remains unsatisfactory in the chemotherapy of *S. japonicum* infection (WHO, 1973).

The development of novel schistosomicides has been urged by the World Health Organization but it has been noted that high development costs as well as "increasingly stringent standards of drug safety introduced by national agencies" may have tended to deter some pharmaceutical manufacturers from investing effort in this field (WHO, 1973).

It may be noted, however, that the role of certain known schistosomicides in *suppressive management* in small, well-disciplined populations should not be ignored. By this is meant giving the drug at weekly or monthly intervals in less-than-curative doses in order to improve tolerance. Such a regimen may reduce egg output and thus relieve the stress on the liver and internal organs, and in theory may affect transmission, since fewer eggs will be available to infect snails.

Such a program may prove to be most acceptable on Khong Island.

4. SANITATION AND HEALTH EDUCATION

It has been noted that man is the active participant in the life-cycle of the human schistosomes. The human-snail interrelationship depends primarily upon man since snails play an essentially passive role in transmission.

Although most control measures are aimed at the parasite (by means of schistosomicidal drugs) or the snails (by killing them or altering their environment so that they no longer thrive), it is clear that any change in human activity that reduces the opportunity for parasites to reach snails or people will have an effect on incidence. The first can be approached by improved sanitation, the second by health education.

Sanitation

Schistosomiasis is a fecally transmitted disease. In nature the life-cycle depends on the habit of promiscuous defecation by the final host. When the host is man, this aspect must be seen as part of the entire cultural milieu. On Khong Island, personal cleanliness is a way of life and people habitually bathe three times a day. Yet many of them lack privies and admit to using the forests and fields for the purpose of defecation. Many also use the river for this purpose, although fewer will admit to it. The habits of children, who represent the portion of the population most heavily infected, are thought to be almost uncontrollable.

It seems clear that people will not be able to alter the life styles imposed by centuries of custom unless they are offered visible and practical alternatives. Most of the adults on Khong would utilize latrine facilities if these were installed. Such facilities, however, may have to await the installation of a safe, piped water supply to the villagers of Khong. While this in itself may only indirectly affect the transmission of the disease, it is nevertheless bound to lead to major changes in sanitary habits. As life styles improve, sanitation becomes a way of life.

Health Education

The above statements imply that public knowledge of the menace of schistosomiasis exists and that methods of controlling it are understood. While health education should play a part in schistosomiasis control programs, the methods to be used will have to be adjusted to local cultural realities. On Khong Island, where villagers know of and are worried by the disease in its later stages, there is a general lack of understanding of its cause. The Lao are receptive to new ideas if they are presented in a suitable or familiar cultural context. While the role of religious leaders in disseminating information should not be ignored, other methods of installing new concepts exist and have proved useful in a variety of situations. Tentative approaches might include any or all of the following:

The Lao are accustomed to receiving education along with entertainment: one of the most popular is the *mohlam ku*, a man-woman team who sing, accompanied by a *khen*, or bamboo harmonica. The song consists of a type of witty and informative dialogue to which the people will listen for hours. *Mohlam ku* could be adapted to incorporate an educational message.

Educational films and/or film strips might be prepared for use in the schools, but the expense of preparing these and the need for a dependable electricity supply to project them must be balanced against the relatively small (less than 10,000 target population. Of more worth would probably be one or two centrally placed *photographic displays*. If the photographs were mounted in plastic they could serve a long time. Such displays would be particularly effective if the photographs included familiar scenes and faces.

Last, booklets might be prepared which describe the disease and its cause in graphic style. Such booklets might be distributed to the children in the intermediate schools.

In all educational efforts, care should be taken to avoid frightening the Khong population by presenting the disease as hopelessly uncontrollable. Rather, the story of transmission should be accompanied by the story of actual or proposed control procedures, and should stress the utility of general cooperation in effecting these. Health education should not be undertaken in advance of control measures but should follow close behind them.

5. SNAIL CONTROL

Snail control in the prevention of schistosomiasis is the subject of a still useful treatise published by the World Health Organization in 1965. Subsequently, WHO issued two technical reports (1967, 1973) which updated certain specific aspects of this subject, including reports on new drugs and molluscicides.

Molluscicides

MolluscRiciding constitutes a major part of most schistosomiasis control programs. It is not immediately evident, however, how snail-killing chemicals will prove useful in the Mekong River.

At the present time, the susceptibility of *Lithoglyphopsis aperta* to available molluscicides has not been determined.

In all cases the efficacy of the chemical to be used must be tested against the target snail under experimental conditions, as well as the amount needed, its toxicity to other life forms, its stability under varying natural conditions of light, turbidity and pH, and its handling properties.

Molluscicides may be generally non-toxic to man when properly handled but are nevertheless general biocides when released into the aquatic environment; because of the dilution factor in the Mekong River, chemicals may prove to be more environmentally acceptable there than in closed water systems, but with conventional methods of application the large dilution factor will also dictate the use of larger amounts of chemical and this may prove too costly on Khong Island, both in terms of money and of associated fish kills.

If molluscicides are to be used in the Mekong River, it is likely that novel methods of application will be required, possibly incorporating the chemical into slow-release rubber strips or rubber-base paints. This subject merits further research.

Engineering Methods

According to Buzo (1972), "engineering methods may be used in the control of schistosomiasis to (1) eliminate or reduce snail habitats, (2) prevent or reduce human contact with potential transmission sites, and (3) facilitate the treatment of water bodies with molluscicides."

The outstanding aspect of human schistosomiasis transmission in the Lower Mekong Basin appears to be its focal nature and the relatively small number of people currently at risk. Whereas in other countries of Asia, Africa and the Americas, schistosomiasis is geographically widespread and affects millions of people, only two important foci of transmission are known at present on the Mekong River, one at Khong Island and the other at Kratié. A third focus probably exists in Northeast Thailand on the Mun River but it has not yet been evaluated (Fig. 16).

In schistosomiasis, control measures must be tailored to meet the realities of the transmission pattern. These appear to be unique at the Khong Island site. The following discussion was based on the situation at Khong Island; transmission conditions at Kratié will probably require different control measures.

Elimination or Reduction of Snail Habitats

The snail, *Lithoglyphopsis aperta,* appears to be restricted in its ecological options. It inhabits shallow to moderately deep water which is warm, moving, has a high pH and high oxygen tension, and where the substrates (leaves, twigs, stones, bottles, *etc.*) support a rich growth of fixed diatoms. Such conditions are characteristic of certain discontinuous sites along the Mekong River from Khemarat to Kratié. At Khong Island, such a site coincides with a popular bathing area in Khong Town. The site is a small peninsula located in the Ban Xieng Wang section of Khong Town. It is a rocky promontory, covered with alluvial deposits during low water periods when it is above water, and supports a heavy growth of rheophytic shrubs (Fig. 15). Besides attracting bathers, the dense cover makes the area popular as a defecation site. The water adjacent to this peninsula has in the past yielded larger numbers of *Lithoglyphopsis aperta* snails than any other site visited on Khong Island.

Most cases of schistosomiasis on Khong Island can be traced to this site.

Deepening and straightening of water margins and removal of vegetation during low water have been proposed as control measures where shorelines of large man-made lakes constitute transmission sites (McJunkin, 1970). During low-water periods, when flow at Khong Town is very slow, the river shore may be likened to that of a very large lake.

It is impractical, however, to attempt destruction of all snail sites at Khong Island; they are too numerous. Moreover most of them are not defecation sites or bathing places for large numbers of people.

Reduction of Human Contact with the Transmission Site

The cultural and sociological aspects of this measure are discussed in Part I, Chapter 4.

The fact of contamination of the Ban Xieng Wang peninsula with human feces has been confirmed by direct observation. The extent to which run-off from drains or discharges from such latrines or septic tanks as may exist along the edge of Khong Town may contribute to the fecal pollution of the river has not been studied and it is not known that these sources contribute to pollution. In this regard, the services of a sanitary engineer conversant with schistosomiasis control will be required at an early stage in any effort to halt transmission on Khong Island.

It is impractical to attempt to prevent the people of Khong from bathing in the river. An alternative might be to offer access to uncontaminated sections of the shore (see below) or even to offshore areas of the river (by the construction of piers) where cercariae should be less numerous (McJunkin, 1970).

In line with the idea of limiting access to the transmission site, the construction of a stone-and-concrete embankment the length of Khong Town and having wide steps leading down to safe bathing areas might do much to reduce transmission. Such stairway embankments are seen at Luang Prabang where they give access to the water from riverside *wats*. At Khong Town, one embankment has already been constructed near the site of the ferry-crossing; it was built in connection with the support for an open-air pavilion at the water's edge which serves as a community meeting place.

A complicating factor in this proposal may be the presen of a number of houses which overhang the river bank in the Bai Xieng Wang section close to the transmission site (Fig. 6); embankment construction might damage the supporting structures of these houses.

Facilitation of Molluscicide Operations

The problem of using available molluscicides in conventional ways against hydrobiid snails on Khong Island is discussed above. Engineering methods will probably be ineffective in aiding such an activity on Khong Island because of the large dilution factor.

Biological Control

Authorities are conservative in their support of biocontrol of snails as a way of preventing schistosomiasis at the present time. There is a general lack of field testing, and thus practical evaluation, of the many incidental observations, casual notes and unconfirmed studies on this subject that have been published.

On the other hand, biocontrol of snails offers, in theory, distinct advantages over other methods. A successful biocontrol scheme would be economically attractive since it would reduce the need for highly specialized equipment, supplies, and personnel.

To date, biocontrol schemes that have received or deserve field trials are few and have all been directed against pulmonate snails.

Marisa

The most thoroughly discussed agent of snail control has thus far been another snail, *Marisa cornuarietis*, a large ampullariid native to northern South America. When introduced into Puerto Rico, this snail has been shown to prey upon and also compete with the transmitter of human schistosomiasis, *Biomphalaria glabrata*. There is evidence that this snail was associated with disappearance of schistosomiasis on the island of Vieques (Ferguson, 1972). However, its habits make it dangerous so that introducing *Marisa* snails into Southeast Asia for any reason probably would be unwise. Although it is hardy and might thrive and compete with disease-bearing snails, *Marisa* is also known to attack seedling rice.

Furthermore, *Marisa* snails are pond-dwellers and thus might not exert any measurable stress on riverine hydrobiid snails in the Mekong River.

Predation

Casual observations of predation on snails by fish, turtles, and birds indicate that such predators would be ineffective for purposes of practical application. Predation by ducks, however, apparently is effective in controlling pulmonate snails in ponds and rice fields in Northeast Thailand. The pulmonate snail, *Indoplanorbis exustus*, which transmits the buffalo schistosome, can rarely be found in significant numbers in ponds visited regularly by domestic ducks. The buffalo schistosome is a main cause of cercarial dermatitis, or paddy-field itch, in Southeast Asia.

Trematode Antagonism

This term means predation by a dominant trematode upon a subordinant species during their larval existence within a snail host. Experimental and field trials on these interactions have been carried out over a period of years by the staff of the Institute for Medical Research, Kuala Lumpur, Malaysia (Lie *et al.*, 1968). Antagonism between species may be direct or indirect, but control may be achieved when antagonism is strong and development of parasites within the snails is rapid. Environmental factors, such as low temperatures or muddy water, which slow or prevent development reduce the effectiveness of this method. During the course of the present studies, a small-scale field experiment was conducted in Khon Kaen Province, Northeast Thailand, to control naturally occurring *Schistosoma spindale* infections in the snail *Indoplanorbis exustus* by dispersing eggs of *Echinostoma malayanum* into the ponds. Complete control of the target parasites was achieved in clear-water ponds, but only after an excessively prolonged period; in ponds with turbid water, echinostome infections did not become established. More field trials of this type should be done in order to familiarize researchers attempting this method of biocontrol with the various complicating factors in nature (Lie *et al.*, 1974a, b).

It must be stressed that as yet there is no basis for proposing trematode antagonism in the control of human schistosomiasis in the Mekong River. However, this possibility should not be ignored in future research.

Sciomyzid Flies

The aquatic larvae of the marsh flies, family Sciomyzidae, are entirely malacophagous. Their use in the biocontrol of snails has been proposed (Ferguson, 1972). Theoretically, the introduction into a foreign environment of very large numbers of marsh flies might result in a significant reduction in snail population. However, there is evidence that one of the most widespread species in Thailand (*Sepedon plumbella*) prefers the flesh of pulmonate snails (Bhuangprakone and Areekul, 1973) to other fresh-water types. Since the snails which transmit buffalo and duck schistosomes are pulmonates, sciomyzid flies might eventually be useful in Southeast Asia in control of paddy-field itch due to the cercariae of these parasites. Their ecological requirements--*i.e.*, preference for ponds and marshes--suggest that they will never be useful in controlling *Lithoglyphopsis aperta* in the Mekong River.

Poisonous Plants

Certain trees and shrubs in Africa have been found to discourage snail populations if planted near transmission sites (Wright, 1968). Such plants have been found to release snail poisons from their leaves and fruits. Although imperfectly evaluated as yet by adequate field trials, the use of such plants appears promising in snail control and should be investigated in Southeast Asia.

Microsporida

Of all theoretical (*i.e.*, untested) biocontrol methods, the most intriguing is the possible use of microsporidan hyperparasites against specific trematode larvae in snails. The Microsporida are protozoan parasites of a variety of invertebrates, including trematodes, insects, and snails. The discovery that microsporidans occurring in nature could interfere with a field trial of trematode antagonism to control *Schistosoma spindale* was reported by Lie *et al.* (1970). The mode of infection of these spore-forming organisms is unknown, but snails presumably pick up the spores by ingesting them. The incorporation of such spores in snail baits might prove an inexpensive and environmentally attractive alternative to molluscicicing in order to control the transmission of schistosomiasis. However, much basic research would be required to discover species of Microsporida that would attack specific target trematodes, to test methods of raising spores in large numbers, to develop suitably inexpensive methods of release, and to evaluate results of such a method by field testing. It must be reiterated that this method of biocontrol of trematodes (not necessarily of the snail hosts) remains entirely hypothetical.

In summary, although biological control appears to be an attractive alternative to molluscicicing, and possibly to engineering approaches, the subject is in its infancy and cannot be suggested as a possibility in the Lower Mekong Basin at present. Nevertheless, basic research into biocontrol methods and field testing of likely methods deserve continued support.

PART IV

OTHER SNAIL-BORNE DISEASES OF THE LOWER MEKONG BASIN

1. OPISTHORCHIASIS

Schistosomiasis is not the only helminth disease which may undergo a change of incidence as a result of water resources development in the Lower Mekong Basin. Other snail-borne helminthiases which merit attention include opisthorchiasis, paragonimiasis, echinostomiasis, angiostrongyliasis (one cause of eosinophilic meningitis) and fascioliasis. Another common helminth disease of humans, but one which is not associated with snail transmission although fresh-water fish figure in the epidemiology, is gnathostomiasis.

No research was done on these parasites during the present study. The following accounts, including considerations of life-cycle, pathogenicity and treatment, distribution and prevalence in the riparian countries, public health importance, and major research needs, were drawn from the available scientific literature.

Life Cycle of the Parasite Causing Opisthorchiasis

In Thailand and Laos, the parasite causing opisthorchiasis is *Opisthorchis viverrini*. It is closely allied with the "classical" Oriental liver fluke, *Clonorchis sinensis* of China, Vietnam, and Japan. The life cycle of *O. viverrini* has been described by Wykoff *et al.* (1965). Eggs are passed in the feces and are eaten by a snail, *Bithynia goniomphalus*, a species found in northeast Thailand. (The species incriminated in the north of Thailand, *B. funiculata*, and in Bangkok, *B. laevis*, may be synonyms of *B. goniomphalus* and represent simply different ecological types [Harinasuta, 1969].) Eggs of *O. viverrini* hatch within the snail, releasing miracidia which metamorphose into sporocysts. These in turn produce a number of rediae which migrate within the snail to the interhepatic lymph spaces. Within each redia several cercariae develop which eventually break out of the parent redia upon maturation, penetrate the tissues of the snail and escape into the surrounding water. The cercariae are able to swim freely. They seek out certain fish as secondary intermediate hosts, penetrate into the flesh beneath the scales and encyst as metacercariae.

In northeast Thailand, the secondary intermediate hosts of *O. viverrini* are cyprinoid fishes (carp minnows or barbs). Wykoff *et al.* (1965) found the highest prevalence rates in *Hampala dispar* (74%), *Puntius orphoides* (65%) and *Cyclocheilichthys siaja* (51%). In addition, six other cyprinoids were incriminated but with much lower infection rates. Of the positive fish, the species containing the largest number of infective larvae was *P. orphoides* (79%).

When the host fish is eaten raw the infective cysts are digested in the stomach and duodenum and the released larvae migrate up the common bile duct into the distal biliary capillaries where they mature.

In nature, *O. viverrini* was originally described as a parasite of the Fishing Cat, *Felis viverrina*, a wild feline that frequents the vicinity of streams where it subsists by eating fish. Today, given the extraordinary prevalence rates detected in humans in Thailand and Laos, particularly in areas associated with tributary stream impoundment, it might be more exact to speak of *O. viverrini* as a human parasite that may sometimes infect the Fishing Cat.

C. sinensis, in North Vietnam, is also a natural parasite of fish-eating mammals.

In northeast Thailand, the transmitting snails (*Bithynia goniomphalus*) occur in small numbers in streams and rivers and in very large numbers in ponds and lakes. Heavy transmission of parasites from humans to snails is apt to be seasonal: affected persons have the habit of promiscuous defecation and during the dry season, the eggs in the feces tend to become desiccated and die. In the rainy season, however, pollution of bodies of water with feces bearing parasite eggs takes place, as feces are flushed by rain action into the streams and ponds. Thus, it has been during the rainy season that infected snails, and consequently infected fish, are present and the risk of human infection is greatest (Harinasuta, 1969).

Pathogenicity and Treatment

The pathological aspects of clonorchiasis and opisthorchiasis are probably similar. The worms are found in the distal bile passages where they cause proliferation of the biliary epithelium, progressive dilatation and thickening of the wall of the ducts, and chronic liver damage. Significant symptoms are displayed only by persons who harbor large numbers of worms: such symptoms may include fever, epigastric pain, tenderness over the liver. Accompanying signs may be eosinophilia, jaundice and progressive hepatomegaly (Hunter *et al.*, 1961).

In cases of infection with extremely large numbers of *O. viverrini*, death may occur (Harinasuta and Vajrasthira, 1960). It has been speculated that large numbers of worms may also be associated with the development of liver cancer (Harinasuta, 1969).

Light infections with *O. viverrini*, on the other hand, apparently do not result in symptoms or changes which can be attributed to this parasite alone. Wykoff *et al.* (1966) examined 921 persons harboring the parasite in northeast Thailand, as well as 191 persons free of infection: signs and symptoms such as edema, anemia, diarrhea, dysentery, nausea, flatulence, malaise, loss of appetite, and enlargement of the liver occurred no more frequently in the infected than in the non-infected groups. These workers also could find no significant differences in blood counts or blood chemistry in these subjects. However, the relative worm burdens were not ascertained in their study, and were thus not correlated with the symptoms. It is possible that their subjects had few worms.

There is at present no drug of choice in the treatment of clonorchiasis or opisthorchiasis. Hetol (1,4-bis-trichloromethylbenzol) has been tried and reported to be promising but the manufacturers have ceased production because this drug produced a hypochromic anemia in dogs during chronic toxicity studies.

Dehydroemetine late-release tablets have proven effective and well tolerated.

Bilevon (Bayer 9015, menichlorpholan), which was originally developed for the treatment of fascioliasis in cattle, has been tested with promising results against *C. sinensis* in Korea and may prove equally effective against *O. viverrini* infections in Laos and Thailand (Rim, 1972).

Distribution and Prevalence of Liver Fluke in the Lower Mekong Basin

Liver flukes of man reported from the riparian countries include *Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrini*. These infections are commonly detected by stool examination: eggs of *C. sinensis* closely resemble those of *O. viverrini* and cannot be easily distinguished under the microscope, whereas eggs of *O. felineus* differ from the other two species by their narrower and more tapering shape (Belding, 1952). In Vietnam, *C. sinensis* and *O. felineus* have been reported from man. In Thailand and Laos, only *C. viverrini* has been reported without question. The pathobiology of these worms in man is apparently similar (Belding, 1952).

Thailand

Prommas (1927) reported *O. felineus from an autopsy case* in northern Thailand but this is now considered to have been *O. viverrini*. According to Sadun (1955) the only agent of liver fluke disease is *O. viverrini*; he estimated that there are as many as 2,000,000 cases in northeast Thailand alone.

The literature has been reviewed by Harinasuta (1969).

Wykoff *et al.* (1966) estimated that more than 3,500,000 people in northeast Thailand harbor this parasite: this would represent more than a third of the entire population (9-10 million).

The prevalence is particularly high in this area, which is inhabited by ethnic Lao, because of the habit of eating raw fish in the form of *koi pla* (raw fish chopped with garlic, lemon juice, chili-pepper, rice and vegetables). According to Harinasuta (1969), this is the most popular of fish foods in the area and people wean their babies with it; indeed, the worm has been detected in infants of three months in certain localities.

Laos

Opisthorchiasis has been reported from Vientiane (15%) and Thakek (23%) (Bédier and Chesneau, 1929). In 1970, the infection was reported from Khong Island (Kitikoon, 1970). Further investigations in Laos probably will produce evidence of even higher prevalence rates since the consumption of raw fish is a common habit of the Lao people.

Khmer Republic

There are no reports of the presence or prevalence of opisthorchiasis or clonorchiasis in the Khmer Republic. The Khmer people do not have the habit of eating uncooked food, and prevalence, if extant, may prove to be limited to exogenous ethnic groups.

Vietnam

Both *C. sinensis* and *O. felineus* have been found in humans in Vietnam (Do Thi Nhuan and Vu Qui Dai, 1969). Verdun and Bruyant (1908) found the cat fluke, *O. felineus*, in humans living in the delta of the Red River.

The following results were recorded from fecal samples submitted to the Pasteur Institute of Saigon for the years 1948 through 1964: of 34,911 samples, 147 (0.04%) contained eggs of *C. sinensis* and 66 (0.02%) contained eggs of *O. felineus* (Do Thi Nhuan and Vu Qui Dai, 1969). It has been noted that the delta of North Vietnam has been highly endemic for liver fluke because of the North Vietnamese habit of eating raw fish, particularly cyprinoids such as *Cyprinus carpio* and *Carassius auratus*.

Public Health Importance

It is difficult to assess the public health importance of liver fluke among people with light infections, as signs and symptoms attributable to the infection are lacking. In cases of heavy infection, the impact of the disease may be strong, involving cirrhosis, cancer, or even death. Heavy infection occurs in areas where raw fish is consumed as a matter of habit. Such areas include North Vietnam and in the Lower Mekong Basin, northeast Thailand and Laos.

The pathobiological role of liver flukes cannot, however, be distinguished from that of other, concomitant worm infections in the areas under discussion.

Research Needs

1. The prime need in liver fluke disease is a study of the pathobiology of the disease in correlation with worm burden and in the absence of other, complicating worm infections. Such a study is not easy to conceive, given the heavy worm burdens of the rural population in the Lower Mekong Basin. Nevertheless, a study of populations under heaviest risk, particularly in northeast Thailand where dam construction has increased the transmission potential of the parasite, could be attempted if sufficient time could be devoted to it.

2. Field trials of promising drugs are needed. Since certain novel drugs are available that seem to show promise (*i.e.*, Bilevon), emphasis should be placed on these.

3. A sociological or anthropological study of the attitudes and practices of the population at risk toward current sanitary practices should be undertaken with a view toward development of new knowledge and changing life styles.

2. PARAGONIMIASIS

Life Cycle of the Parasite

Species of *Paragonimus* are widespread in mountainous areas of Asia from Siberia to New Guinea. In the Lower Mekong Basin, two species have been reported from man, *P. westermani* and *P. heterotremus*. With the exception of specific identification of the intermediate hosts, the following description of the life cycle is taken from that of *P. westermani*. There is reason to believe that *P. heterotremus* is the more common parasite in the Mekong watershed, but there is no reason to believe that the basic elements of the life cycle differ between the species.

The undeveloped eggs are expectorated or are passed in the feces. If conditions are satisfactory they hatch in approximately three weeks, releasing a miracidium capable of finding and penetrating a suitable snail host.

Within the snail, successive larval stages termed sporocyst, redia, and cercaria are formed. The cercariae leave the snail and crawl on the bottom until they are able to penetrate a suitable crustacean host, where they encyst as metacercariae. The final, vertebrate host acquires the infection by eating the crustacean raw. The metacercariae excyst in the duodenum. The released larval trematode passes actively across the wall of the intestine and the diaphragm and comes to lie in the pleural cavity of the lungs (parenchyma or bronchioles). In the lungs the worms become enclosed in cystic capsules and grow to maturity. Eggs are laid within the cysts and are released when the cysts break. They are then passed up with the sputum in order to again reach the outside environment (Belding, 1952).

Pathogenicity and Treatment

The most characteristic pathological changes are found in the lungs (although migrating worms can also encyst in the intestinal mucosa where they may produce inflammation and ulceration). In the lungs, the parasites stimulate a host reaction which consists of inflammation, necrosis of surrounding tissue, and fibrous encapsulation. Although located in the parenchymal tissue, many of the cysts are in contact with radicles of the bronchial tree.

The cysts contain a reddish-brown exudate ("anchovy sauce necrosis") which eventually comes to include viable eggs as well as dead tissue. When the cysts break, they release the dark fluid and the eggs into the bronchioles, where the symptoms of bronchitis and a productive cough develop. The sputum becomes brownish or reddish, the so-called "endemic hemoptysis," and there may be additional symptoms of pain or lack of breath. Bronchopneumonia may ensue. With heavy infections there may be a lung abscess.

The chronic disease with small numbers of worms is seldom fatal but the prognosis in heavy infections is grave (Hunter *et al.*, 1961).

Bithionol (2,2'-thio-bis-4,6-dichlorophenol) is a promising therapeutic agent with a high efficacy and lower toxicity than emetine hydrochloride (which was previously used). At the present time, Bithionol is considered the drug of choice in paragonimiasis and, in Asia, has been used extensively since 1962 (Rim, 1972).

Distribution and Prevalence in the Lower Mekong Basin

Paragonimiasis in Asia is reported from Japan, Korea, Taiwan, Philippines, Thailand, and Laos. In Malaysia, the worms have been found in members of the cat family but not in man.

Thailand

Six species of *Paragonimus* have been reported from Thailand. They are *P. bangkokensis*, *P. harinasutai*, *P. heterotremus*, *P. macrorchis*, *P. siamensis*, and *P. westermani*. All are natural parasites of cats, dogs, or rodents. Only two of these species, *P. heterotremus* and *P. westermani*, are known to be able to mature in man. The subject has been reviewed by Vajrasthira (1969).

To date, *P. westermani*, the most widely distributed of the Oriental Lung Flukes in man, has not been found in man in the Mekong Basin, although natural infections have been recovered in tigers in Thailand. Since man is susceptible, it is perhaps only a matter of time before human infections with this species are detected in this country.

The first case of human infection with *P. heterotremus* in the world was reported from Thailand (Miyazaki and Harinasuta, 1966). This species also occurs in leopards, cats and dogs. Thus far, most cases have been reported from Saraburi Province, which is on the border between the Mekong and the Chao Phya drainages. The second intermediate host has been shown experimentally to be the fresh-water crab, *Potamon smithianus*, an inhabitant of streams and inundated paddy fields. The molluscan first intermediate host is unknown (Miyazaki and Vajrasthira, 1967).

Laos

The first cases of human paragonimiasis in Laos were reported by Constant and Lagard in 1962. Since that time, a small but steady number of cases have been seen in the hospitals of Vientiane (Fontan, 1971). In 1970, a rare autopsy permitted recovery of adult *Paragonimus* from a Lao soldier and the worms proved to be *P. heterotremus* (Miyazaki and Fontan, 1970). At the refugee hospital in Ban Son, 46 cases of active pulmonary paragonimiasis were treated from September 1971 through March 1973; it has been speculated that the large refugee population introduced paragonimiasis to this area. If it was rare before the war, it has now become established in the water system adjacent to Ban Xon (Kirkley, 1973).

Public Health Importance of Paragonimiasis

In Thailand and Laos, human cases of paragonimiasis often go undetected or unconfirmed. In Thailand, authorities accord the condition a rather low priority as a health problem. The situation in Laos is becoming more defined; here, the incidence of paragonimiasis may be increasing in the area just north of Vientiane as a result of the large numbers of war refugees settling there. If these people have, indeed, brought the parasite from their traditional mountain homes to the north, it is likely that they left the natural reservoir hosts behind and have themselves become, in effect, the new reservoirs for the parasite. This concept is best understood in the light of the Lao habit of eating uncooked or undercooked crabs.

Since the snail host is unknown in the region, it is difficult to predict the effect of major water-management schemes on the incidence of paragonimiasis, but it seems unlikely that the realization of such schemes would *reduce* the incidence.

In Laos, this problem may be increasing. Cases are now being diagnosed in children who were born on the Vientiane Plain and who have never visited the mountains (Kirkley, 1973). Increased incidence is no doubt due to refugee movements and indirectly to the war.

Research Needs in Paragonimiasis

The most pressing problem is the identification of the natural first intermediate, or snail, host. Snails of the genus *Oncomelania* can be infected experimentally with *P. heterotremus* but no snail has been found infected in the natural state. Elsewhere in Asia, snails most commonly found infected are members of the family Melaniidae. Some of these might eventually prove to be the first intermediate hosts in Thailand and Laos, where members of this family are common and numerous.

There is also a need to know the prevalence of infection in the second intermediate, or crab, host (*Potamon smithianus*) in endemic areas, as well as to discover other crustaceans capable of transmitting the parasite.

3. ECHINOSTOMIASIS

Life Cycle of the Parasite

The echinostomes are intestinal trematode parasites of waterfowl and small mammals. Man is not essential to the maintenance of these parasites in nature and is probably infected only accidentally, except when his eating habits parallel those of the natural hosts.

Eggs pass in the feces of the vertebrate host in the uncleaved stage and embryonation takes place in the external environment. Depending on species, the miracidia develop in pulmonate snails such as *Lymnaea rubiginosa* (Michelin), *Indoplanorbis exustus* (Deshayes), and *Gyraulus convexiusculus* (Hutton). Such snails are ubiquitous throughout Southeast Asia and are found in ponds and lakes which afford shallow water, sunlight, and abundant food. In Northeast Thailand they have been collected in the Nam Phong Reservoir, Lam Pao Reservoir, and the Lam Dom Noi Reservoir.

Mature cercariae leave the snail (first intermediate host) and encyst as metacercariae in other species of snails (including species eaten by man), fish, and/or tadpoles.

The final vertebrate hosts, including man, acquire the infection by ingesting the second intermediate host.

Pathogenicity and Treatment

The adult echinostome attaches itself by means of its oral circlet of spines to the mucosa of the small intestine, typically the jejunum. The worms, if present in small numbers, are thought to cause little damage to the host apart from slight local irriation. However, heavy infections have been reported to cause diarrhea, abdominal distress, anemia, and edema.

Infected individuals respond readily to a number of common vermifuges, including hexylresorcinol, tetrachlorethylene or oleoresin of aspidium, which cause the adult worms to be expelled (Rim, 1972). The identification of echinostome species must be based on the recovery of the adult worm as the eggs possess no clear-cut recognition characters.

Distribution and Prevalence in the Lower Mekong Basin

Figures on distribution and prevalence are generally lacking for this parasite in South Vietnam, Khmer Republic and Laos. Human echinostomiasis is common in certain areas of Northeast Thailand. The situation has been reviewed (Sornmani, 1969). Of 257 individuals in five villages of Kalasin Province, 130 (50.6%) were positive for echinostome eggs by single stool examination. When adult worms were expelled by means of tetrachlorethylene, they were identified as *Hypoderaeum conoideum* (Bloch) (Yokogawa *et al.*, 1965) and *Echinostoma malayanum* (Leiper) (Harinasuta *et al.*, 1963). Human infection with *E. revolutum* (Froelich) has also been reported in neighboring Mahasarakham Province (Bhiabulaya *et al.*, 1965).

Public Health Importance

In cases of light infection the echinostomes do not produce clinical disease. However, where raw snails and fish are eaten as a matter of course it is possible for individuals to become so heavily infected that symptoms develop. Such a situation exists in Northeast Thailand where raw snails are customarily eaten.

A problem to be faced by the clinician is the similarity between symptoms due to echinostomiasis and those caused by opisthorchiasis (liver fluke, see above). In many areas which had high echinostome prevalence rates the villagers also showed heavy (60-80%) infections with liver fluke, and it was difficult to state with certainty which worms were responsible for the symptoms. According to Harinasuta *et al.* (1963), the general experience in Thailand is that the flukes do not cause much trouble or, if they do, the symptoms are vague. But their observation may have been derived from observations of mild or moderate infections.

Research Needs

There is a question regarding the correct identification of the echinostome reported from Northeast Thailand. According to Lie *et al.* (1973) those previously identified as *H. conoideum* were morphologically similar to *H. dingeri* when re-examined. Likewise, certain morphological features of the worms identified as *E. revolutum* had more in common with *E. lindoense*. More work clearly is needed on this group.

Their life-cycles are intimately associated with the life-histories of certain snails favored by impoundment. When it is considered that the echinostomes occur in an area largely populated by individuals devoted to the consumption of raw foods, it is easy to forecast an increasing incidence and increasing worm-burden in the individual in areas destined for water-management schemes. At present, Northeast Thailand is one of the dryest areas of Southeast Asia. The process of dam construction and water impoundment is advancing well, however, in this region. One can confidently predict an increased incidence of infection with echinostomes and a concomitant increased worm-burden in the individual, with accompanying symptoms of disease.

4. ANGIOSTRONGYLIASIS

The Rat Lungworm, *Angiostrongylus cantonensis,* is a nematode parasite of rodents. Man is also susceptible. In man, the immature stages of the worm can produce eosinophilic meningitis.

Life Cycle

In the natural rat host, the infective larvae which reach the stomach are thought to pass to the duodenum and actively penetrate its wall to enter the intestinal veins. They then migrate via the portal system, liver, and posterior vena cava to the right side of the heart, pass through the pulmonary artery, pulmonary capillaries, and pulmonary vein to the left side of the heart, and thence by means of the common cartoid artery to the brain and spinal cord, where development occurs to young adulthood in four weeks. The larvae then enter the venous system of the brain, travel via the jugular vein to the heart and lung, and develop to sexual maturity in the lungs in another two weeks.

Eggs are laid in the lungs, where they develop and hatch. The first-stage larvae actively migrate out of the pulmonary capillaries, reach the trachea and leave the body in the feces.

Subsequent development is indirect in that it requires certain mollusks as intermediate hosts. The first-stage larvae are ingested by the mollusk, which may be a snail or a slug, and reach the infective third stage in about two weeks. At this stage of development, oral contamination with the raw snail or slug, or even mucus extruded by them, is responsible for passing the parasite on to humans and, of course, to other rats (Alicata, 1965). The principal molluscan host appears to be the Giant African land snail, *Achatina fulica*; most rat infections are probably passed on by this snail. In Thailand, however, the large aquatic snail, *Pila ampullacea*, which is often eaten raw in certain regions, has been incriminated (Punyagupta, 1965).

Pathogenicity and Treatment

The symptoms of larval migration through the brain of the human host include severe, unbearable headache, lasting sometimes for several days before the victim decides to seek medical help. There is frequent vomiting. The neck may be stiff. There may be tingling sensations of the skin and shooting pains in the limbs but such symptoms are variable. Some victims suffer nothing but mild headache. Others, at the opposite extreme, may develop cranial nerve palsy and even intracranial bleeding and blocking of blood vessels.

Most cases seem to be mild and only a few as severe as pictured above. However, fatalities (attributed to very heavy infection) have been recorded.

The disease, eosinophilic meningitis, can be produced by a number of parasites whose immature stages migrate through the human body (see Part IV, Chapter 6). It can also be induced by other conditions such as bacterial infection or tumors. According to Alicata (1969), the disease caused by the rat lungworm should be more properly called *cerebral angiostrongylosis*.

Treatment is symptomatic. Cortisone and antibiotics have been used to reduce the severity of the symptoms (Brumpt *et al.*, 1968).

Distribution and Prevalence

A. cantonensis occurs naturally in a wide tropical belt extending from Madagascar to Hawaii. In the Pacific region, human cases of infection have been reported from Guam, Philippines, Taiwan, and Indonesia (Sumatra) (Alicata, 1969).

In Southeast Asia, the parasite has been reported in animal hosts from Thailand, Khmer Republic, and Vietnam, and is known to occur in Laos.

Vietnam

One case of parasitic eosinophilic meningoencephalitis in Vietnam, presumed to have been caused by *A. cantonensis* infection, was reported (Jindrák and Alicata, 1965). The case occurred in Haiphong, involved an 18-year-old girl, and had a fatal outcome. No information has been published on the prevalence of *A. cantonensis* in rats in Vietnam.

Jindrák and Alicata (1965) reported observing children eating land snails raw as they were collecting them for pig food.

Khmer Republic

The Khmer people do not, in general, have the habit of consuming uncooked snails. This may explain the rarity of cases of eosinophilic meningitis. Two cases, both in young children, were described by Brumpt *et al.* (1968). Both exhibited purely meningeal symptoms which were mild and disappeared after two weeks. The children, brother and sister, had access to infected *Achatina fulica* in the garden of their home.

The prevalence of infected rats in the city of Phnom Penh is high. Brumpt *et al.* (1968) reported 42 of 213 *Rattus norvegicus* positive (19.7%) in the city proper, and five of 93 (5.4%) positive in neighboring rural areas. They also found one of seven *Bandicota indica* positive in the countryside.

Thailand

Eosinophilic meningitis is an important disease in Thailand. Proven cases (supported by laboratory studies) have been reported in 18 central provinces, 13 northeast provinces, two northern provinces, and one southern province. According to Punyagupta *et al.* (1967), there is good reason to suspect that many cases go unrecognized or are misdiagnosed. Two types of clinical disease occur in Thailand, namely, the "typical" eosinophilic meningitis characterized by severe headache, fever, and signs of meningeal irritation, and secondly, a "myeloencephalitis" type, typified by generalized agonizing pain and often paraplegia. The etiology of the second type is unknown, but the principal cause of the "typical" form is *A. cantonensis*. The main source of human infection seems to be the consumption of raw snails of the genus *Pila*, which are ubiquitous in ponds and lakes in Thailand.

Laos

A. cantonensis has been found in rats in Vientiane and infective third-stage larvae have been recovered from *Pila* snails and also from land snails of the genus *Hemiplecta* (Brandt, personal communication to CRS).

Public Health Importance

According to Punyagupta *et al.*(1967), eosinophilic meningitis is an important public health problem in Thailand. Proven cases have been reported from 34 provinces; 18 of these in the central region, 13 in the northeast, two in the north and one in the south. Many cases go unrecognized or are misdiagnosed.

The disease occurs in all age groups but is more common in the second and third decades. Twice as many males as females are involved. The highest attack rate seems to be during the rainy season.

The main source of infection in Thailand comes from eating raw *Pila* snails containing infective third-stage larvae of *A. cantonensis*. The role of other raw animal food has not been assessed.

The discovery that the infective third-stage larvae may pass out of the infected mollusk and contaminate the external mucus and thus the vegetation on which the mollusk is crawling has put the role of raw snail ingestion in a different light. Two cases of angiostrongyliasis recorded in Khmer children (Brumpt *et al.*, 1968) may have been contracted by playing with infected land snails in the children's backyard.

Research Needs

1. Determination, on a local basis, of the true prevalence of eosinophilic meningitis caused by *A. cantonensis* (cerebral angiostrongyliasis) in the human population.

2. Survey of local rat populations for infection rates.

3. Survey of local mollusks, including land forms (slugs and land snails) and aquatic snails (*Pila*, Viviparidae) for infection rates.

4. Establishment of the public health importance of this disease in relation to other parasitic diseases and in relation to incapacitating illness in general.

5. FASCIOLOPSIASIS

The etiological agent is the large intestinal fluke, *Fasciolopsis buski*, a ubiquitous parasite of pigs and man in many tropical areas. The first intermediate hosts are small planorbid snails of the genus *Segmentina*, in which larval stages mature, producing free-swimming cercariae. Upon leaving the snail, the cercariae encyst upon certain types of aquatic vegetation; the resulting metacercaria is the infective stage for the final host.

Plants which commonly serve as second intermediate hosts for *F. buski* in Asia include the water caltrop, *Trapa natans*, and the water morning-glory, *Ipomoea reptans*. If the fresh caltrop is peeled with the teeth, or if the morning-glory is eaten without adequate cooking, the infective stages are ingested. When moist, the metacercariae can survive for many months; desiccation, however, kills them.

When eaten, the flukes excyst in the intestine and begin to produce eggs within a month.

A light infection with these worms may show no symptoms, or there may be a slight diarrhea. In heavy infection, with thousands of worms, there is inflammation and ulceration at the site of attachment. The patient may suffer nausea and loss of appetite. Ascites may occur. Such subjects are particularly prone to intercurrent diseases which may prove fatal (Hunter *et al.*, 1961).

F. buski has a wide distribution in Asia but has been little studied in Southeast Asia. In Thailand, it occurs chiefly in the central part (Daengsvang and Mangalasmaya, 1941; Sadun and Maiphoom, 1953), but seems to be much less common in northeast Thailand and the Thai portion of the Mekong watershed (Manning, 1969). In central Thailand, the snail *Segmentina hemisphaerula* has been found naturally infected and *S. (Trochorbis) trochoideus* has been experimentally infected (Harinasuta, 1969). The parasite occurs in pigs in central and northeast Thailand in areas where pig manure is used as fertilizer (De Jesus and Waramontri, 1961).

In Laos, the parasite has been reported in humans but prevalence rates are not known (Segal *et al.*, 1968).

In the Khmer Republic, one study in the vicinity of Phnom Penh revealed that 5% of pigs were infected but very few humans (0.04%) (Brumpt and Kong-Kim-Choun, 1965).

In Vietnam, the infection was studied in the north during French colonical times: it was found in humans (Galliard, 1948), pigs, and dogs (Galliard and Dang-Van-Ngu, 1940, 1941). In one study, nearly 15% of Asians and 3% of Europeans were found carrying the parasite (Baugé, 1954).

Reports from South Vietnam differ considerably with regard to prevalence but are in agreement that the infection is primarily a rural one (Barrois and Noc, 1908; Bernard *et al.*, 1924; Colwell *et al.*, 1971).

Since the parasite can produce serious pathological changes and even death when present in large numbers in the human host, it is surprising that so little epidemiological information is available. It is, at present, accorded a low or non-existent position in terms of public health priorities, possibly because the diagnosis is missed. Cross (1969) has noted that this parasite merits the interest of parasitologists in Southeast Asia: it is "large, easily obtained in numbers from pigs in endemic areas, and would offer those interested in biochemistry, physiology, and immunology of helminths a ready source of material."

6. GNATHOSTOMIASIS

Life Cycle of the Parasite

The worms are natural parasites of the stomach wall of dogs and cats in Thailand; adults lie coiled in tumors in the tissue (Prommas and Daengsvang, 1933; 1936, 1937). Eggs pass in the feces and develop and hatch on reaching fresh water. In order to continue the life cycle, the liberated larva must be ingested by the first intermediate host, a microscopic swimming crustacean (*Cyclops*). In this host, development continues to a stage capable of infecting the second intermediate host, a fish. In Thailand, the most heavily infected fish are Walking Catfish (*Clarias batrachus*), and Snakehead (*Ophicephalus striatus* and *O. micropeltes*). The fish *Monopterus albus* and the frog *Rana rugulosa* have also been recorded with natural infections. Experimentally, chickens, rats, and lizards can be infected (Daengsvang and Tansurat, 1938).

When the fish (or frog) is eaten by a cat, development to the adult stage begins when the larva burrows into the stomach wall. Maturation is reached and eggs from the new generation begin to appear in about six months.

Pathogenicity and Treatment

Human gnathostomiasis is an accidental infection with *Gnathostoma* larvae. When a human eats raw fish containing the larvae, the latter are unable to develop naturally. Instead, they move through the viscera and/or the subcutaneous tissues. In the dermis they can cause intense itching, swelling abscesses, or subcutaneous nodules (Daengsvang, 1949). The migration time may be relatively short (10 days or so) but, in the form of creeping eruption in the skin, the symptoms can be alarming and are often incapacitating. If they reach the head, there may be impaired vision or even blindness. In more complicated cases, the brain or spinal cord may be invaded, producing meningoencephalitis with headache, convulsions, or partial coma. The outcome may be fatal (Chitanondh and Rosen, 1967).

When migration is limited to the viscera, symptoms may be absent, but there is a customary high eosinophilia that may climb to 80% (Khwanmitra *et al.*, 1957).

If the signs and symptoms are limited to eosinophilia and surface swellings, the diagnosis may be missed or confused with angiostrongyliasis or sparganosis (Khwanmitra *et al.*, 1957). However, clinicians who see many cases soon learn to distinguish the swelling and itching of gnathostomiasis.

The condition is self-limiting, barring reinfection. General health is not permanently impaired and prognosis is usually good in uncomplicated cases (Daengsvang, 1949).

Drugs are not recommended. If conditions permit, the worms should be surgically removed (Daengsvang, 1949).

Distribution and Prevalence in the Lower Mekong Basin

It is thought that human gnathostomiasis is limited to regions where raw fish is consumed. There are no figures for the prevalence of human gnathostomiasis in Laos, Khmer Republic, or Vietnam. In Thailand, the condition is said to be heavily endemic but exact figures on prevalence are unavailable (Daengsvang, 1949).

Public Health Importance

Human gnathostomiasis must be considered to represent an unusual accident under any circumstances, with a certain unregistered frequency among people who have the habit of eating raw fish. If it often represents a diagnostic problem in the clinic, it occupies a low position of priority in the thinking of public health authorities.

Research Needs

Since human infection is acquired by eating raw fish, simple cooking of the fish would remove the hazard. The infective larvae are killed by exposing to boiling water for 5 minutes. Immersion of the fish in vinegar for 5.5 hours also kills larvae (Hunter *et al.*, 1961). They are not, however, killed by chilling in the refrigerator (4°C/one month) (Prommas and Daengsvang, 1937).

The prevalence of fish infection in established and newly formed lakes should be studied by field investigations, in order to determine the true degree of hazard presented by gnathostomiasis in the riparian communities.

REPORTS, PUBLICATIONS, AND LABORATORY DEMONSTRATIONS RESULTING FROM THE WATERBORNE DISEASES PROJECT

1. "The human schistosome of the Mekong River: Guesswork and facts." Paper presented by C. R. Schneider at the Ninth SEAMEO-TROPMED Seminar on Epidemiology, Prevention, and Control of the Endemic Diseases in Southeast Asia and the Far East, Tokyo, Osaka, July 5-7, 1971.

2. "Life cycle of *S. japonicum*-like trematode from Khong Island, South Laos." by S. Sornmani, V. Kitikoon, and C. R. Schneider. Laboratory demonstration presented at the Tenth SEAMEO-TROPMED Seminar, "Symposium on Chemotherapy in Tropical Medicine of Southeast Asia and the Far East," Bangkok, October 26-30, 1971. Abstract in the *Southeast Asian Journal of Tropical Medicine and Public Health*, volume 2, number 4, page 575.

3. "Infection of aquatic hydrobiid snails and animals with *Schistosoma japonicum*-like parasites from Khong Island, Southern Laos." by C. Harinasuta, S. Sornmani, V. Kitikoon, C. R. Schneider, and O. Pathammavong. Published in the *Transactions of the Royal Society of Tropical Medicine and Hygiene*, volume 66, number 1, pages 184-185 (1972).

4. "Snail Transmission of Human Schistosomiasis in the Lower Mekong Basin." Secretariat note presented as Agenda Item 5(iii) at the 55th Session (Plenary) of the Committee for Coordination of Investigations of the Lower Mekong Basin, January 26-31, 1972, Bangkok, Thailand.

5. "Bilharziose ou schistosomiase humaines dans le bassin du Mékong." Lecture presented by C. R. Schneider at the Faculté de Médecine, Phnom Penh, Khmer Republic, February 22, 1972.

6. "New knowledge of the *Schistosoma japonicum*-like parasite of the Lower Mekong River." Paper presented by C. R. Schneider at the 41st Annual Meeting of the Japanese Society of Parasitology, April 5-6, 1972, Okayama, Japan.

7. "The human schistosome of the Mekong River." Lecture presented by C. R. Schneider at the Faculty of Medicine, Saigon, Republic of South Vietnam, April 14, 1972.

8. "Mekong schistosomiasis: 1. Life cycle of *Schistosoma japonicum*, Mekong strain, in the laboratory." by S. Sornmani, V. Kitikoon, C. R. Schneider, C. Harinasuta, and O. Pathammavong. Published in the *Southeast Asian Journal of Tropical Medicine and Public Health*, volume 4, number 2, pages 218-225 (1973).

9. "Life cycle of *Schistosoma japonicum*-like trematode from Khong Island, Southern Laos." by S. Sornmani, C. R. Schneider, and V. Kitikoon. Laboratory demonstration presented at the Malaysian Society of Parasitology and Tropical Medicine, January 27-28, 1973, at the Institute for Medical Research, Kuala Lumpur, Malaysia. Abstract in *Southeast Asian Journal of Tropical Medicine and Public Health*, volume 4, number 2, page 279 (1973).

10. "Mekong schistosomiasis: 2. Evidence of the natural transmission of *Schistosoma japonicum*, Mekong strain, at Khong Island, Laos." by V. Kitikoon, C. R. Schneider, S. Sornmani, C. Harinasuta, and G. R. Lanza. Published in the *Southeast Asian Journal of Tropical Medicine and Public Health*, volume 4, number 3, pages 350-358 (1973).

11. "Activities of the Smithsonian-AID Waterborne Diseases Project in the Lower Mekong Basin." Lecture presented by C. R. Schneider to the Epidemiological Discussion Group, National Institute of Medical Research, Jakarta, Indonesia, November 5, 1973.

12. "Biological control by trematode antagonism. I. A successful field trial to control *Schistosoma spindale* in northeast Thailand." by Lie Kian Joe, C. R. Schneider, S. Sornmani, G. R. Lanza, and P. Impand. Published in the *Southeast Asian Journal of Tropical Medicine and Public Health*, volume 5, number 1, pages 46-59 (1974).

13. "Biological control by trematode antagonism. II. Failure to control *Schistosoma spindale* in a field trial in northeast Thailand." by Lie Kian Joe, C. R. Schneider, S. Sornmani, G. R. Lanza, and P. Impand. Published in the *Southeast Asian Journal of Tropical Medicine and Public Health*, volume 5, number 1, pages 60-64 (1974).

14. "Mekong schistosomiasis: 3. A parasitological survey of domestic water buffalo (*Bubalus bubalis*) on Khong Island, Laos." by C. R. Schneider, V. Kitikoon, S. Sornmani, and S. Thirachantra. In press, *Annals of Parasitology and Tropical Medicine* (Liverpool) (1975).

15. "*Lithoglyphopsis aperta* (Gastropoda): functional morphology, evolutionary relationships, and the transmission of Mekong schistosomiasis." by G. M. Davis, V. Kitikoon, C. R. Schneider, and P. Temcharoen. In preparation, to be submitted to *Malacologia* (1975).

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APPENDIX I

SURVEY OF FRESH-WATER MOLLUSKS OF NORTHEAST THAILAND

FRESH-WATER MOLLUSK SURVEY OF THE CHI-MUN TRIBUTARY SYSTEM OF THE MEKONG RIVER IN NORTHEAST THAILAND

The Mekong River's extensive Chi-Mun tributary, draining much of northeast Thaialnd, was sampled for fresh-water snails and clams at 101 localities. Thirty-seven of these sites were sampled at least twice to assure more complete collections.

Thirty-one species and subspecies of snails and 14 species and subspecies of clams were found; animals of *Stenothyra* (snails) and *Corbicula* (clams) were not identified to species owing to taxonomic chaos in these groups.

Except for *Stenothyra* and *Corbicula,* the geographical distribution of the mollusk species and subspecies was plotted according to several arbitrary divisions (*cf.* Table 1, Fig. 1), and field notes were used to delineate the general ecological distribution of the various taxa.

The geographical distribution of the mollusks collected in this survey is recorded according to general drainage division (Table 2) and also specific localities (Figs. 2-46).

Medically Important Mollusks

The following lists are modified from the snails that Brandt (1971) reported as intermediate hosts of various human trematodes. Of special significance in northeastern Thailand are the widespread *Bithynia siamensis, Indoplanorbis exustus* and *Radix rubiginosa,* and also species of *Idiopoma* and *Pila,* since natives eat these large snails uncooked. In these lists, the specific trematode is noted above the names of the snails.

Echinostomatidae (*Echinostoma ilocanum, E. malayanum,*
Hypoderaeum sp.)
Hippeutis umbilicatus
Idiopoma bengalensis
Idiopoma ingallsiana
Radix rubiginosa
Indoplanorbis exustus
Gyraulus convexiusculus
Pila ampullacea
Pila polita

Fasciola gigantica
Radix rubiginosa

Opisthorchis viverrini
Bithynia siamensis

Fasciolopsis buski
Hippeutis umbilicatus
Segmentina hemisphaerula
Segmentina trochoideus

Paragonimus heterotremus
Melanoides tuberculata?
Tarebia granifera?

"snail-itch" (= *Schistosoma incognitum, S. indicum,*
S. nasale, and mainly, S. spindale;
Orientobilharzia sp.; others?)
Indoplanorbis exustus
Radix rubiginosa
Trichobilharzia brevis
(*Adamietta housei* suggested by natives, but
not confirmed)

TABLE 1

ARBITRARY DIVISIONS OF THE CHI AND MUN TRIBUTARY SYSTEMS OF THE MEKONG RIVER, IN NORTHEASTERN THAILAND

Name	Geographic Area
Upper Chi drainage	Upstream from Kosum Phisai, Maha Sarakham Province.
Lower Chi drainage	Downstream from Kosum Phisai, Maha Sarakham Province, to the junction of the Chi and Mun rivers at the border of Sisaket and Ubon Ratchathani provinces.
Upper Mun drainage	Upstream from Phuttisong, Buriram Province.
Middle Mun drainage	Downstream from Phuttisong, Buriram Province, to the junction of the Chi and Mun rivers at the border of Sisaket and Ubon Ratchathani provinces.
Lower Mun drainage	Downstream from the junction of the Chi and Mun rivers, at the border of Sisaket and Ubon Ratchathani provinces, to the Mekong River.

Systematic Section

In the following account, the mollusks are listed alphabetically by family, by genera within each family, and by species and subspecies within each genus. Taxonomic determinations are according to Brandt (1971) unless specified otherwise.

GASTROPODA (snails)

Family Bithyniidae Walker

1. *Bithynia siamensis* Lea (= *B. s. siamensis* Lea and *B. s. goniomphalus* [Morelet] *sensu* Brandt).
   Occurring throughout the Chi-Mun drainage (Fig. 2). Most commonly found in small streams, but also recovered from six reservoirs, four natural lakes with permanent water and 11 other kinds of habitats (one to two sites each); a successful invader of reservoirs and *klongs* (canals), and occasionally present in seasonally drying lentic and lotic waters. Present largely on muddy sand, and sandy mud, with rooted aquatic vegetation abundant to absent.

2. *Wattebledia crosseana* (Wattebled).
   Occurring throughout the Chi system, but present only in the Upper and Lower Mun system (Fig. 3). Found mostly in standing waters (natural lakes with permanent water and reservoirs), and only rarely recovered from streams. Present on muddy sand and sand mud, with profuse rooted aquatic vegetation typically present.

Family Bucciniidae Fleming

3. *Clea helena* (Philippi). Occurring throughout the Chi-Mun drainage (Fig. 4). Most commonly found in streams (mainly of small size), four reservoirs and five other kinds of habitats (one-two sites each). Present mainly on muddy sand, less often on sandy mud, and only infrequently on mud, sand or fine gravel; rooted aquatic vegetation more commonly sparse, to common. Only a partially successful invader of man-made habitats: present in reservoirs, but not recorded from canals.

Family Hydrobiidae Troschel

Many genera and species of this family are known from rocky areas of the Mekong River proper, but comparatively few extend "inland" into northeastern Thailand.

4. *Hydrorissoia munensis* Brandt.
Occurring only in the lower Mun system (Fig. 5). Found at just two localities: at and near Phibun Mangsahan, Ubon Ratchathani Province. Evidently confined to large streams with rocky substrates; algae present, but rooted aquatic vegetation absent.

5. *Jullienia munensis* Brandt.
Found only in the Lower Mun at Phibun Mangsahan, Ubon Ratchathani Province (Fig. 6), a large stream with rock substrate; algae present, but rooted aquatic vegetation absent.

6. *Lacunopsis massiei* Bavay. Found only in the Lower Mun near Phibun Mangsahan, Ubon Ratchathani Province (Fig. 7), a large stream with rock substrate; algae present, but rooted aquatic vegetation absent.

7. *Manningiella expansa* Brandt. Found only in the Lower Mun near Phibun Mangsahan, Ubon Ratchathani Province (Fig. 8), a large stream with rock substrate; algae present, but rooted aquatic vegetation present.

8. *Pachydrobia munensis* Brandt. Found at only one site each in the Upper Mun (very rare) and in the Middle Mun (very abundant) (Fig. 9). Seemingly favoring muddy sand, with only sparse algal vegetation present; very rare on sand, with algae common. This is the only hydrobiid encountered upstream from the rock substrate at Phibun Mangsahan in the Lower Mun.

9. *Pachydrobia zilchi* Brandt. Found at only one site each in the Lower Chi (small stream) and in the Lower Mun near Phibun Mangsahan (large stream) (Fig. 10). Occurring as dead shells among muddy sand in the Chi, but alive on rocks in the Mun; algae present at the latter site, but rooted aquatic vegetation absent.

Family Lymnaeidae Gray

10. *Radix rubiginosa* (Michelin) (= *Lymnaea auricularia rubiginosa* [Michelin] *sensu* Brandt, 1971).
Found commonly in the Upper Chi and Mun, and only rarely in the Lower Chi and Middle Mun (Fig. 11). Occurring principally in small streams; less commonly encountered in natural lakes with permanent water, permanent water in canals and reservoirs, and only very rarely in other kinds of habitats. Associated with muddy and sandy mud substrates; abundant rooted aquatic vegetation typically present.

Family Pilidae (= Ampullariidae)

11. *Pila gracilis* (Lea) (= *P. g. gracilis* and *P. pesmei* [Morlet] *sensu* Brandt, 1971).
Found throughout the Chi-Mun system, but mainly in the upper parts (Fig. 12). Occurring most commonly in small and medium-sized streams, but also found in a variety of lentic habitats (some seasonally drying). Substrate usually mud, sometimes with sand; rooted vegetation usually present.

12. *Pila polita* (Deshayes).
Found mainly in the Upper Chi, but also in the Middle Chi, Upper Mun and Middle Mun (Fig. 13). Occurring largely in small streams, and less often in various lentic habitats (some seasonally drying). Substrate typically muddy, interspersed with sand; rooted aquatic vegetation usually present.

Family Planorbidae Gray

13. *Camptocercas jiraponi* Hubendick.
Found only as a few shell specimens in just one locality in the Upper Chi (Fig. 14): a small stream with muddy sand substrate and sparse rooted aquatic vegetation.

14. *Gyraulus convexiusculus* (Hutton).
Found largely in the Upper Chi and Upper Mun (Fig. 15). Occurring just in standing waters of various types, but mainly in natural lakes with permanent water and reservoirs. Associated with muddy sand and sandy mud substrates; abundant rooted aquatic vegetation typically present.

15. *Hippeutis umbilicatus* (Benson).
Found only rarely in the Upper and Lower Chi (Fig. 16). Occurring in natural lakes with permanent water, and pools in a seasonally drying small stream. Substrate muddy, with rooted aquatic vegetation present.

16. *Indoplanorbis exustus* (Deshayes). Found in the Upper Chi and Lower Chi, Upper Mun, and as shells only in the Lower Mun (Fig. 17). Occurring mainly in lentic waters (especially reservoirs) and small streams. Associated with sandy mud and muddy sand substrates, and abundant rooted aquatic vegetation.

17. *Segmentina hemisphaerula* (Benson).
Found in the Chi system at two localities (Fig. 18): a reservoir, and isolated pools in a seasonally drying small stream. Associated with muddy sand substrate, and algae (drying stream) and rooted aquatic vegetation (reservoir).

18. *Segmentina trochoideus* (Benson).
Found in the Chi system, and at one locality in the Upper Mun (Fig. 19). Occurring only in lentic waters: a canal, a permanent and seasonally-drying lake, and two reservoirs. Muddy sand substrate; moderately abundant rooted aquatic vegetation present.

Family Thiaridae Gray

19. *Adamietta housei* (Lea).
Found throughout the Chi-Mun system (Fig. 20). Occurring largely in lotic waters (especially small streams), and only rarely in standing waters. Associated with muddy sand substrate, and but sparse algal and rooted aquatic vegetation.

20. *Melanoides jugicostis* (Hanley and Theobald).
Found at only two localities in the Upper Mun and at one site in the Upper Chi, all small streams (Fig. 21). Occurring on sand and muddy substrate; algal and rooted aquatic vegetation absent.

21. *Melanoides tuberculata* (O.F. Müller).
Found throughout the Chi-Mun system, but most often in the Mun; most abundant in the Upper Chi and Upper Mun (Fig. 22). Found very largely in streams, especially those of small size; recorded just once from standing water: permanent water in a small, man-made excavation. Associated with a variety of substrates: muddy sand, sand, gravel, and rocks and boulders below a small dam; algae sparse, rooted aquatic vegetation absent.

22. *Tarebia granifera* (Lamarck).
Found at one locality, in the upper Mun (Fig. 23): a small stream with sand and sandy muddy substrate devoid of vegetation.

Family Viviparidae Gray

23. *Annulotaia forcarti* Brandt.
Found only in the Lower Chi and Middle Mun (Fig. 24). Occurring in small and medium-sized streams, on muddy sand and muddy clay substrates; algae sparse to absent, rooted aquatic vegetation lacking.

24. *Idiopoma bengalensis* (Lamarck) (= *Filopaludina sumatrensis polygramma* [von Martens] and *F.s. speciosa* [Deshayes] *sensu* Brandt, 1971).
Found frequently in the Upper Chi, and only rarely (and mostly as shells only) in the Lower Chi, Upper Mun and Lower Mun (Fig. 25). Occurring mainly in small streams, less frequently in medium- and large-sized streams; also recorded, but uncommonly, from roadside ditches (some with permanent water, and some seasonally drying), reservoirs and one natural lake with permanent water. Associated with muddy sand substrate; algae sparse, to abundant (in seasonally drying habitats), and rooted aquatic vegetation sparse to absent.

25. *Idiopoma ingallsiana* (Lea) (= *Filopaludina martensi* s.l. *sensu* [Brandt, 1971]).

The most commonly encountered snail, found throughout the Chi-Mun system (Fig. 26). Occurring mainly in small streams (some seasonally drying), less frequently in medium-sized and large streams; also recorded from road-side ditches and canals (with both permanent and seasonal water), natural lakes (with both permanent and seasonal water) and reservoirs (two to five localities each). Substrate highly variable: sandy mud, mud, muddy sand, sand, gravel and rocks; algae usually sparse, to abundant (in seasonally drying habitats), with rooted aquatic vegetation typically sparse, to absent.

26. *Idiopoma trochoides* (von Martens) (= *Trochoides trochoides* [von Martens] *sensu* Brandt, 1971).

Found only rarely in the Upper and Lower Chi (Fig. 27). Occurring mainly in seasonally drying lakes, as well as a medium- and a small-sized stream. Associated with muddy substrate, with abundant rooted aquatic vegetation typically present.

27. *Idiopoma umblicata* (Lea).

Found throughout the Chi-Mun system (Fig. 28). Occurring largely in small streams, with only one collection made from a large stream; also recovered from three kinds of lentic habitats, two of which were seasonally drying. Associated with muddy sand and sandy mud; algae rarely present, and rooted aquatic vegetation sparse, to usually absent.

28. *Mekongia pongensis* Brandt.

Found in the Upper and Lower Chi and in the Middle and Lower Mun (Fig. 29). Found only in streams: three of small size, two of medium size and one large stream. Associated with muddy sand, or rarely just sand; sparse algae present at one locality, rooted aquatic vegetation at only one other.

29. *Mekongia swainsoni lamarcki* (Deshayes) (= *M. lamarcki* [Deshayes] *sensu* Brandt, 1971).
Found in the Lower Chi (shells only) and Lower Mun (Fig. 30). Occurring alive in a small stream on muddy sand substrates, with algae and rooted aquatic vegetation lacking. Shell specimens recorded from small-, medium-, and large-sized streams from other localities.

30. *Mekongia swainsoni spnaericula* (Deshayes) (= *M. sphaericula* s.l. *sensu* Brandt, 1971).
Found in the Lower Chi (shells only) and in the Middle and Lower Mun (Fig. 31). Occurring mainly in small streams, with one collection from a medium-sized stream; absent from lentic habitats. Live animals recorded mainly from muddy sand, less often from sandy mud; algae and rooted aquatic vegetation usually absent, sparse when present.

31. *Mekongia swainsoni swainsoni* (Lea).
Found in the Lower Chi (shells only) and Middle Mun (Fig. 32). Occurring in small (shells) and medium-sized (live) streams only; absent from lentic habitats. Associated with muddy sand substrate, with both algae and rooted aquatic vegetation absent.

PELECYPODA (clams)

Family Amblemidae Rafinesque

1. *Ensidens ingallsianus* (Lea).
Found throughout the Chi-Mun system (Fig. 33). Occurring largely in small streams, uncommonly in medium-sized streams, rarely in large streams; in lentic habitats: one collection each from a natural lake with permanent water, and a reservoir. Present mainly in muddy sand and sand and sandy mud substrates, less commonly in sand and fine gravel. Algae uncommon, and rooted aquatic vegetation typically absent.

2. *Pilsbryoconcha exilis* (Lea) (= *P. exilis* [Lea] and *P. exilis compressa* [von Martens] *sensu* Brandt).
Found throughout the Chi-Mun system (Fig. 34). Occurring mainly in small streams, only rarely in medium-sized and large streams; in lentic habitats: one collection each from a seasonally drying and permanent roadside canal, and a lake with permanent water. Associated with sandy mud and muddy sand substrates; algae and rooted aquatic vegetation rare, usually lacking.

3. *Pilsbryoconcha lemeslei* (Morelet).
Found alive at just one locality in the Lower Chi (a small stream with sandy-clay substrate, and devoid of vegetation), and as shells only in the Upper Chi and Middle Mun (Fig. 35).

4. *Pseudodon cambodjensis* (Petit de la Saussaye) (= *P. cambodjensis* [Petit] and *P. c. tenerrimus* Brandt *sensu* Brandt).
Found throughout the Chi-Mun system, except in the Upper Chi (Fig. 36). Occurring mainly in small streams, less commonly in medium-sized streams and not in lentic waters. Present mainly in sandy mud, as well as in muddy sand, sand and fine gravel, and sandy clay. All vegetation ordinarily absent, algae rarely present.

5. *Pseudodon mouhotii* (Lea).
Found throughout the Chi-Mun system (Fig. 37), but as shells only in the Upper Chi. Occurring largely in small streams, less frequently in medium-sized and large streams and not in lentic waters. Present mainly in muddy sand substrate, and also sandy mud, sand and fine gravel, and sandy clay. Algal and rooted aquatic vegetation lacking.

6. *Pseudodon vondembuschianus ellipticus* Conrad.
Found throughout the Chi-Mun system (Fig. 38). Present principally in small streams, rarely in medium-sized streams and not in large streams; in lentic habitats: one collection each from isolated pools in a seasonally drying small stream and in a seasonally drying canal, and permanent water in a roadside ditch and a canal; not recorded from lakes and reservoirs. Occurring in a wide variety of substrates, but most often muddy sand; algal and rooted aquatic vegetation usually lacking, sparse when present.

7. *Scabies crispata* (Gould) (= *S. crispata* [Gould] and *S. phaseolus* [Lea] *sensu* Brandt).
Found throughout the Chi-Mun system (Fig. 39). Occurring mainly in small and medium-sized streams, rarely in large streams; in lentic habitats: only one record, in a natural lake with permanent water (shells only). Associated largely with sandy mud substrate, and also in muddy sand and sand and fine gravel; algae typically absent; rooted aquatic vegetation often present, but sparse.

8. *Unionetta nucleus* (Lea).
Found throughout the Mun, but absent in the Chi (Fig. 40). Of seven collections of live animals, the first specimens known, other than the holotype which was described in 1856, were all from small streams with sandy mud and muddy sand substrates; no vegetation present.

Family Mytilidae Rafinesque

9. *Limnoperna siamensis* (Morelet).
Found in the Upper Chi, Middle Mun and Lower Mun (Fig. 41). Occurring primarily in medium-sized streams, rarely in small and large streams, lakes and reservoirs. Typically byssally attached to submerged twigs and logs, as well as to other clam shells.

Family Unionidae Rafinesque

10. *Cristaria plicata bellua* (Morelet).
Found at just one locality (in the Upper Chi: Fig. 42), a natural lake with permanent water. Present on very soft mud substrate, a seemingly limiting factor since the large animal's very small foot probably prevents burrowing into firmer, coarser substrates in other kinds of habitats. Algal and rooted aquatic vegetation absent.

11. *Hyriopsis bialatus* Simpson.
Found throughout the Chi-Mun system (Fig. 43). Occurring commonly in small and medium-sized streams, rarely in large streams and absent from lentic waters. Present in various substrates: muddy sand and fine gravel (mainly), muddy sand, sandy mud, and sandy clay. Algal and rooted aquatic vegetation typically absent, sparse when present.

12. *Physunio cambodiensis* (Lea) (= *P. cambodiensis* [Lea] and *P. modelli* Brandt *sensu* Brandt).
Found throughout the Chi-Mun system (Fig. 44). Occurring mainly in small streams, only rarely in medium-sized streams, and absent from lentic habitats. Associated with muddy sand and sandy mud substrates; algae and rooted aquatic vegetation sparse to common when present, but usually lacking.

13. *Physunio eximius* (Lea).
Found throughout the Chi-Mun system (Fig. 45). Occurring principally in small streams, less commonly in medium-sized streams and only rarely in large streams; absent from lentic habitats. Associated mainly with muddy sand substrate, less frequently with sandy mud and sand; algae and rooted aquatic vegetation usually absent, to moderately abundant.

14. *Trapezoideus exolescens pallegoixi* (Sowerby).
Found in the Lower Chi, and Middle and Lower Mun (Fig. 46). Occurring most frequently in medium-sized streams, rarely in small and large streams, and not in lentic habitats. Present mainly in sandy mud substrate, less commonly in sand and fine gravel, mud and fine gravel, and muddy sand. Algae usually absent, to common; rooted aquatic vegetation typically absent, sparse when present.

TABLE 2

GENERAL DISTRIBUTION OF FRESH-WATER MOLLUSKS IN THE CHI-MUN WATERSHED IN NORTHEASTERN THAILAND; ALPHABETICAL LIST BY GENUS AND SPECIES

Taxon	Upper Chi	Lower Chi	Upper Mun	Middle Mun	Lower Mun	Fig. No.
GASTROPODA						
Adamietta housei	x	x	x	x	x	20
Annulotaia forcarti		x		x	x	24
Bithynia siamensis	x	x	x	x	x	2
Camptocercas jiraponi	x[a]					14
Clea helena	x	x	x	x	x	4
Gyraulus convexiusculus	x	x	x			15
Hippeutis umbilicatus	x	x				16
Hydrorissoia munensis					x	5
Idiopoma bengalensis	x	x	x		x	25
Idiopoma ingallsiana	x	x	x	x	x	26
Idiopoma trochoides	x	x[a]				27
Idiopoma umbilicata	x	x	x	x	x	28
Indoplanorbis exustus	x	x	x		x[a]	17
Jullienia munensis					x	6
Lacunopsis massiei					x	7
Manningiella expansa					x	8
Mekongia pongensis	x	x		x	x	29
Mekongia swainsoni lamarcki		x[a]			x	30
Mekongia swainsoni sphaericula		x[a]		x	x	31
Mekongia swainsoni swainsoni		x[a]		x		32
Melanoides jugicostis	x		x			21
Melanoides tuberculata	x	x	x	x[a]	x	22
Pachydrobia munensis			x	x		9
Pachydrobia zilchi		x[a]			x	10
Pila gracilis	x	x	x	x	x	12
Pila polita	x	x	x	x	x[a]	13
Radix rubiginosa	x	x	x	x		11
Segmentina hemisphaerula	x	x				18
Segmentina trochoideus	x	x	x			19
Tarebia granifera			x			23
Wattebledia crosseana	x	x	x		x	3

[a]Records from shells only (no live animals found).

TABLE 2.--*Continued*

Taxon	Upper Chi	Lower Chi	Upper Mun	Middle Mun	Lower Mun	Fig. No.
PELECYPODA						
Cristaria plicata bellua	x					42
Ensidens ingallsianus	x	x	x	x	x	33
Hyriopsis bialatus	x	x	x	x	x	43
Limnoperna siamensis	x			x	x	41
Physunio cambodiensis		x	x	x	x	44
Physunio eximius	x	x	x	x	x	45
Pilsbryoconcha exilis	x	x	x	x	x	34
Pilsbryoconcha lemeslei	x[a]	x		x[a]		35
Pseudodon cambodjensis		x	x	x	x	36
Pseudodon mouhotii	x[a]	x	x	x	x	37
Pseudodon vondembuschianus ellipticus	x	x	x	x	x	38
Scabies crispata	x	x	x	x	x	39
Trapezoideus exolescens pallegoixi		x	x[a]	x	x	46
Unionetta nucleus			x	x	x	40

[a]Records from shells only (no live animals found).

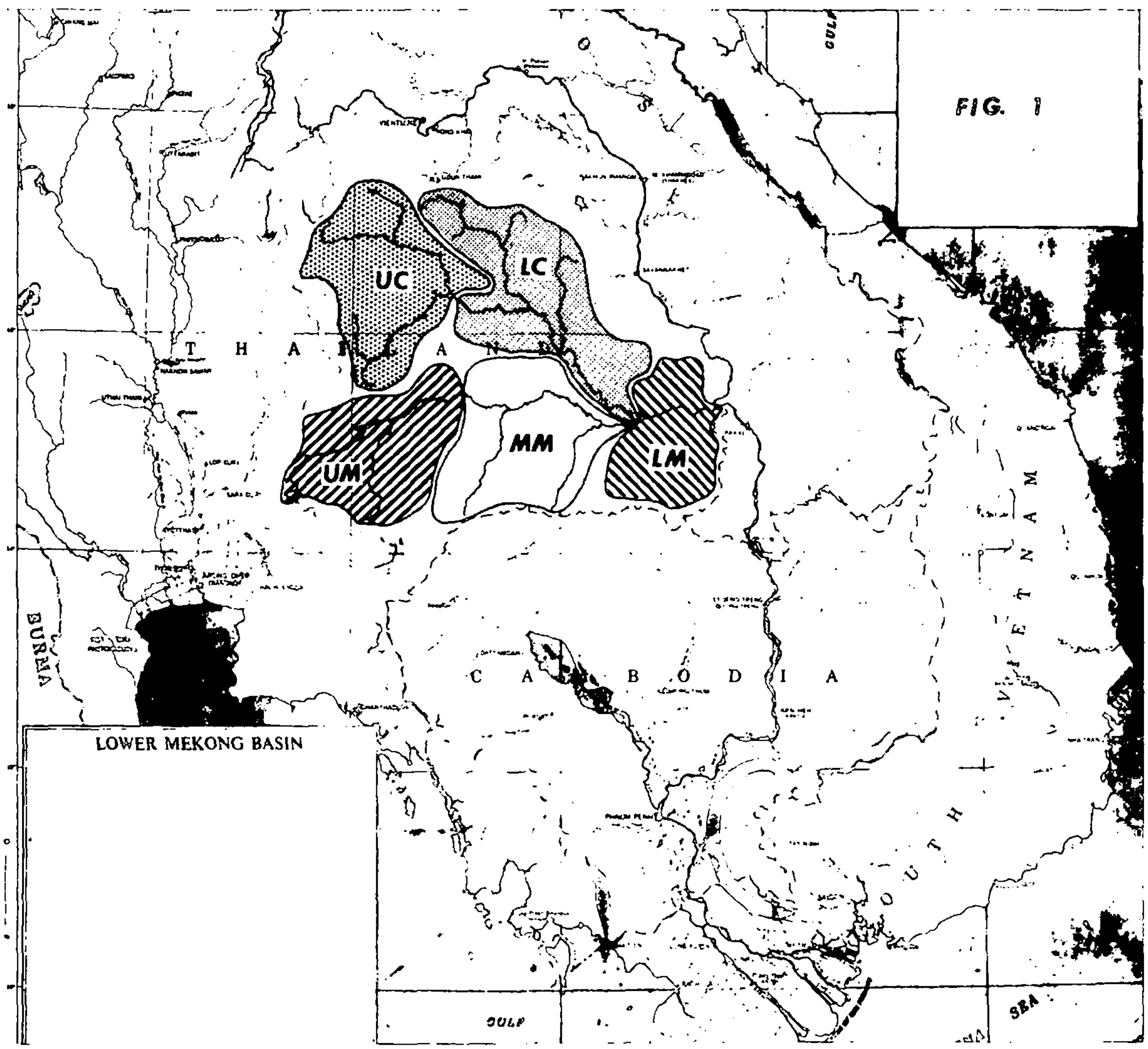


FIG. 1
UC
LC
UM
MM
LM
T H A I L A N D
C A M B O D I A
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
GULF
SEA

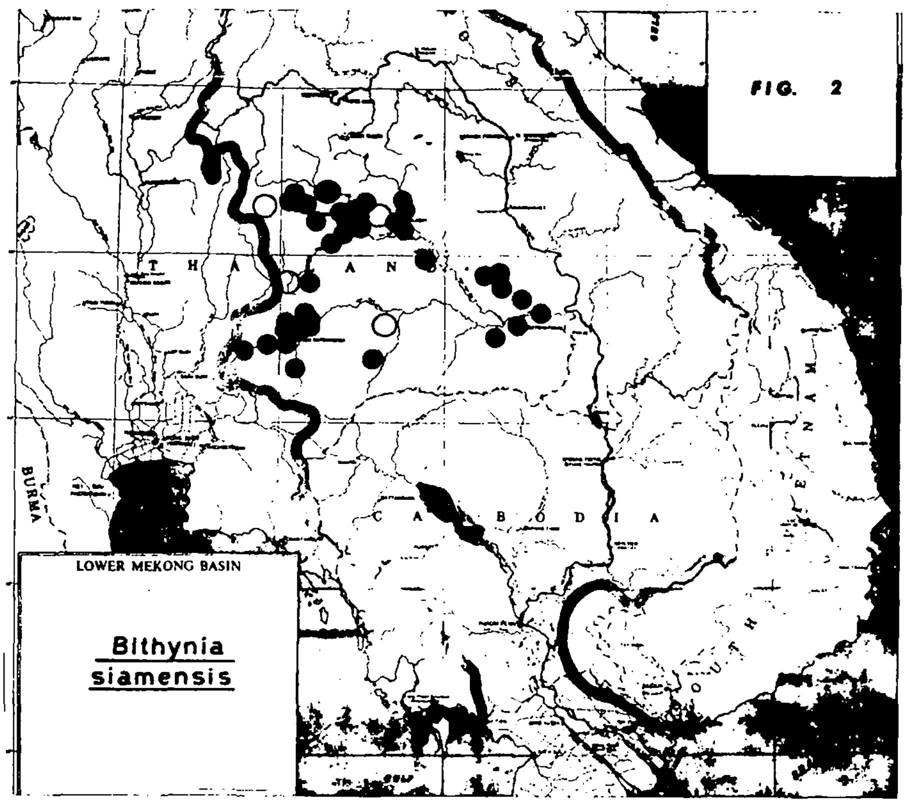


FIG. 2
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Bithynia siamensis

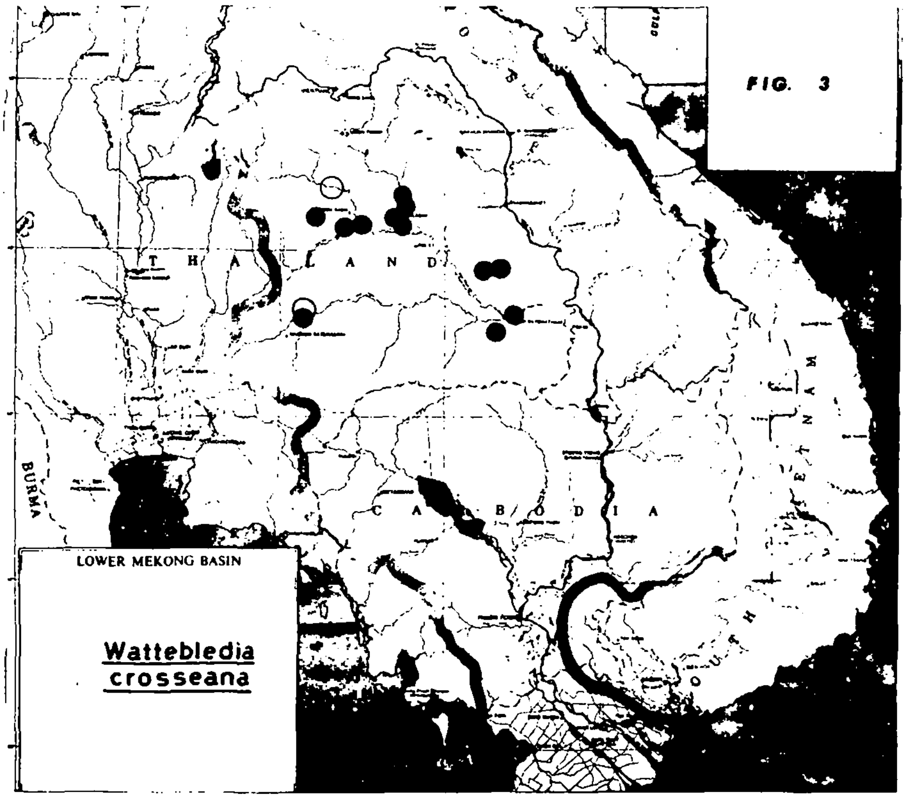


FIG. 3
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Watttebledia crosseana

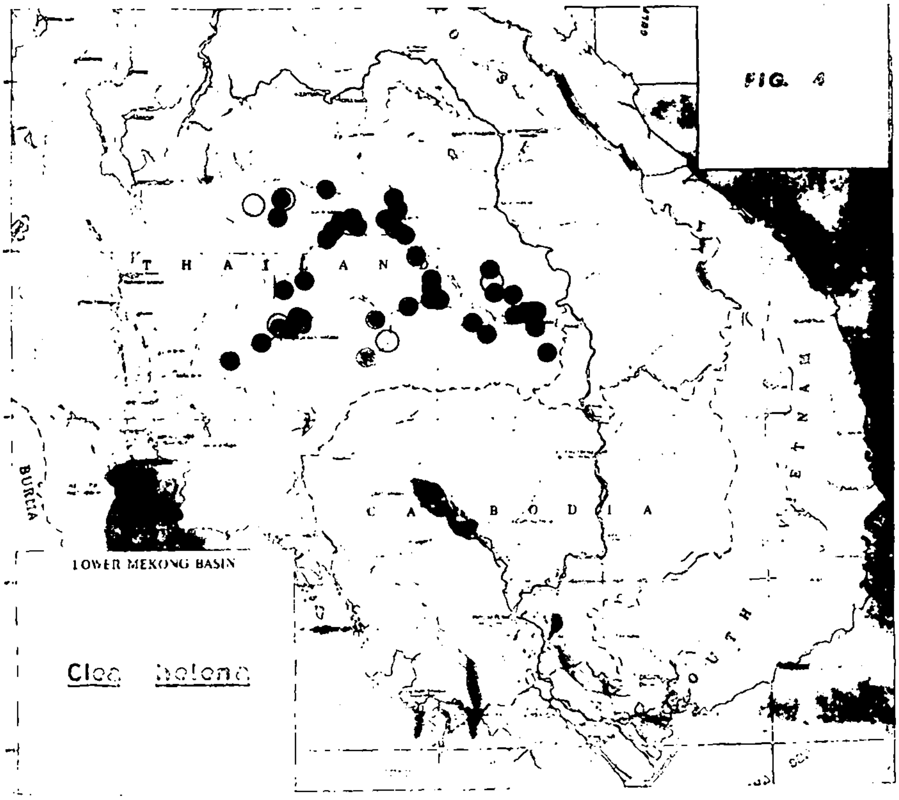


FIG. 4
THAILAND
CAMBODIA
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Clea helena

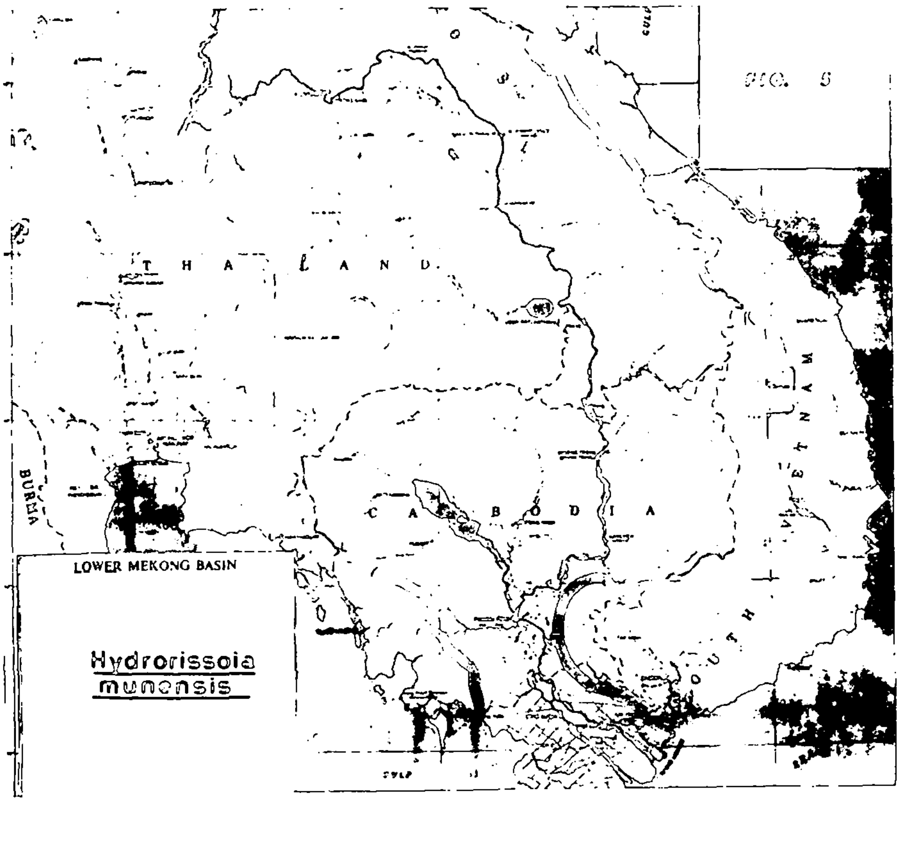
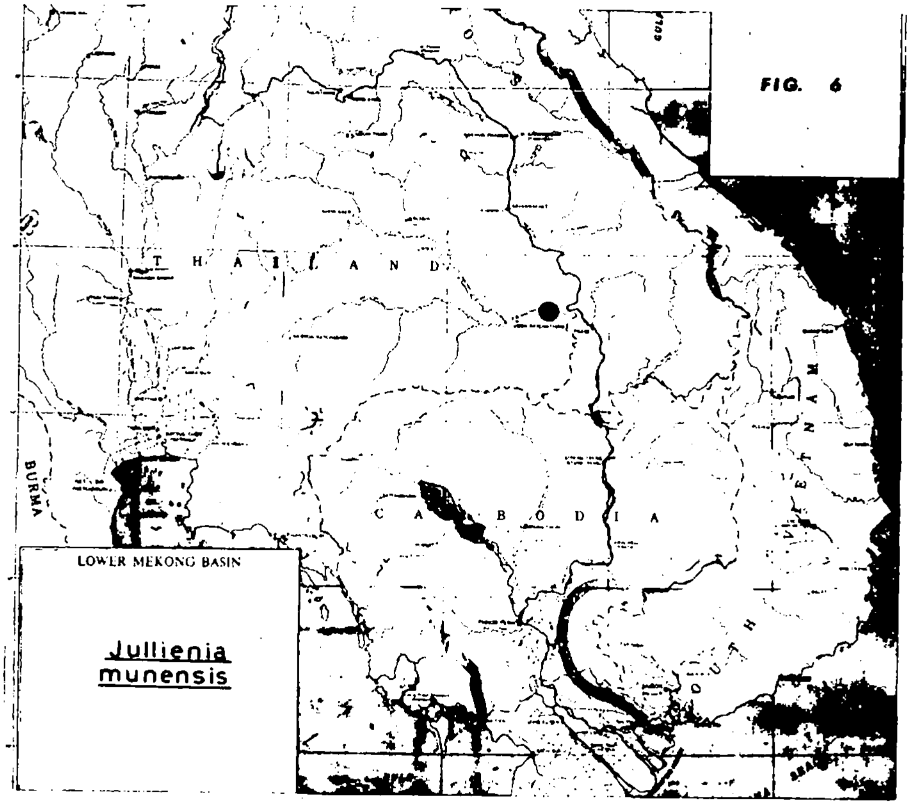
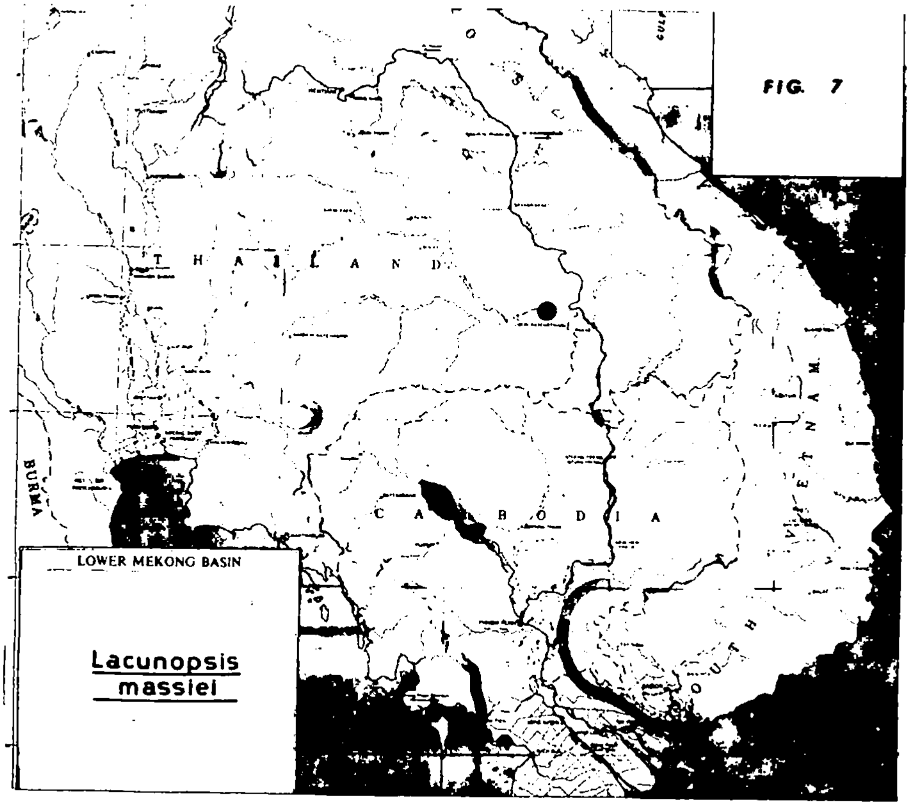


FIG. 5
THAILAND
CAMBODIA
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Hydrorissoia munensis


THAILAND
CAMBODIA
BURMA
LOWER MEKONG BASIN
Jullienia munensis
FIG. 6


THAILAND
CAMBODIA
BURMA
LOWER MEKONG BASIN
Lacunopsis massiei
FIG. 7

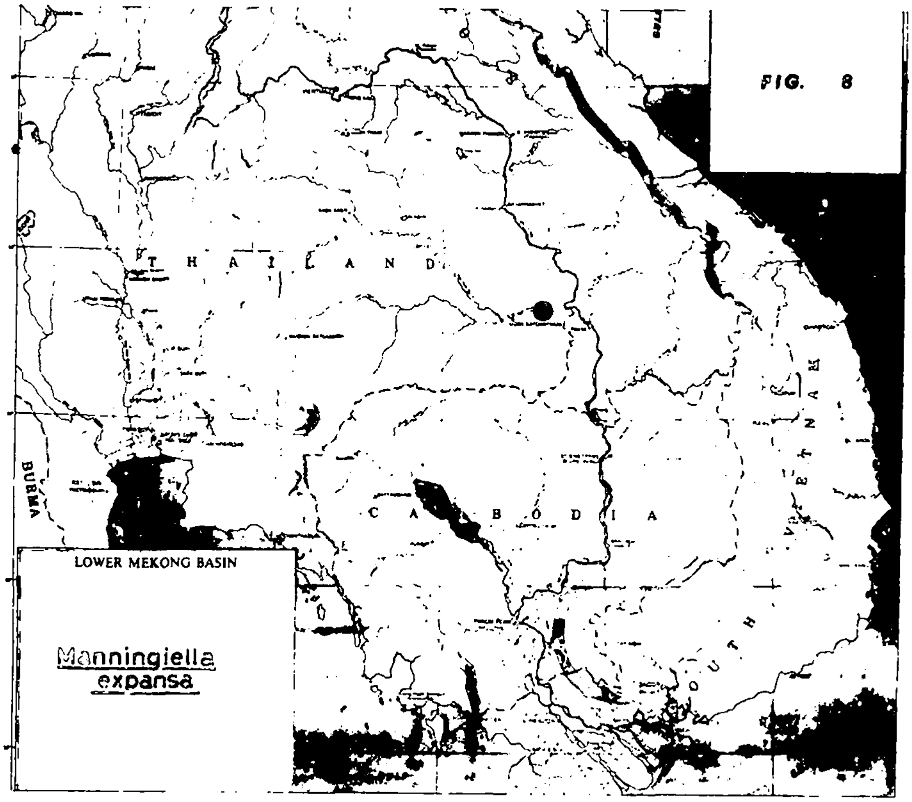


FIG. 8
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Manningiella expansa

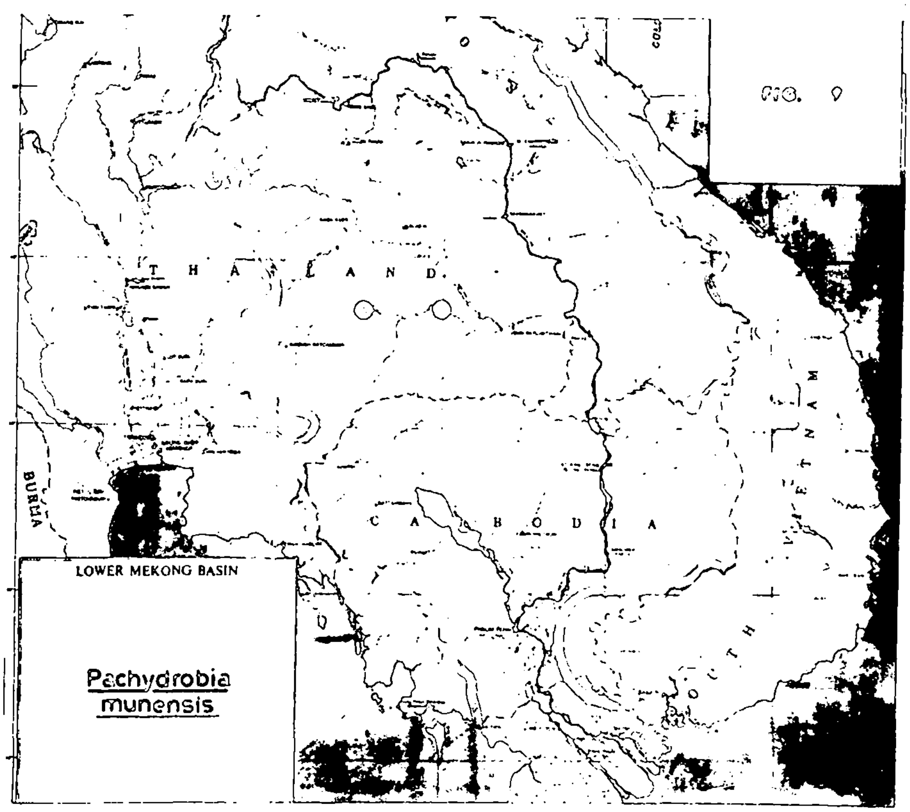


FIG. 9
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Pachydrobia munensis

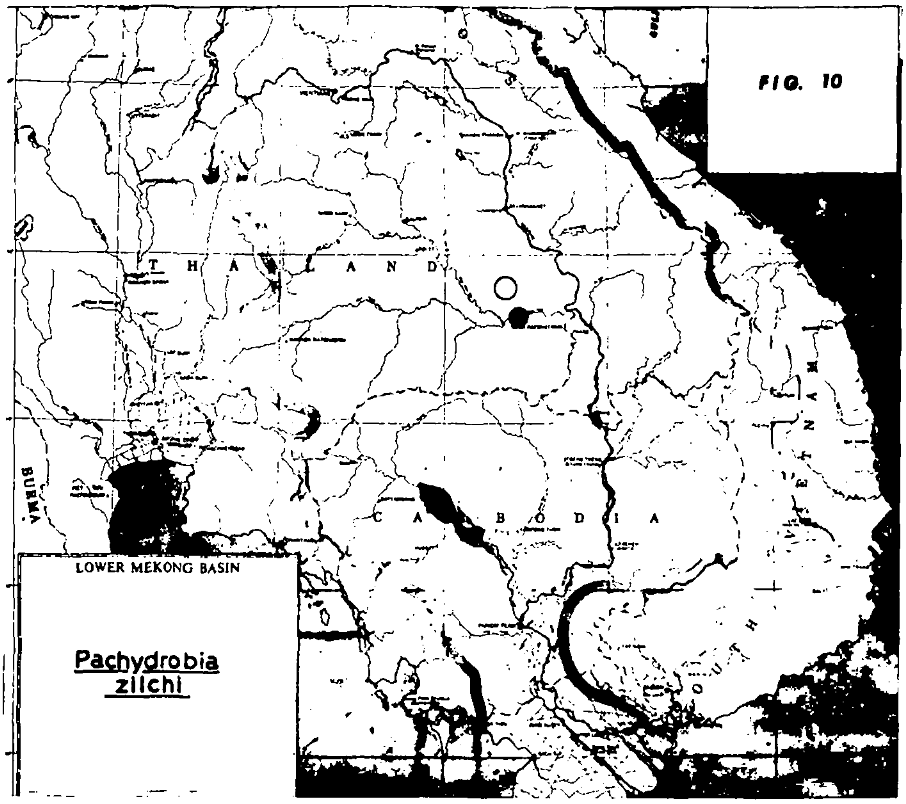


FIG. 10
THAILAND
CAMBODIA
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Pachydrobia zilchi

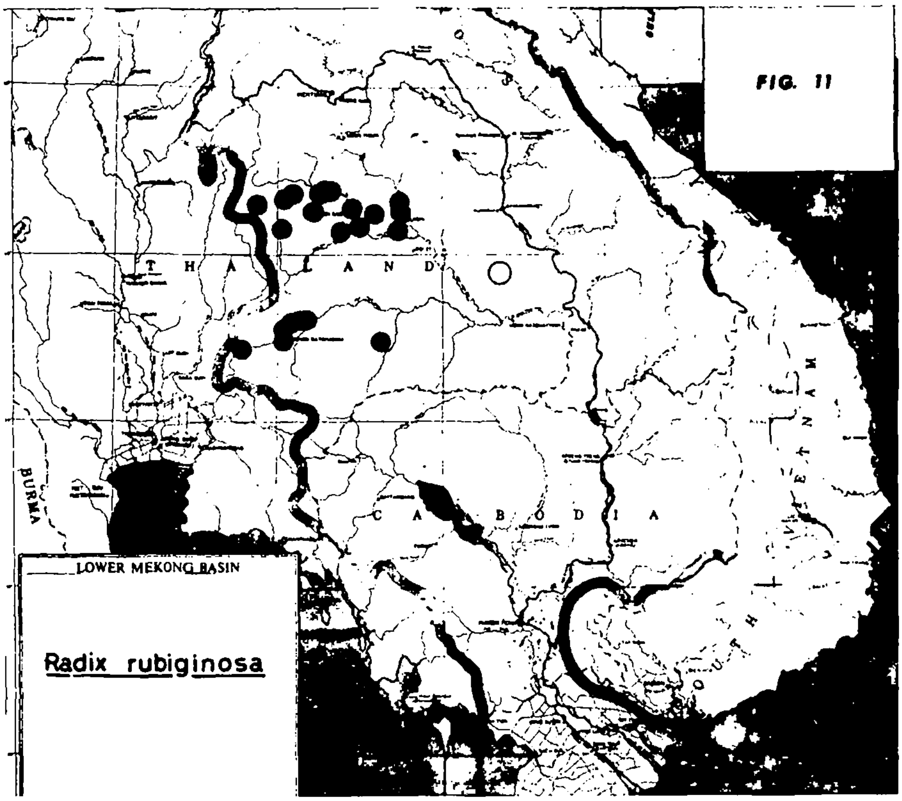


FIG. 11
THAILAND
CAMBODIA
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Radix rubiginosa

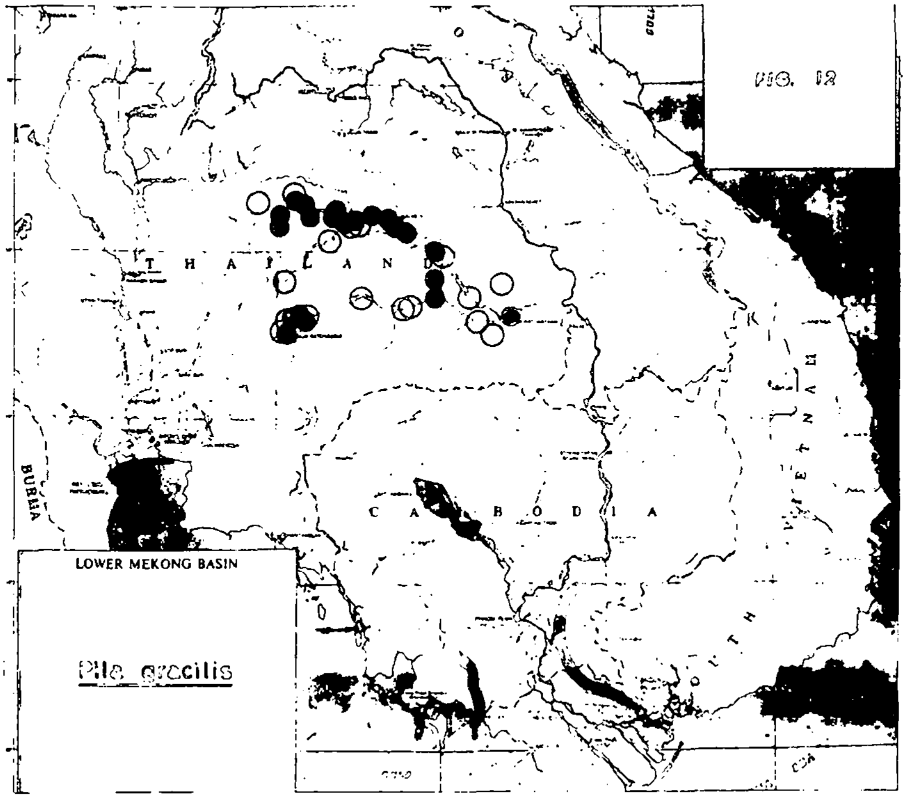


FIG. 12
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Pila gracilis

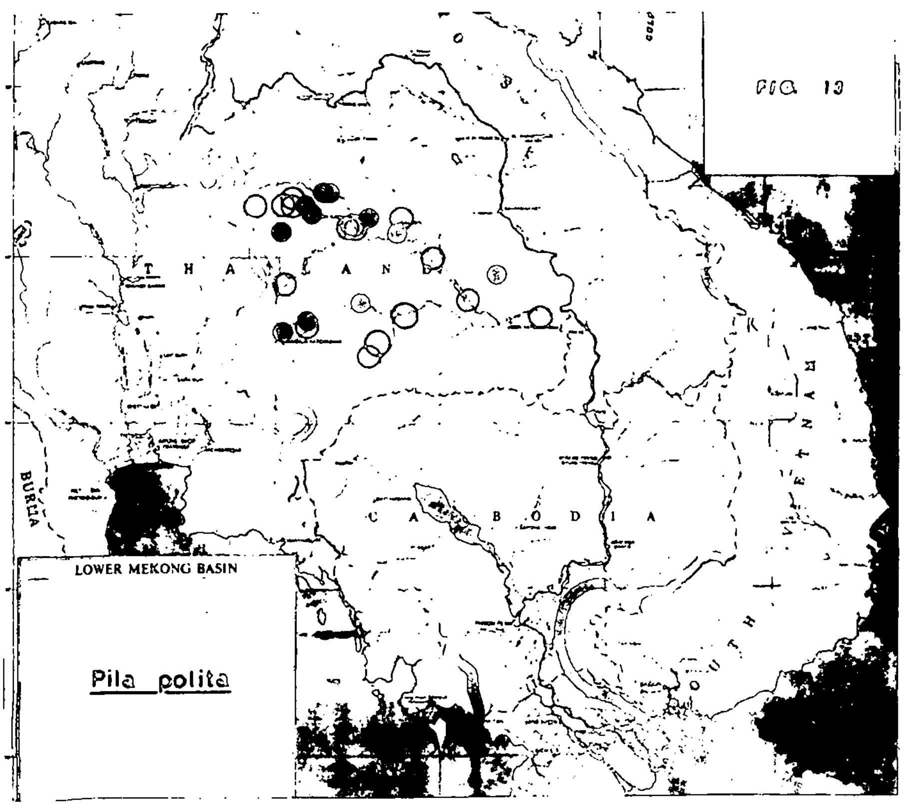


FIG. 13
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Pila polita

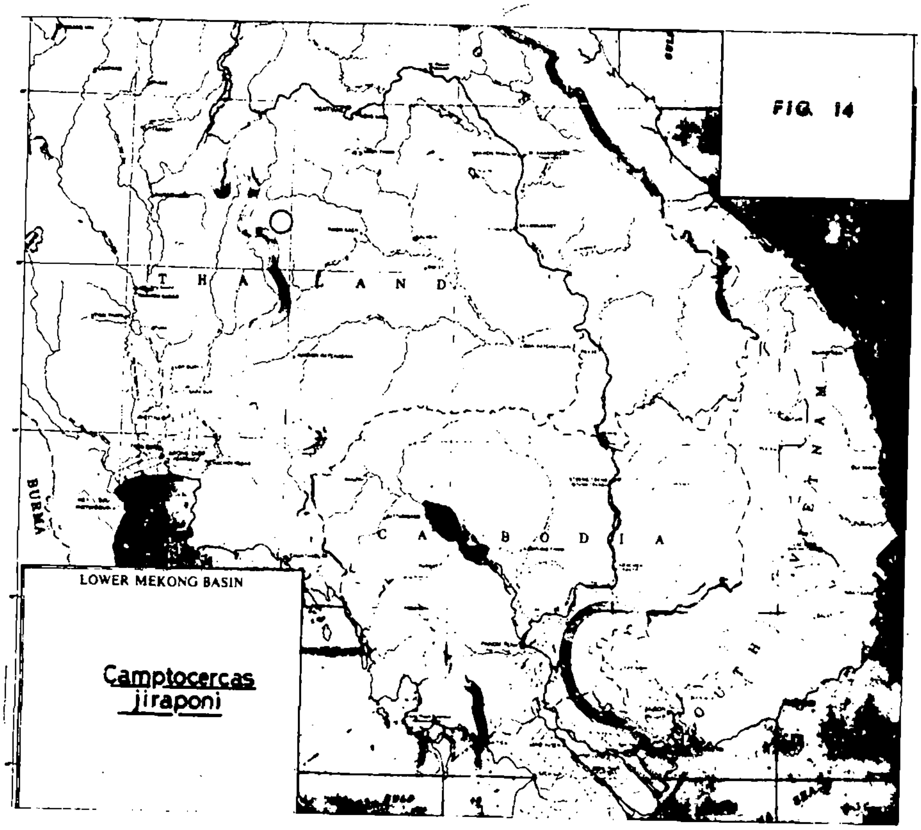


FIG. 14
T H A I L A N D
BURMA
C A M B O D I A
VIETNAM
LOWER MEKONG BASIN
Camptocercas jiraponi

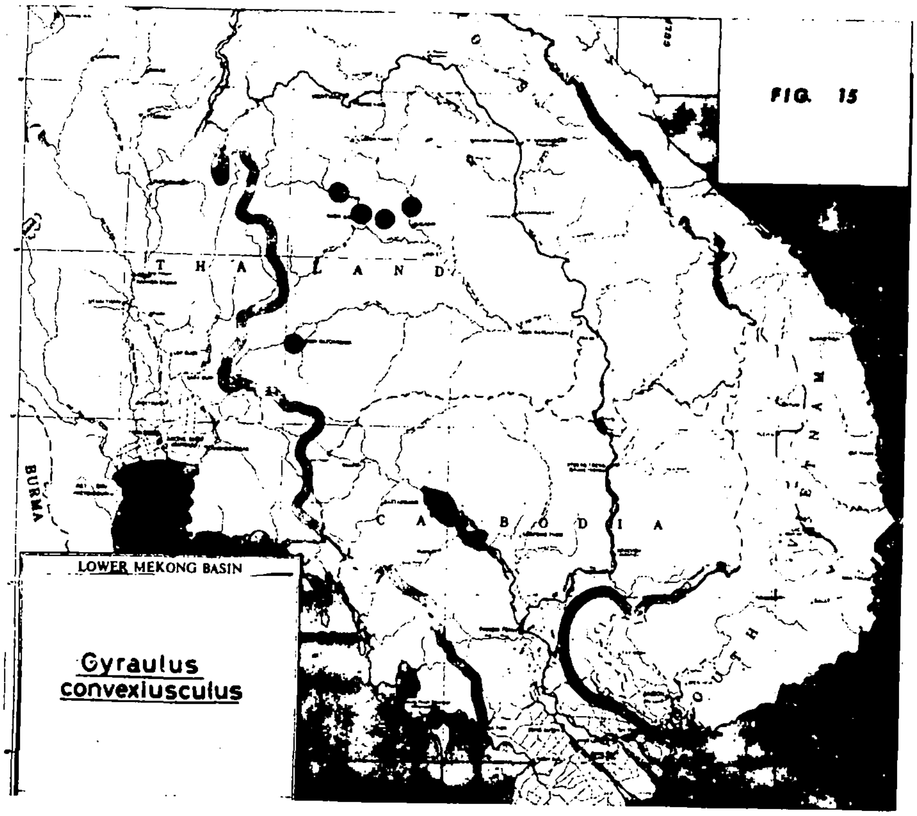


FIG. 15
T H A I L A N D
BURMA
C A M B O D I A
LOWER MEKONG BASIN
Gyraulus convexiusculus

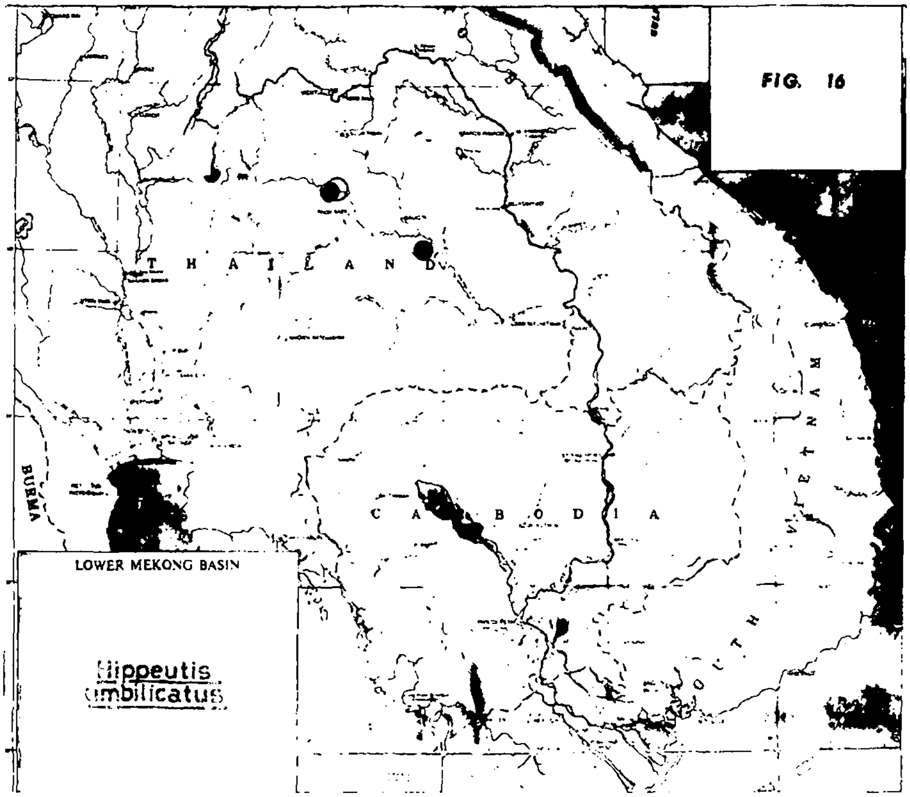


FIG. 16
THAILAND
BURMA
CAMBODIA
SOUTH VIETNAM
LOWER MEKONG BASIN
Hippeutis umbilicatus

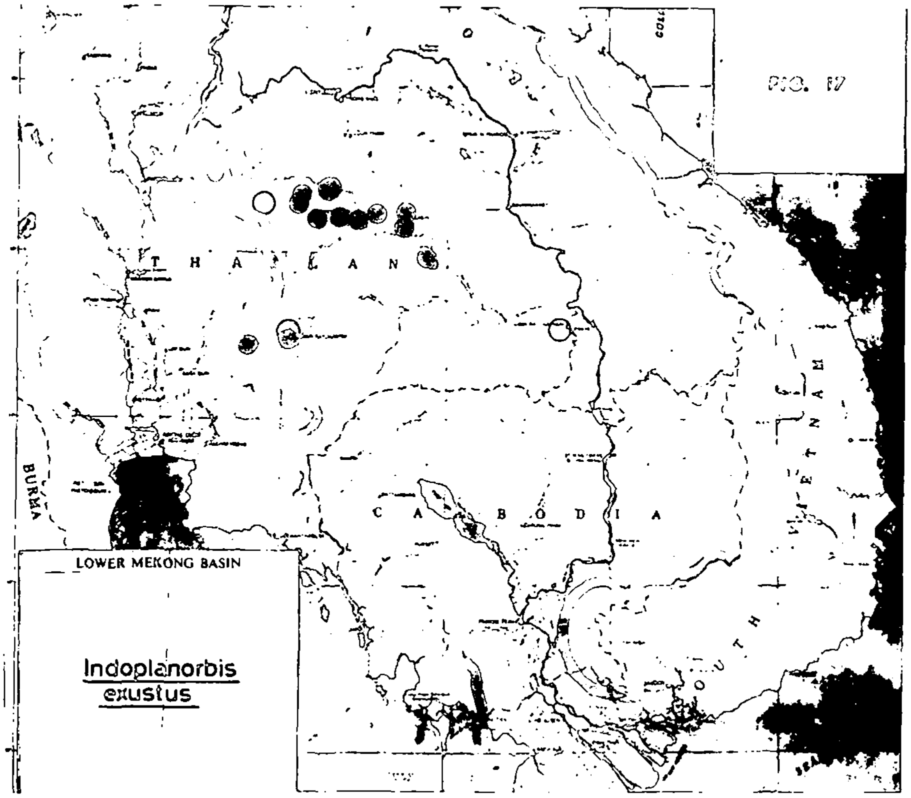


FIG. 17
THAILAND
BURMA
CAMBODIA
SOUTH VIETNAM
LOWER MEKONG BASIN
Indoplanorbis exustus

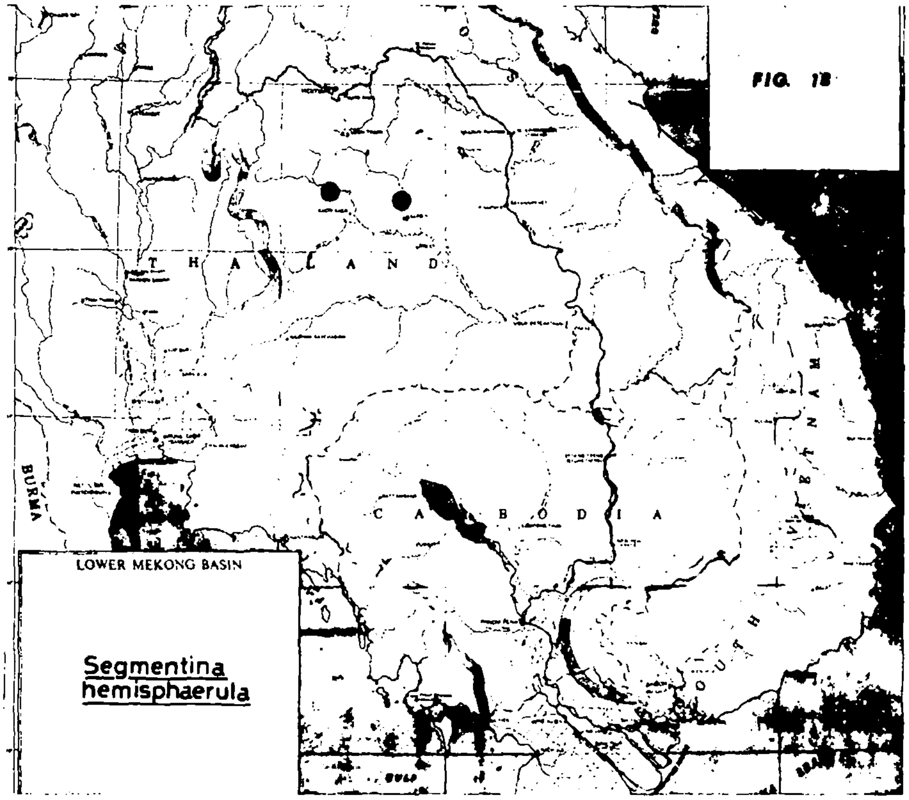


FIG. 18
THAILAND
BURMA
CAMBODIA
SOUTH VIETNAM
LOWER MEKONG BASIN
Segmentina hemisphaerula

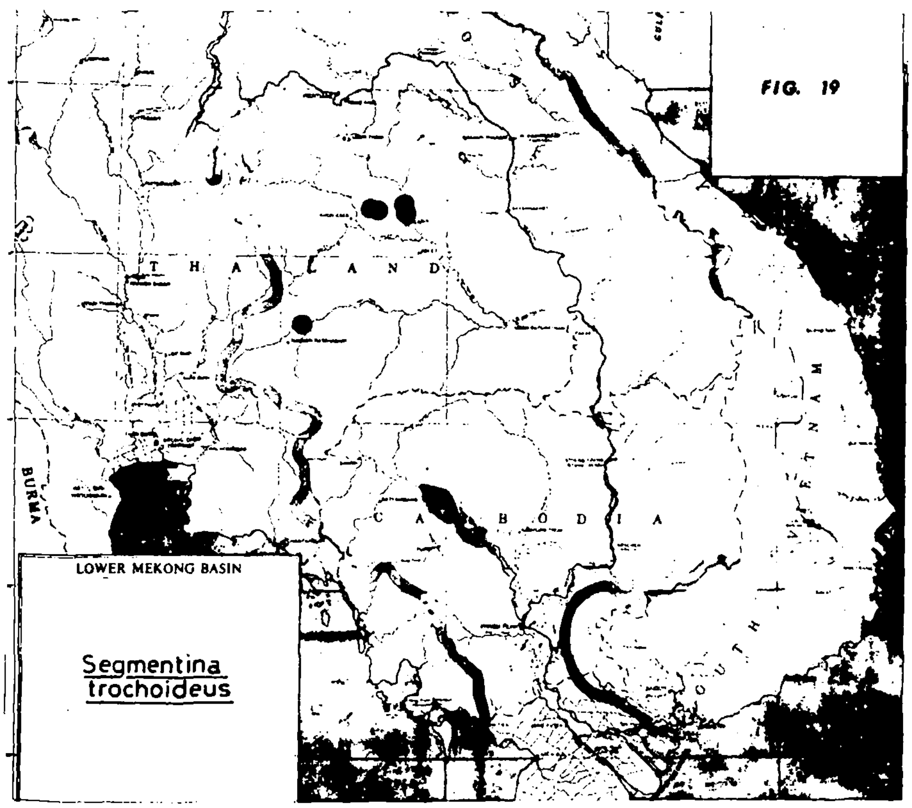


FIG. 19
THAILAND
BURMA
CAMBODIA
SOUTH VIETNAM
LOWER MEKONG BASIN
Segmentina trochoideus

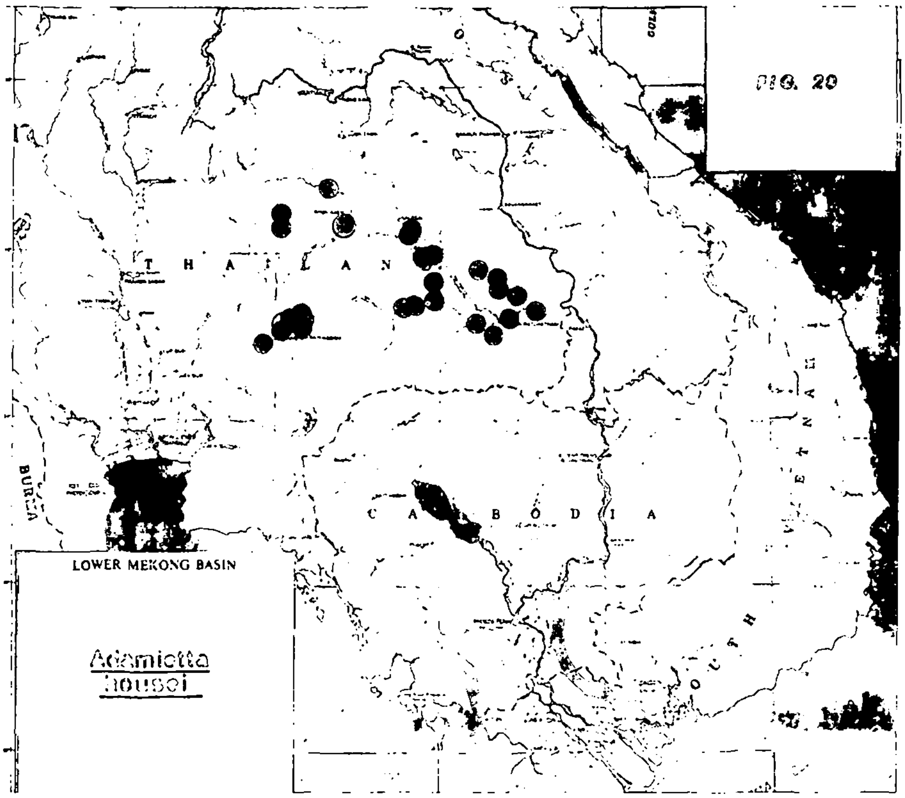


FIG. 20
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Adamietta housei

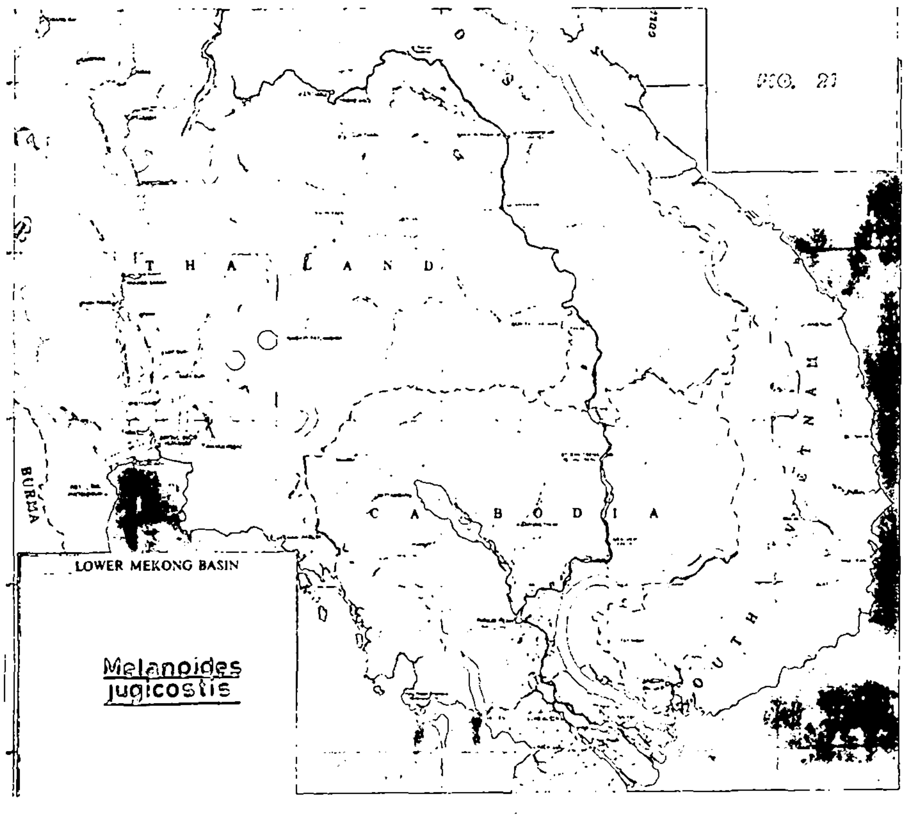


FIG. 21
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Melanoides jugicostis

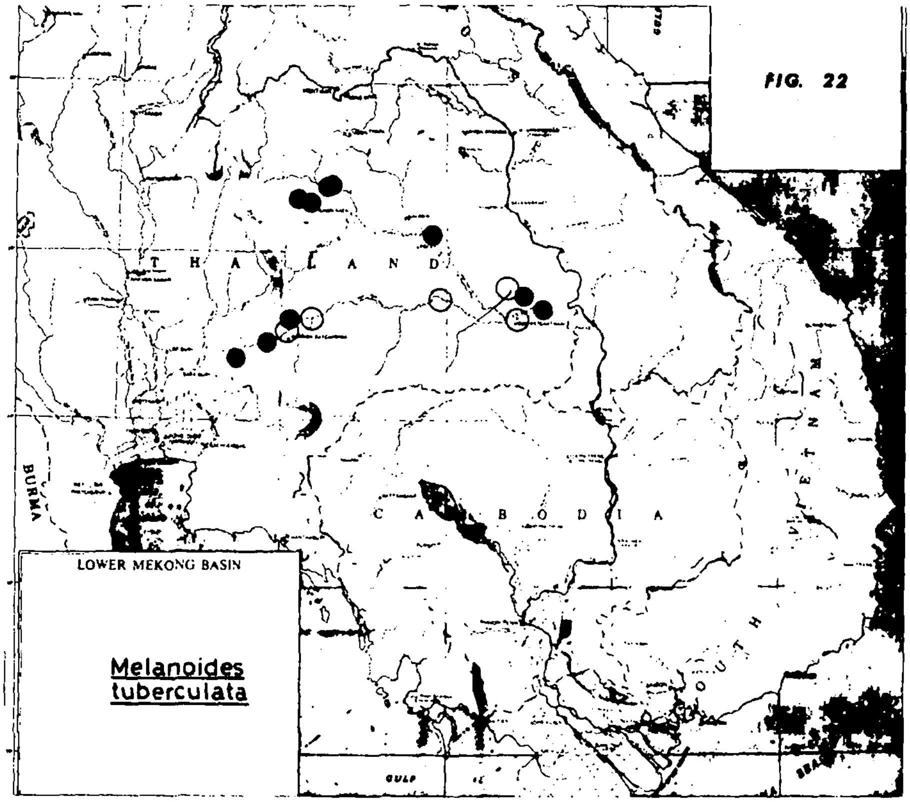


FIG. 22
THAILAND
CAMBODIA
BURMA
SOUTH VIETNAM
GULF
LOWER MEKONG BASIN
Melanoides tuberculata

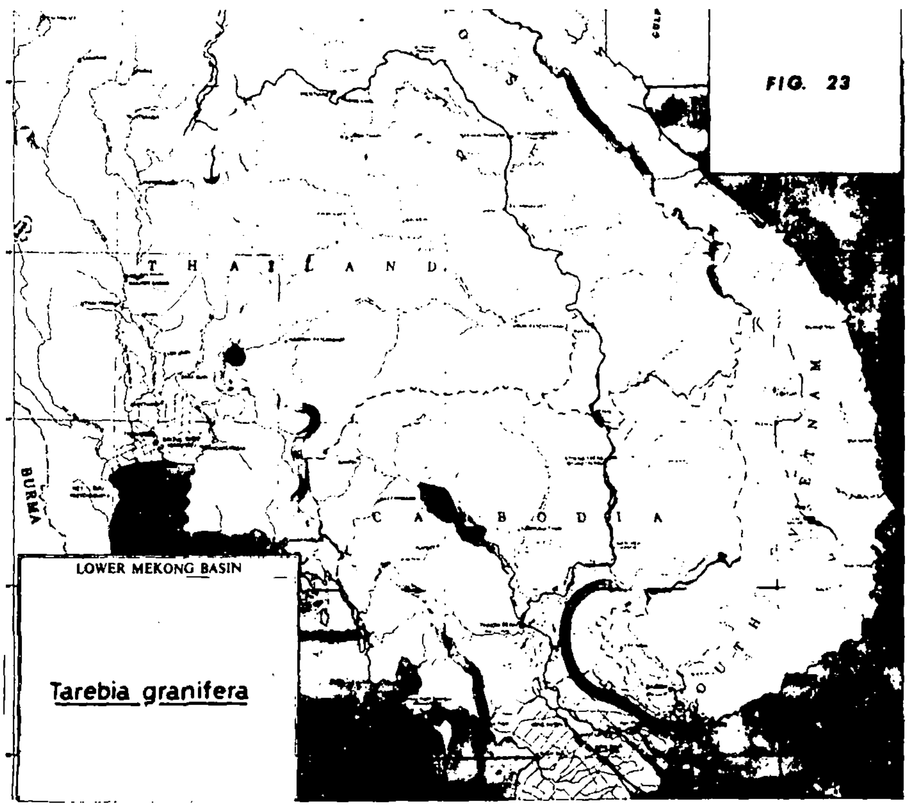


FIG. 23
THAILAND
CAMBODIA
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Tarebia granifera

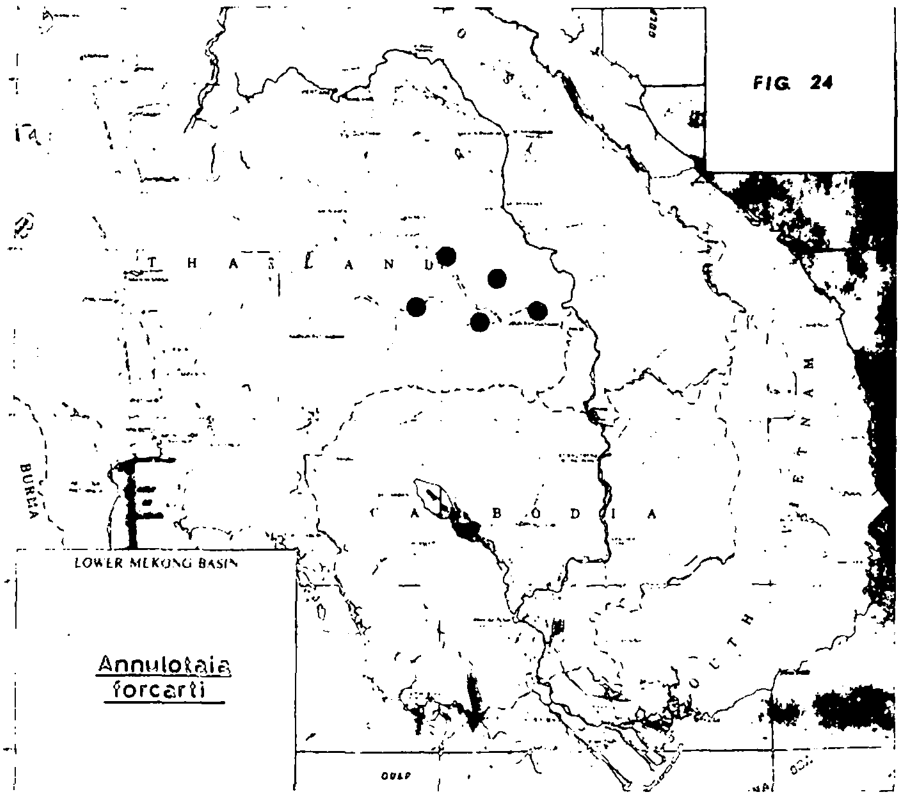


FIG. 24
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Annulotaia forcarti

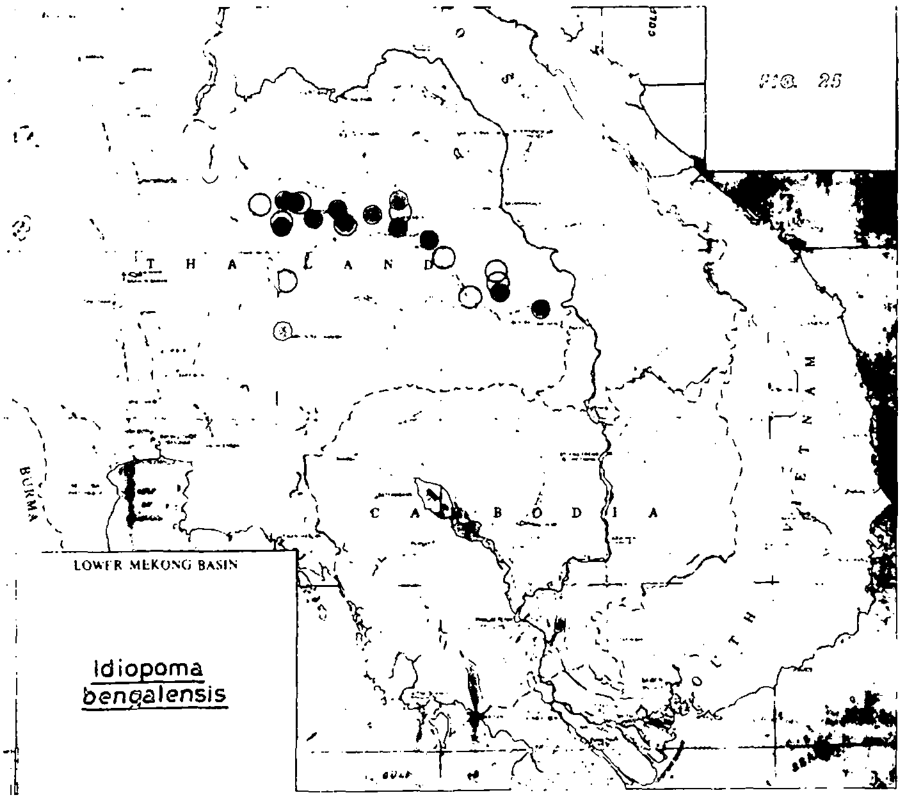


FIG. 25
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Idiopoma bengalensis

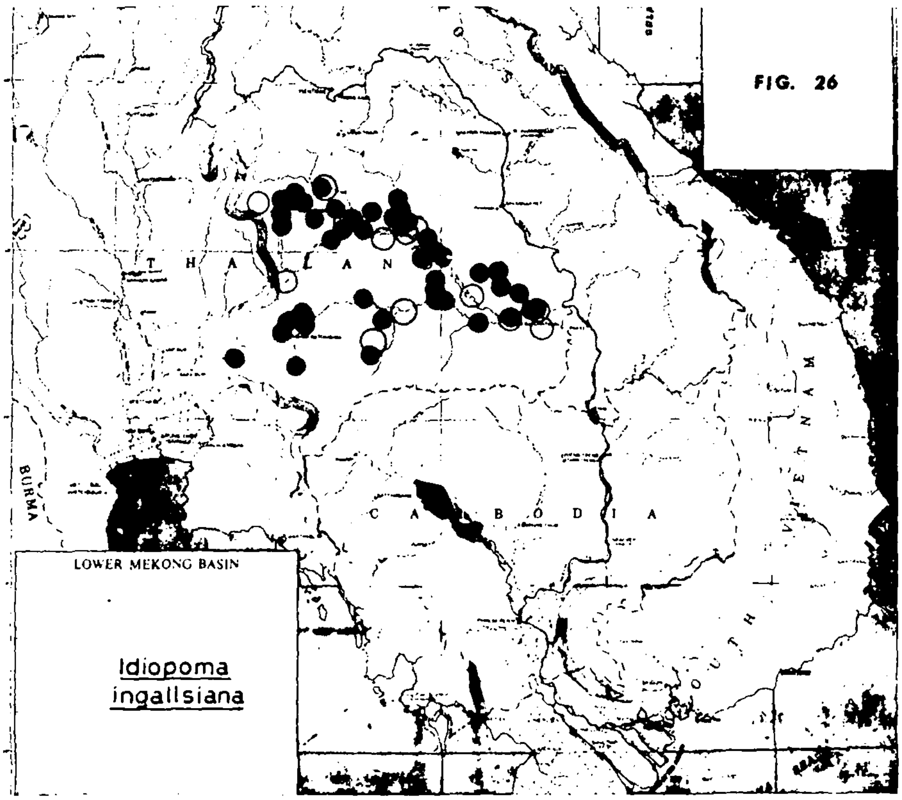


FIG. 26
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Idiopoma ingallsiana

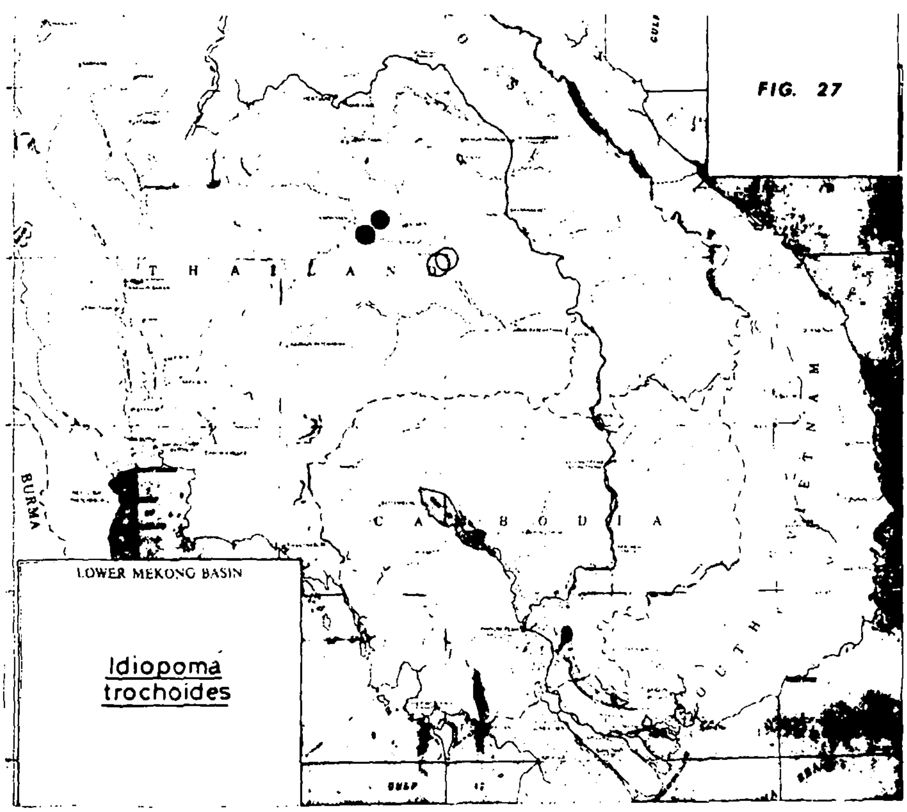


FIG. 27
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Idiopoma trochoides

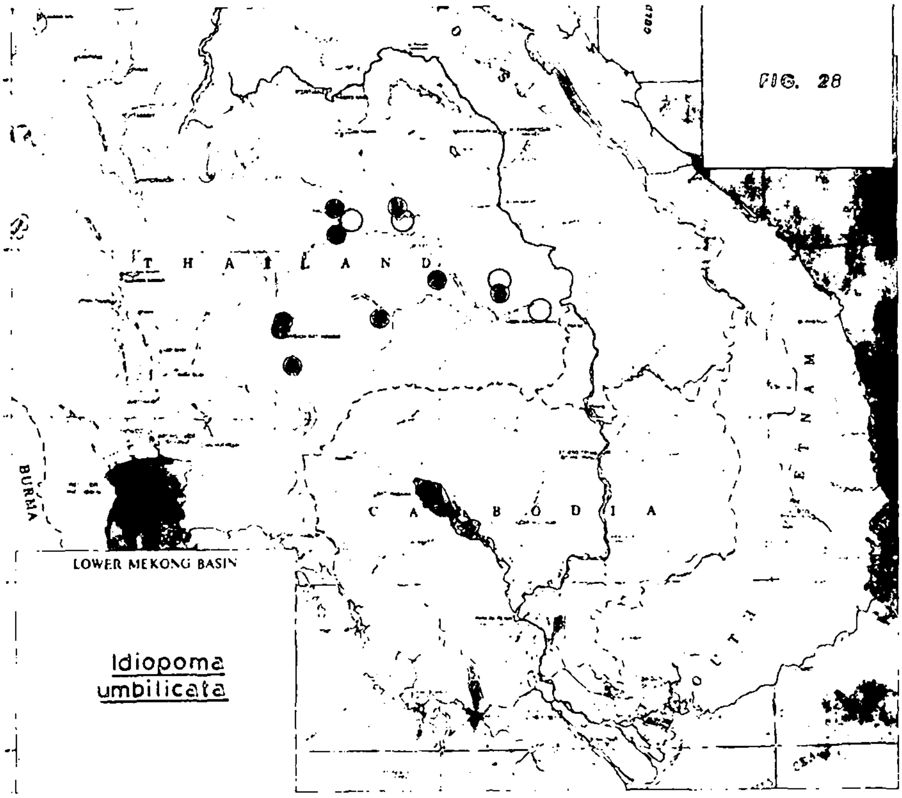


FIG. 28
THAILAND
CAMBODIA
BURMA
VIETNAM
LOWER MEKONG BASIN
Idiopoma umbilicata

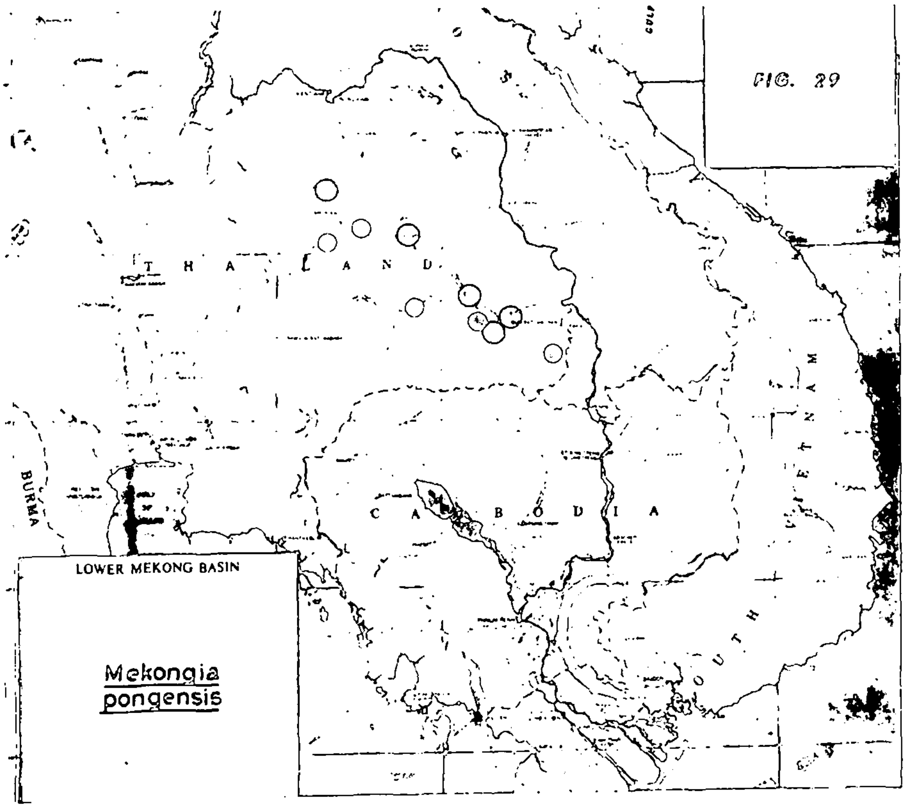


FIG. 29
THAILAND
CAMBODIA
BURMA
VIETNAM
LOWER MEKONG BASIN
Mekongia pongensis

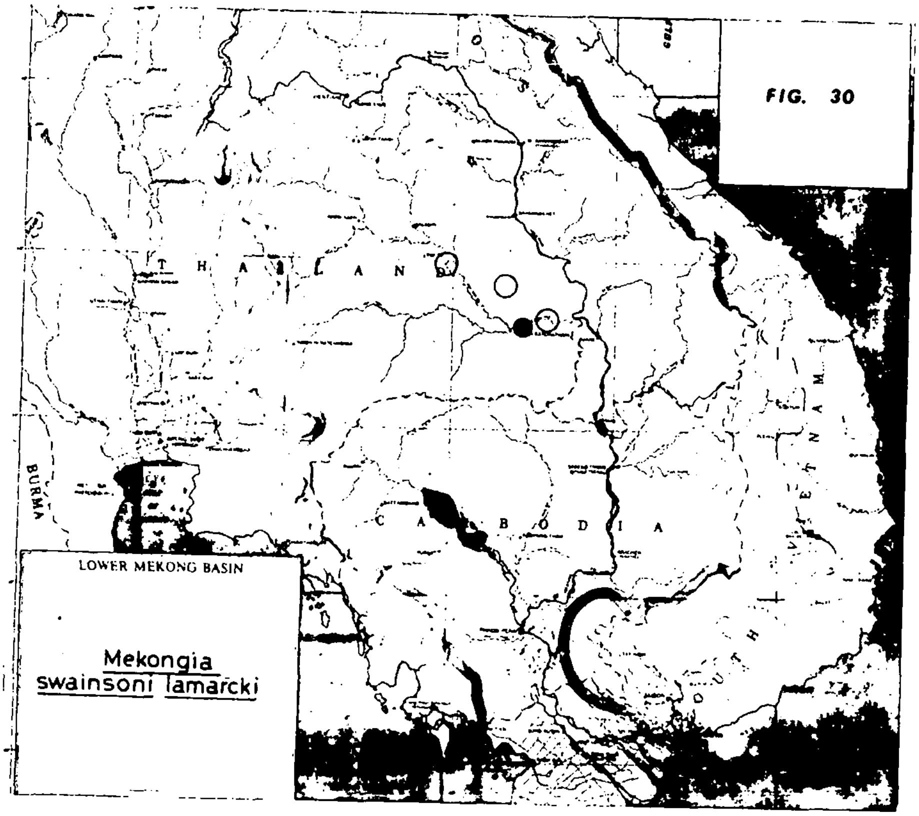

THAILAND
BURMA
CAMBODIA
SOUTH VIETNAM
LOWER MEKONG BASIN
Mekongia swainsoni lamarcki

FIG. 30

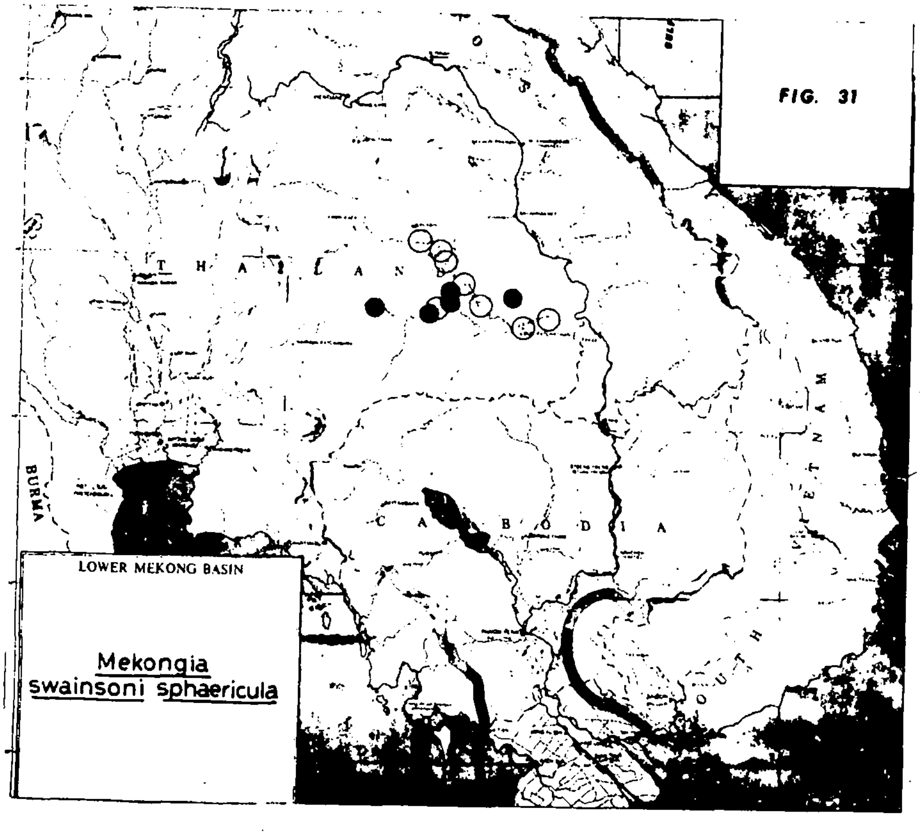

THAILAND
BURMA
CAMBODIA
SOUTH VIETNAM
LOWER MEKONG BASIN
Mekongia swainsoni sphaericula

FIG. 31

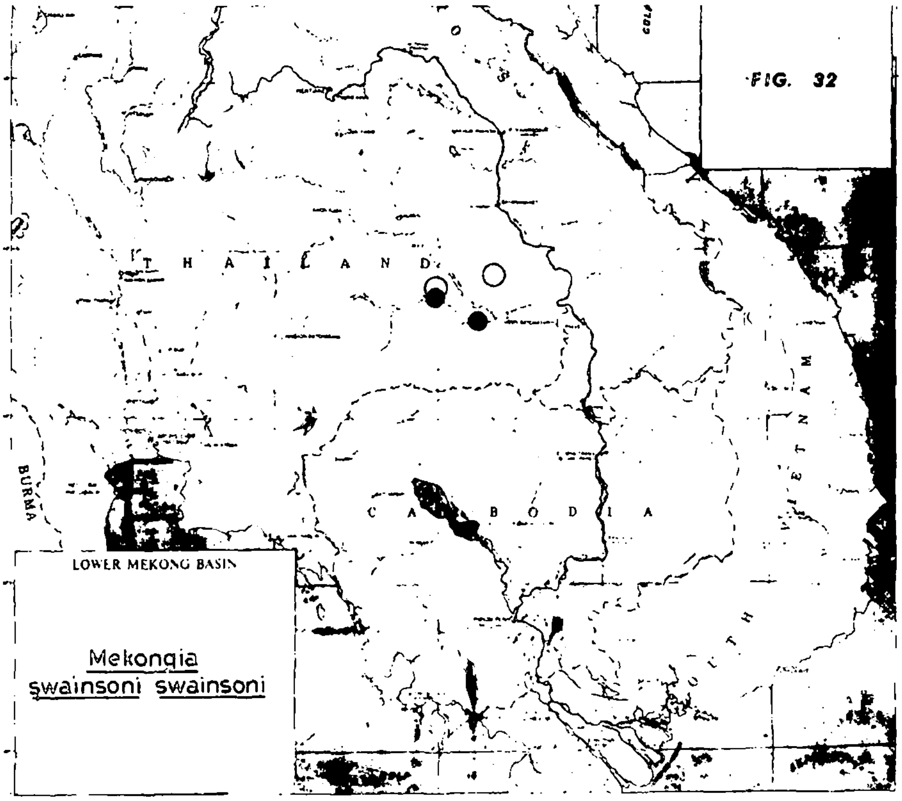


FIG. 32
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Mekongia swainsoni swainsoni

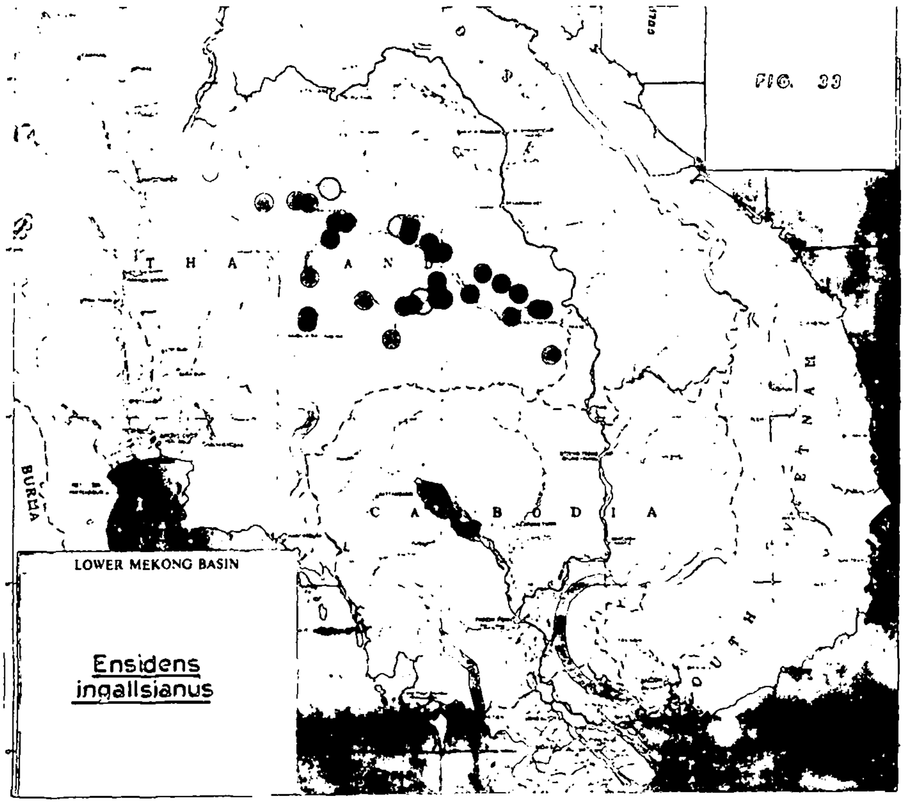


FIG. 33
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Ensidens ingallsianus

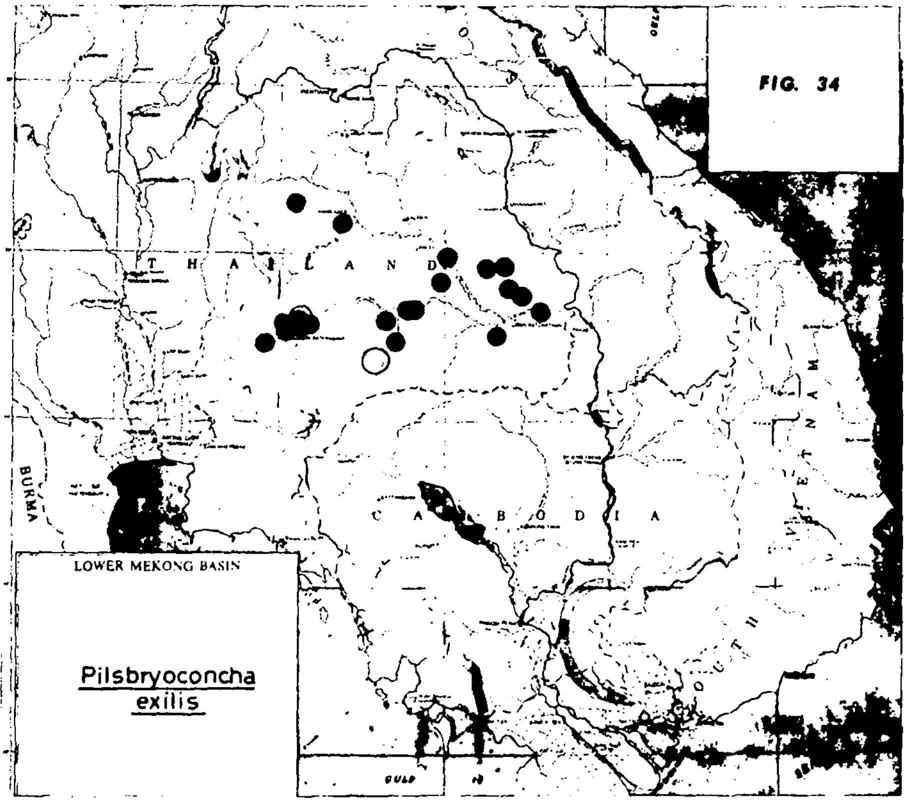


FIG. 34
THAILAND
CAMBODIA
BURMA
VIETNAM
LOWER MEKONG BASIN
Pilsbryoconcha exilis

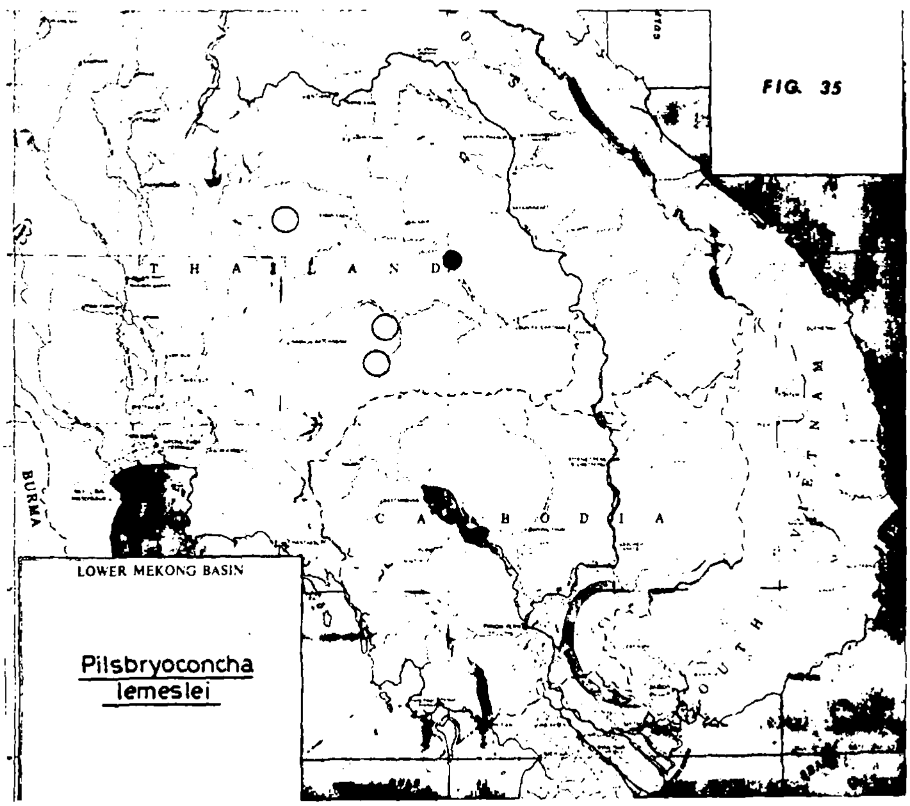


FIG. 35
THAILAND
CAMBODIA
BURMA
VIETNAM
LOWER MEKONG BASIN
Pilsbryoconcha lemeslei

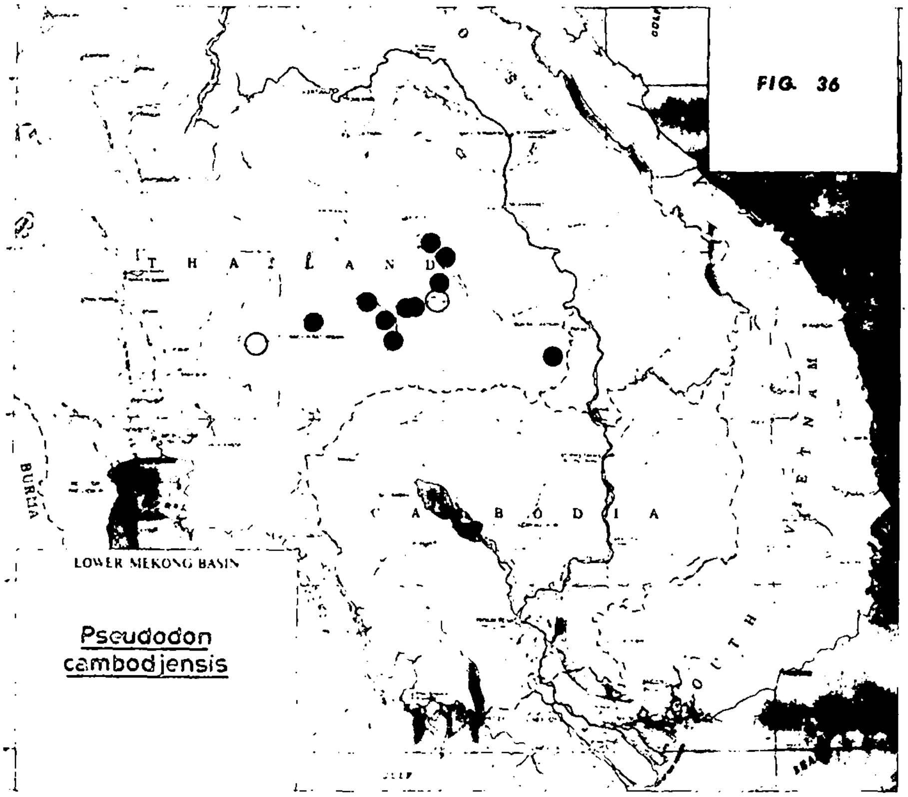


FIG. 36

Pseudodon cambodjensis

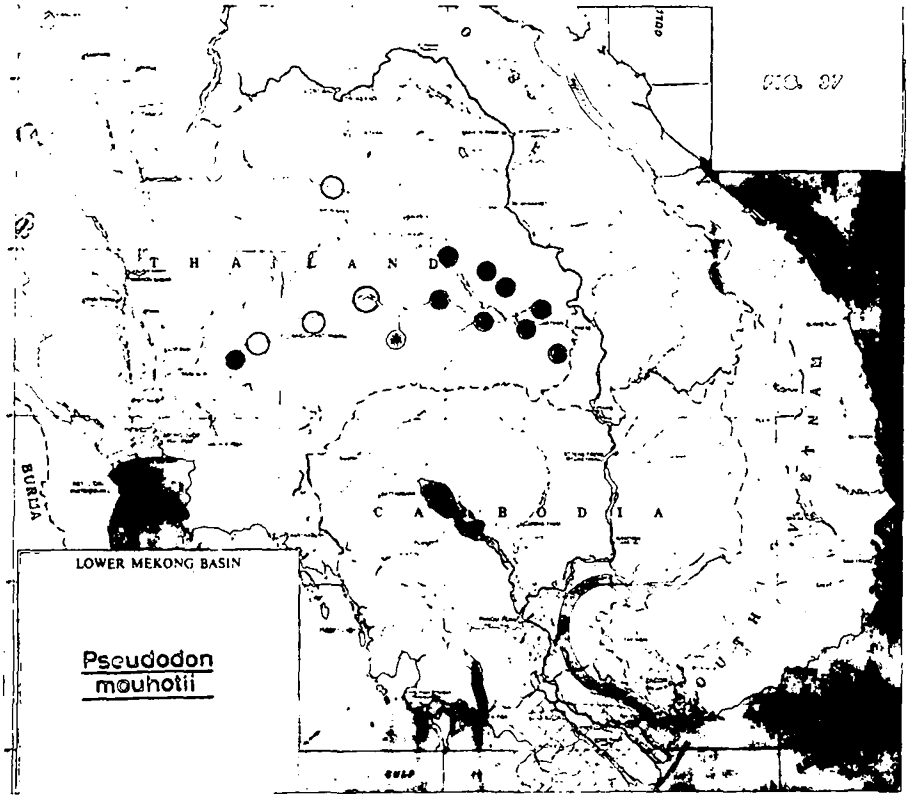


FIG. 37

Pseudodon mouhotii

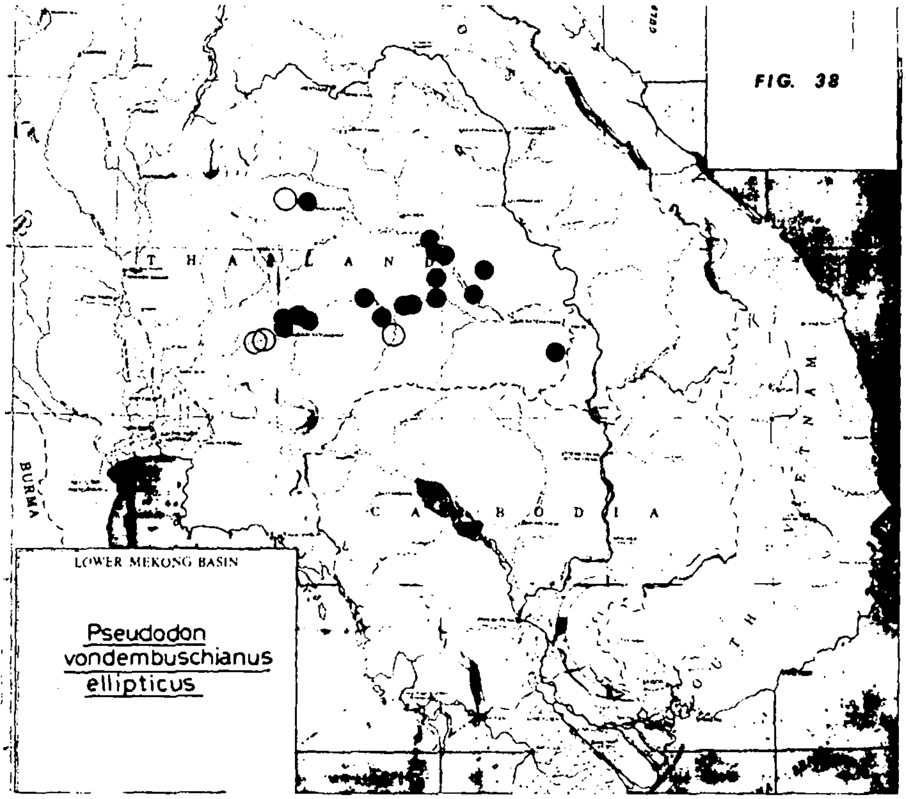


FIG. 38
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Pseudodon vondembuschianus ellipticus

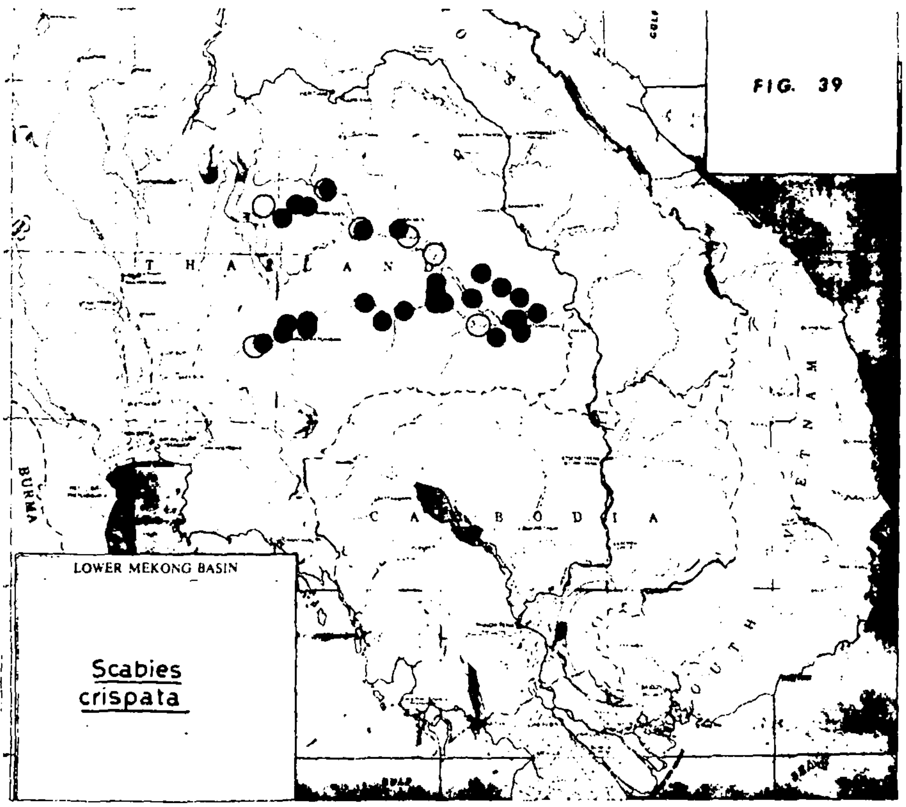


FIG. 39
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Scabies crispata

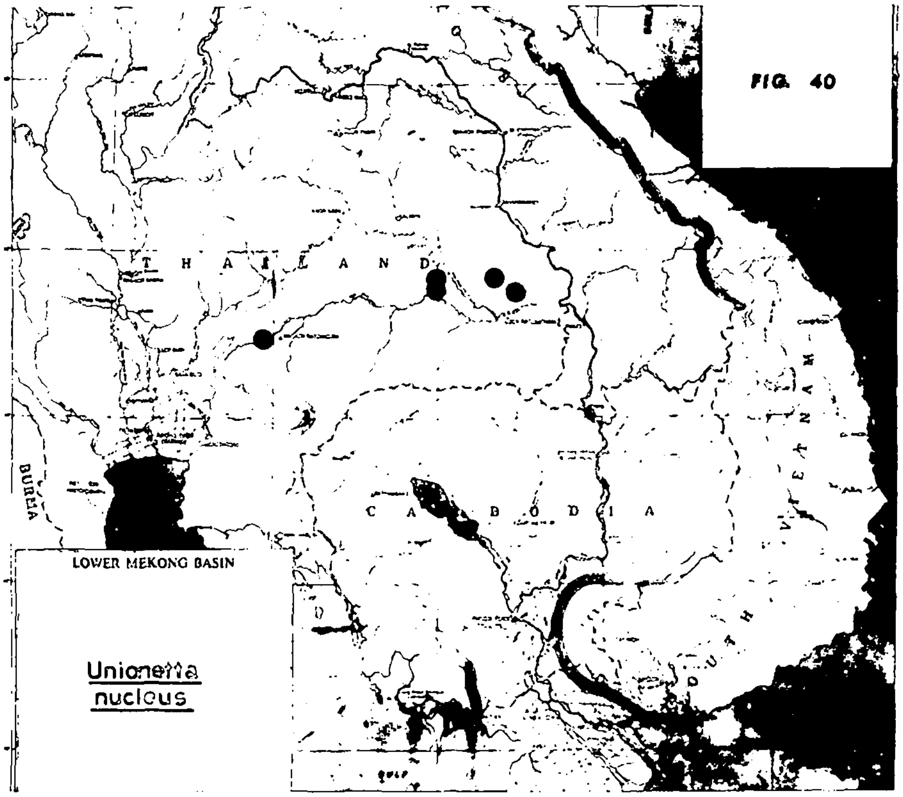


FIG. 40
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Unionetta nucleus

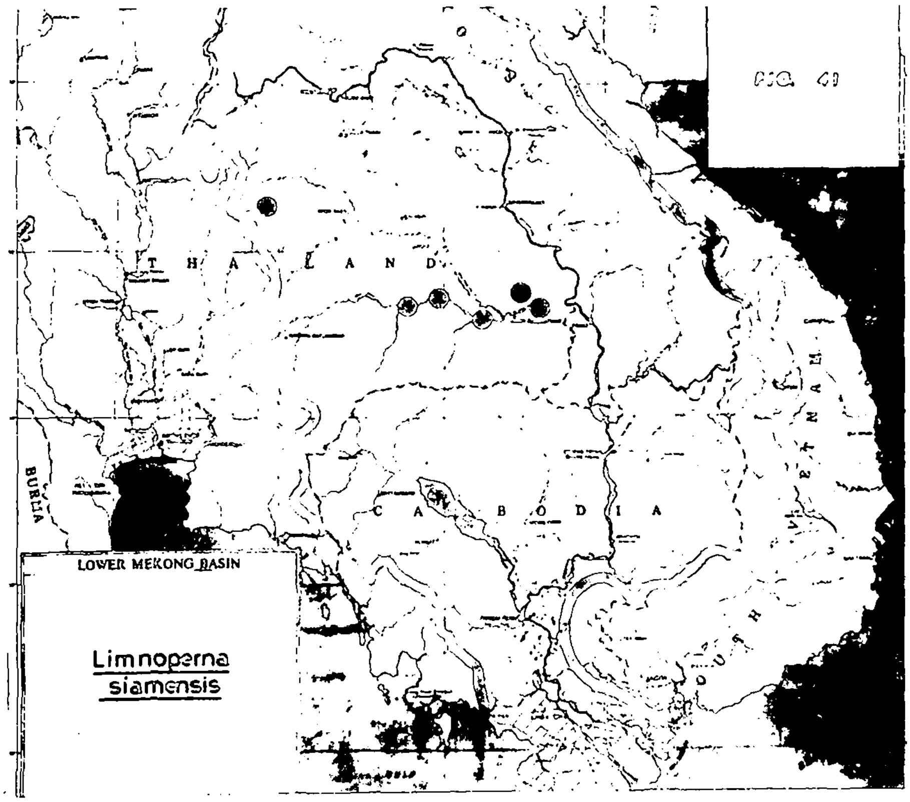


FIG. 41
THAILAND
CAMBODIA
SOUTH VIETNAM
BURMA
LOWER MEKONG BASIN
Limnoperna siamensis

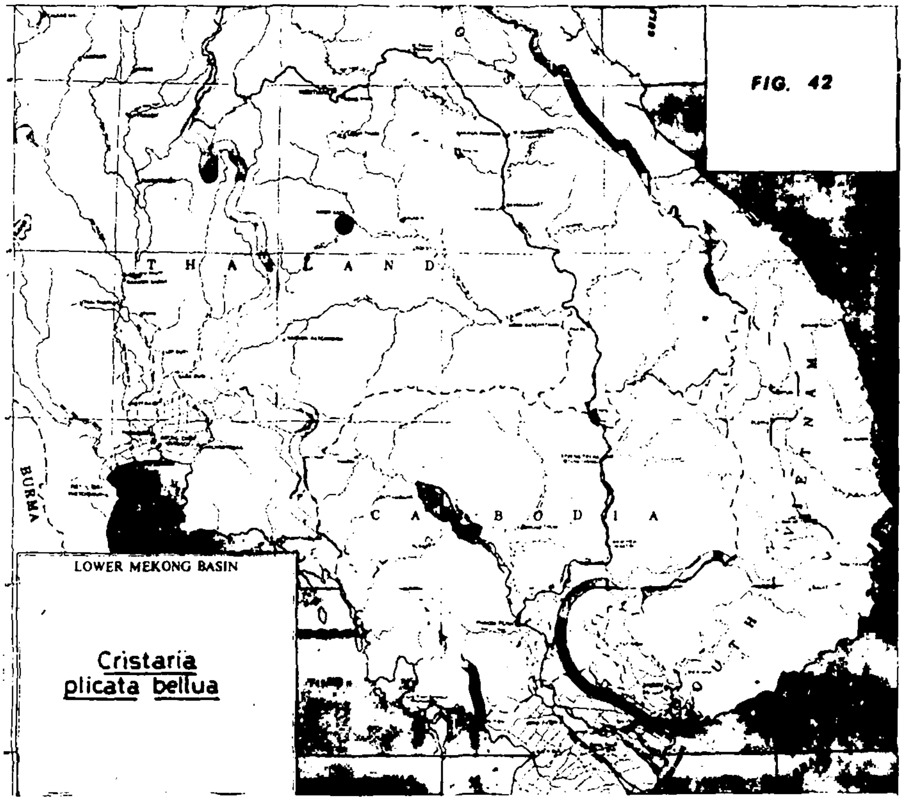


FIG. 42

Cristaria plicata bellua

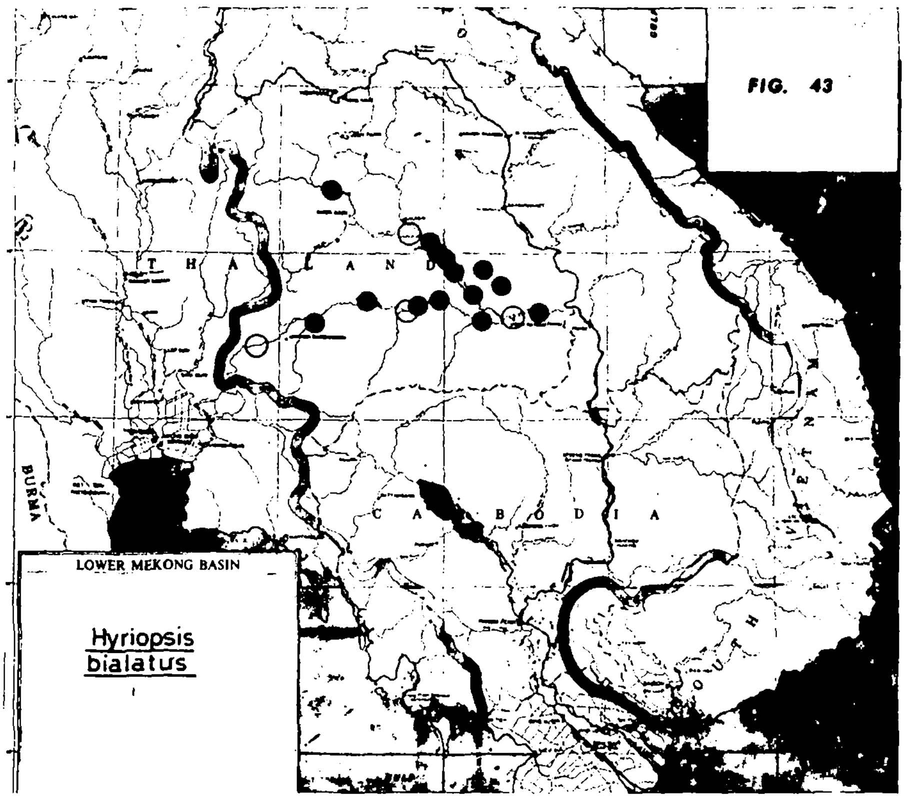


FIG. 43

Hyriopsis bialatus

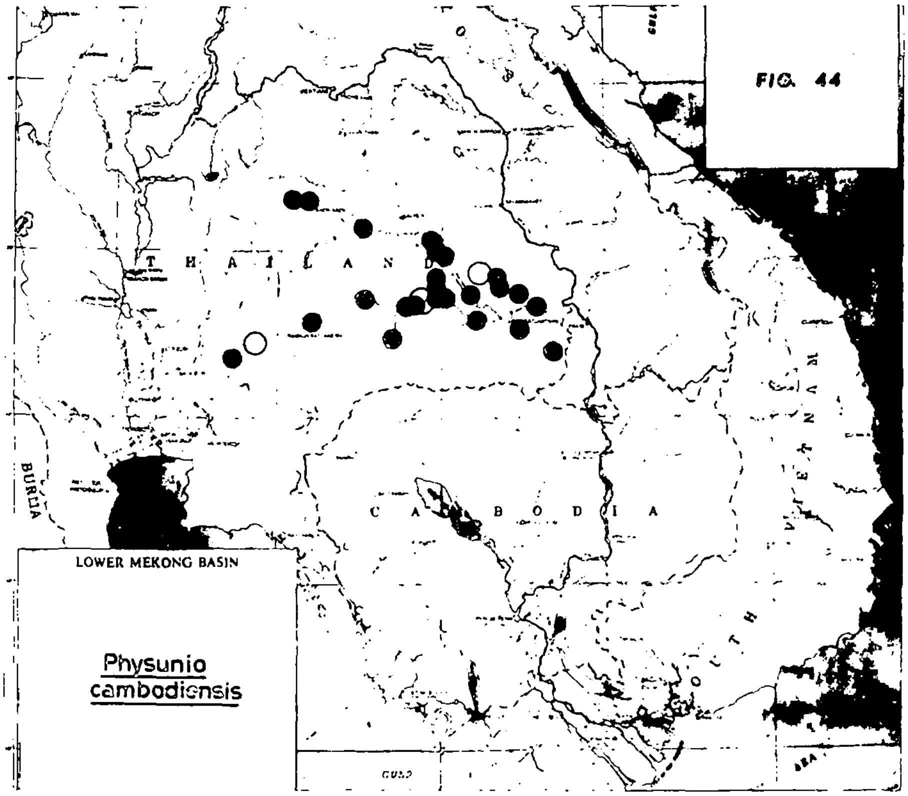


FIG. 44
T H A I L A N D
C A M B O D I A
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Physunio cambodiensis

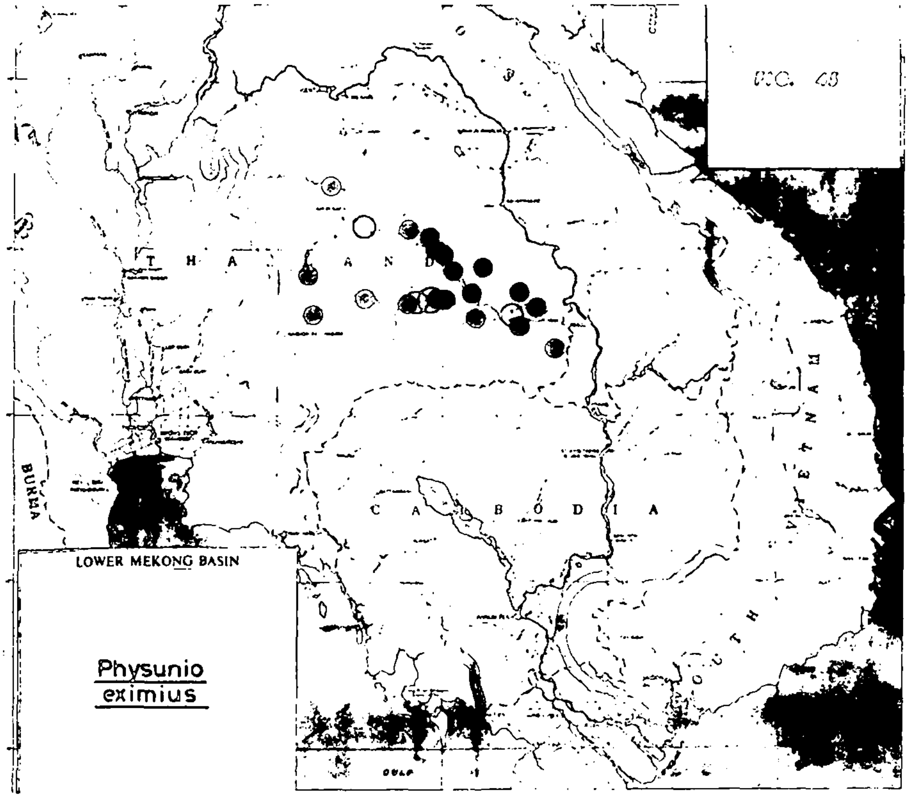


FIG. 43
T H A I L A N D
C A M B O D I A
BURMA
SOUTH VIETNAM
LOWER MEKONG BASIN
Physunio eximius

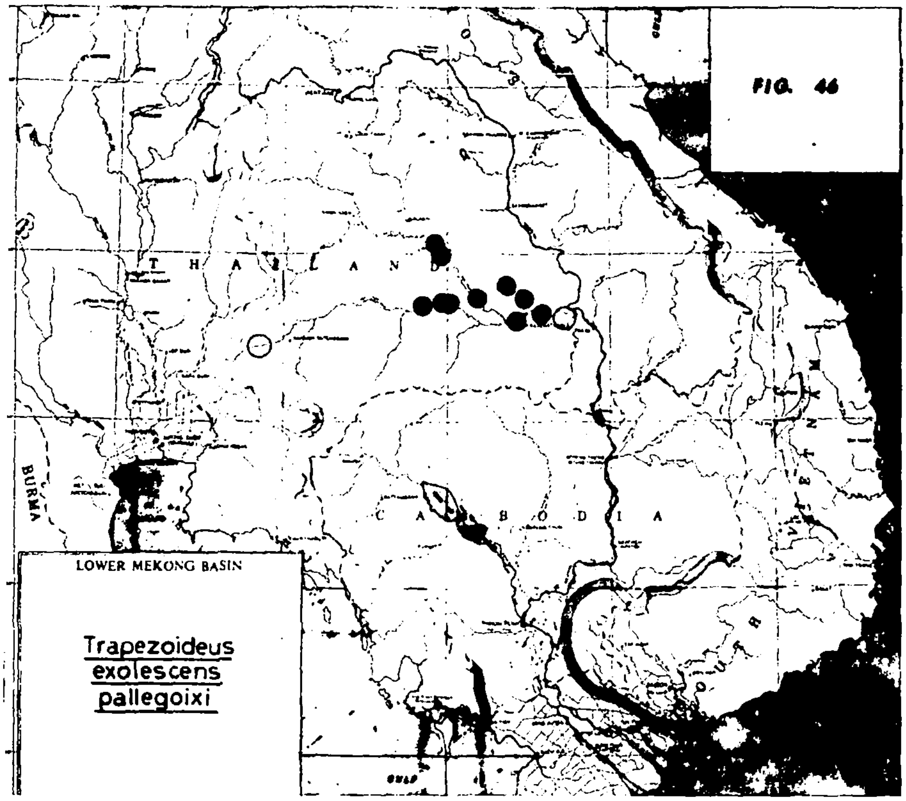


FIG. 46
T H A I L A N D
C A M B O D I A
BURMA
LOWER MEKONG BASIN
Trapezoideus
exolescens
pallegoixi

APPENDIX II

QUESTIONNAIRE ON KNOWLEDGE-ATTITUDES-PRACTICE
ON KHONG ISLAND

QUESTIONNAIRE

Ethnic group ____________

Date _______ Village ________ Town _______ District _______

Province ______________ Number of questionnaire __________

List of the members of the household

Number	Name	Relationship with the person interviewed	Sex	Age	Married

I. 1. Indicate precisely where the people in this household go to bathe (well? shore of the river? in what area of the river?)

2. Indicate where the water is drawn for use in this household (drinking water, wash water, etc.)

3. Where do the people in the household ordinarily go to defecate?

4. Does it sometimes happen that children in this house defecate in the river?

5. If an indoor latrine were added to this house, would it be used?

6. Do you think it would be possible to teach, train, or force children to use an indoor latrine instead?

7. Do you go fishing? If so, where?

8. What style of fishing do you use? Do you have your feet in the water while you fish?

II. 1. Let us imagine that the government was interested in making certain collective improvements around Khong. Which of the following would you select?

______ Build new roads or improve existing ones?

______ Build or improve irrigation canals?

______ Build a temple or redecorate an existing one?

______ Build or enlarge a medical dispensary?

______ Install running water?

______ Bring electricity to all the villages of Khong?

______ Build a latrine for each house?

______ Improve the bathing areas along the river with an embankment and putting in a sandy bottom?

2. Of these choices, pick 2 or 3 that seem the most useful to you.

3. Besides the types of improvement mentioned above, are there others that you would like to see come about? What are they?

4. If such improvements are made, would the villagers be willing to accept the cost of maintenance?

5. If electricity is installed, would the members of the household be willing to pay for use?

6. If running water is installed, would the members of the household be willing to pay for use?

7. If running water is installed, would the people in this household continue to use water from former places or would they be willing to change?

8. If the government were to use explosives to remove some of the rocks in the river, causing you to have to go farther away to find fish, would you object?

III. DISCUSSION OF SYMPTOMS OF SCHISTOSOMIASIS

(List of acute and chronic symptoms and signs of schistosomiasis)

When you felt the symptom(s) listed above, did you consider that you were ill? If you had a disease, what did you call it and what was its cause? What did you do for it? Did you pay a visit to a Lao herb-doctor, or a magician or some other kind of doctor, or did you visit a temple?

Did you go to the hospital? Did you take care of the disease by yourself? Can the disease be cured?

Does anyone in the house have these symptoms at present? Who?

Did anyone ever have these symptoms? How were they treated? What was the result of treatment: cure, no change, or death?

If the individual is still sick, what further treatment can be applied?

APPENDIX III

GENERA AND SPECIES OF HYDROBIIDAE OF THE LOWER MEKONG RIVER

GENERA AND SPECIES OF HYDROBIIDAE
OF THE LOWER MEKONG RIVER

	Reported during the present study from the: Mekong River	Mun River
Genus *Hydrorissoia*		
cambodiensis		
elegans	x	
elongata		x
gracilis	x	
hospitalis	x	
munensis	x	x
paviei	x	
trispiralis		
waltoni		
Genus *Hubendickia*		
crooki		
coronata		
gochenouri		
rolfbrandti		
schlickumi		x
schuetti	x	
siamensis	x	x
spiralis	x	
sulcata	x	
tuberculata		
Genus *Jullienia*		
acuta	x	x
crooki	x	
flava		
harmandi		x
microsculpta		
munensis	x	x
nodulosa		
nucula		
poirieri	x	
rolfbrandti		

		Mekong River	Mun River
Genus	*Lacunopsis*		
	conica	x	
	coronata		
	deiecta		
	fischerpiettei	x	
	globosa	x	
	harmandi	x	
	jullieni		
	levayi		
	massiei		
	monodonta		
	rolfbrandti	x	
	sphaerica	x	
	ventricosa		
	sp.	x	x
Genus	*Lithoglyphopsis*		
	aperta	x	x
	sp.	x	
Genus	*Manningiella*		
	cambodiensis		
	conica	x	
	expansa	x	
	incerta		
	microsculpta		
	pellucida	x	
	polita	x	
	rolfbrandti		
	subulata		x
Genus	*Pachydrobia*		
	bavayi	x	
	bertini	x	
	crooki	x	
	dubiosa		
	fischeriana		
	harmandi	x	
	mcmulleni		

	Mekong River	Mun River
Genus *Pachydrobia* *(cont'd)*		
munensis		
paradoxa	x	
poirieri	x	
scalaroides		
spinosa	x	
variabilis	x	
wykoffi		
zilchi		x
Genus *Pachydrobiella*		
brevis	x	
Genus *Paraprososthenia*		
acicula		
adami		
brandti		
bollingi		
davisi	x	
fischerpiettei		
hanseni		
hydrorissoidea		
iijimai	x	
levayi	x	
taylori	x	
vivonai		
sp.		x
Genus *Saduniella*		
planispira		
Genus *Wykoffia*		
costata		
minima	x	
tricostata		

APPENDIX IV

WATER ANALYSIS SHEETS: MEKONG AND MUN RIVER SITES

REPORT OF WATER ANALYSES, LOWER MEKONG AND MUN RIVERS[a]

Lab. No.	Location[b]	Sampling Temp. °C Air	Sampling Temp. °C Water	Sampling Date 1973	Analyzed ppm TS	Analyzed ppm TRS TC	TDS ppm	pH@ 25°C	ECx10^6 @25°C	Ca+Mg	Ca	Mg	K	Na	CO_3	HCO_3	Cl	SAR
4609	MeB			6/23	300	416	116	7.4	172	0.90	0.66	0.24	0.04	0.66	0.00	1.93	0.33	1.0
4610	MuB			6/23	196	376	180	6.7	252	0.44	0.27	0.17	0.10	1.87	0.00	1.80	1.60	4.0
4611	MuP			6/23	306	532	226	7.4	305	0.37	0.24	0.13	0.10	2.80	0.00	1.10	2.13	6.5
4618	MeB#2			7/12	460	582	122	8.4	186	1.25	0.94	0.31	0.04	0.80	0.00	1.76	0.29	1.0
4619	MuB			7/12	154	286	132	7.4	136	0.36	0.21	0.15	0.10	1.03	0.00	0.82	0.84	2.4
4620	MuP#2			7/10	174	342	168	7.7	174	0.49	0.31	0.18	0.11	1.39	0.00	0.89	1.13	2.8
4621	MuP			7/12	214	436	222	8.8	198	0.39	0.25	0.14	0.09	1.79	0.00	1.30	0.91	4.0
4622	MeB	30	27	8/12	624	786	162	9.7	158	0.30	0.25	0.05	0.04	1.75	0.59	0.98	0.28	4.5
4623	MuB			8/12	148	324	176	9.7	155	0.24	0.19	0.05	0.06	1.70	0.39	1.10	0.48	4.9
4624	MuP	30	29	8/12	160	362	202	10.0	172	0.26	0.22	0.04	0.06	1.80	0.65	0.92	0.50	5.0
4625	MeB	28		9/19	960	1112	152	8.8	175	1.20	0.95	0.25	0.04	1.03	0.19	1.74	0.21	1.3
4626	MuB	30		9/19	102	268	166	9.3	124	0.35	0.24	0.11	0.06	1.12	0.27	0.93	0.49	2.7
4627	MuP	30.5		9/18	160	306	146	8.0	116	0.39	0.26	0.13	0.06	0.92	0.00	1.03	0.56	2.1
4628	MeK			11/8	354	496	142	8.1	217	1.15	1.10	0.05	0.04	1.08	0.16	2.00	0.44	1.4
4629	MeB			11/9	184	318	134	8.3	189	1.55	1.10	0.45	0.04	0.67	0.00	1.91	0.25	0.8
4630	MuB			11/9	30	182	152	7.7	208	0.62	0.41	0.21	0.07	1.70	0.23	1.34	1.05	3.0
4631	MuP			11/9	36	196	160	7.9	218	0.66	0.43	0.23	0.07	1.75	0.26	1.27	1.12	3.0
4632	MeK	22	20	12/7	456	598	142	9.4	221	0.69	0.30	0.39	0.04	0.73	0.08	1.91	0.37	1.2
4633	MeB	24	22	12/8	228	368	140	8.4	214	0.10	0.09	0.01	0.03	0.72	0.16	1.91	0.38	3.2
4634	MuB	26	24	12/8	20	134	114	9.6	174	0.16	0.15	0.01	0.06	0.95	0.00	3.23	0.98	3.4
4635	MuP	30	28	12/8	32	170	138	9.7	217	0.20	0.19	0.01	0.08	1.20	0.21	0.87	1.25	3.8

[a] Samples 4609-4611 received June 25, reported July 31, 1973. Samples 4618-4621 received July 17, reported August 31, 1973. Samples 4622 received August 15, reported Setpember 5, 1973. Samples 4625-4627 received September 21, reported October 31, 1973. Samples 4628-4631 received November 15, reported November 30, 1973. Samples 4632-4635 received December 11, 1973, reported January 15, 1974.

[b] MeB = Mekong River at Ban Dan; MuB = Mun River at Ban Dan; MuP = Mun River at Phibun Mangsahan; MeK = Mekong River at Khemrat.

REPORTS OF WATER ANALYSES, LOWER MEKONG AND MUN RIVERS[a]

Lab. No.	Location[b]	Sampling Temp. °C Air	Sampling Temp. °C Water	Sampling Date 1974	Analyzed ppm TS	Analyzed ppm TRS TC	TDS ppm	pH@ 25°C	ECx10^6 @25°	Ca+Mg	Ca	Mg	K	Na	CO_3	HCO_3	Cl	SAR
4636	MeK	28	21	1/20	40	282	242	9.9	379	0.85	0.36	0.49	0.03	2.40	0.34	2.30	0.44	3.7
4637	MeB	30	22	1/18	40	206	166	9.2	274	2.02	1.40	0.62	0.03	1.20	0.00	2.48	0.38	1.2
4638	MuB	30	25	1/18	22	204	182	10.0	360	1.27	0.99	0.28	0.07	2.09	0.16	1.24	1.51	2.6
4639	MuP	33	31	1/18	62	342	280	10.3	518	1.00	0.89	0.11	0.10	3.22	0.46	1.46	2.34	4.6
4640	MeK	26	24.5	2/25	54	216	162	8.6	336	1.97	1.36	0.61	0.03	0.57	0.00	2.06	0.40	0.6
4641	MeB	31.5	25.5	2/23	34	178	144	9.7	317	1.52	0.92	0.60	0.03	0.87	0.00	1.81	0.43	1.0
4642	MuB	31.5	27	2/23	104	236	132	10.1	485	1.16	0.75	0.41	0.11	2.55	0.05	1.46	2.30	3.4
4643	MuP	35.2	30	2/23	42	212	170	9.8	384	1.02	0.65	0.37	0.14	1.80	0.00	1.37	1.66	2.5
4644	MeK	34	26	3/11	52	238	186	7.7	278	2.09	1.43	0.66	0.04	0.58	0.00	2.10	0.39	0.6
4645	MeB	30	26	3/13	14	180	166	8.7	271	1.95	1.33	0.62	0.04	0.82	0.00	2.16	0.47	0.8
4646	MuB	35	29	3/13	24	192	168	8.5	317	1.03	0.67	0.36	0.12	1.25	0.00	1.42	1.08	1.7
4647	MuP	35		3/13	54	230	176	9.3	274	0.85	0.56	0.29	0.10	1.25	0.00	1.29	0.97	1.9

[a]Samples 4636-4639 received January 25, reported February 28, 1974. Samples 4640-4643 received February 28, reported March 29, 1974. Samples 4644-4647 received March 15, reported April 30, 1974.

[b]MeB = Mekong River at Ban Dan; MuB = Mun River at Ban Dan; MuP = Mun River at Phibun Mangsahan; MeK = Mekong River at Khemarat.

REPORT OF WATER ANALYSES, PA MONG DAM SITE[a]

Lab. No.	Location	Sampling		Analyzed		TDS ppm	pH@ 25°C	ECx10^6 @25°C	Ca+Mg	Ca	Mg	K	Na	CO_3	HCO_3	Cl	SAR
		Date 1974	Water Level (m)	ppm TS	ppm TRS TC												
4648	Ban Khoke Sauk	April 6 11:30 a.m.	1.58	52	146	94	7.5	266	2.28	1.53	0.75	0.06	0.52	0.00	2.10	0.45	0.5
4651	Ban Khoke Sauk	April 16	1.81	30	142	112	7.8	237	2.04	1.37	0.67	0.07	0.46	0.00	1.87	0.44	0.5
4652	Ban Khoke Sauk	April 30 12:00 a.m.	1.92	98	188	90	7.8	262	2.23	1.49	0.74	0.06	0.52	0.00	2.00	0.42	0.5
4649	Police Station	May 5		52	166	114	7.6	247	2.12	1.43	0.69	0.06	0.47	0.00	1.88	0.46	0.5
4650	Wat	May 5		116	224	108	7.6	245	2.12	1.43	0.69	0.06	0.47	0.00	1.90	0.47	0.5

[a]Samples 4648-4652 received April-May, 1974, reported May 15, 1974. Samples 4649 and 4650 received May 7, 1974, reported May 14, 1974.

APPENDIX V

SCHISTOSOMIASIS CONTROL IN CONNECTION WITH WATER MANAGEMENT PROGRAMS

SCHISTOSOMIASIS CONTROL IN CONNECTION WITH WATER MANAGEMENT PROGRAMS

A number of countries in Asia, Africa, and the Americas have undertaken schistosomiasis control projects in connection with national development. However, few control programs have been directly associated with water management schemes.

In Asia, programs on a national level have been supported by the governments of Japan and the Philippines. They are summarized briefly below. Although of necessity integrated with irrigation, they have not been associated with dam construction or man-made lakes. Since the transmitting snail, *Oncomelania hupensis*, is amphibious, transmission is not associated with lakes.

In Africa, a number of governments support schistosomiasis research and control, sometimes in conjunction with anti-malaria programs, because schistosomiasis is recognized as an indigenous and permanent public health problem. The threat of increased incidence as a result of the creation of impounded waters is widely recognized, but there are few examples of control activities incorporated into dam construction programs.

The transmission of Mekong schistosomiasis is entirely aquatic. Control schemes designed to cope with transmission by amphibious *Oncomelania* snails cannot be redesigned for aquatic snails. The nearest analogue to the pattern of transmission that has been identified in the Lower Mekong Basin is probably to be found in snail control in bodies of running water in Iran.

Nowhere in the world has an attempt been made to control schistosomiasis transmission in a major river the size of the Mekong in advance of impoundment.

The following paragraphs are based largely on the summary of Farooq (1973).

A number of countries throughout the tropical world have recognized that schistosomiasis represents an important public health problem. Like malaria, tuberculosis, and other infectious diseases with known transmission patterns, schistosomiasis has been attacked by these countries as part of their continuing public health responsibilities.

Transmission by aquatic snails.--Of the three recognized species of human *Schistosoma,* two (*S. mansoni* and *S. haematobium*) are transmitted by pulmonate snails that are entirely aquatic. Control measures developed against these aquatic snails are of interest in considering a plan of attack against *Lithoglyphopsis aperta,* which is also totally aquatic.

The third human schistosoma, *S. japonicum,* is transmitted by amphibious snails. Control measures developed against these snails cannot be applied to control of *Lithoglyphopsis aperta,* even though *L. aperta* is more closely related to *Oncomelania* than to any of the pulmonate snails.

Egypt.--Two species of human schistosomes exist side by side in the Nile Delta; *S. mansoni*, transmitted by *Biomphalaria* snails, and *S. haematobium*, transmitted by *Bulinus* snails. These snails are relatively large, aquatic species that inhabit ponds, canals, and drains. A few may be found on occasion in the mainstream of the Nile but they do not flourish there. Humans contract schistosomiasis from snails in irrigation canals and ponds. In the Delta, schistosomiasis of both kinds is potentiated by overpopulation, lack of sanitation, lack of health education, and lack of alternatives to daily exposure to contaminated waters. Such conditions have existed for millenia.

Attempts by modern governments to control schistosomiasis in Egypt are now relatively old. Mass treatment campaigns, using tartar emetic, began in 1920. In 1928, a country-wide program for control was organized at the national level. In 1941, treatment was made compulsory.

At the present time, control in Egypt is based on a multidisciplinary approach, including mass treatment, snail control, health education, and sanitation. These activities are coordinated by the Ministry of Health.

Thus far, success in the Delta, where *perennial* irrigation is practiced, has been elusive. With many different agencies engaged in the operation, reports of local success are offset by other reports of failure.

Treatment, using antimonials, has been reported to give cure rates of 25 to 100%, according to different mobile units.

Snail control using copper sulphate is extremely costly and must be repeated year after year.

During the past 10 years nearly 60% of rural communities in the Delta have been supplied with piped water. Nevertheless, control has not been achieved. And, although recent data cannot always be exactly compared with earlier data, trends indicate that there has been a downward shift in the age at which prevalence is greatest. This suggests that the rate of transmission has *increased* between the mid-1930's and the present.

In the past, schistosomiasis mansoni has not occurred in the Nile Basin south of Cairo. The reason was that *basin* irrigation was practiced here, which depended on the annual Nile flood. For many months of the year the irrigation canals were empty, resulting in massive snail kills. Now, the presence of the High Dam at Aswan assures that perennial irrigation will replace basin irrigation in the valley south of Cairo. Increased prevalence of schistosomiasis has already been noted in this region. An already intolerable health situation has in fact been extended.

The situation has not gone unattended. A number of projects sponsored by WHO, UNICEF, and the Egyptian Government, have been developed to identify some form of effective methodology for control of schistosomiasis in Egypt. Transmission dynamics have been studied, preliminary field screening of newer biocides has commenced, and research on snail biology and genetics with a view to development of novel control measures is now being supported by various international agencies.

Sudan.--The Sennar reservoir was created by damming the Blue Nile about 250 kilometers above its confluence with the White Nile. The resulting irrigation tract, in the Gezira, provided a good breeding ground for *Bulinus* and *Biomphalaria* snails. In an area where schistosomiasis already existed, incidence could only increase. The irrigation system went into use in 1925. The snails were found throughout the system (covering 900,000 acres) by 1928. By 1949, 27.5% of the population had schistosomiasis of one type or the other.

Copper sulphate was selected to control snails. Because of its high cost, experiments were performed using continuous application of small amounts. Such amounts were effective and were also found to kill weeds, which further hindered snail populations. Early in the control program, mobile teams carried out treatment of all infected persons in the area. The consequent reduction in prevalence of *S. haematobium* and, to a lesser degree, *S. mansoni*, has been credited to the treatment program (Farooq, 1973). The Gezira scheme in general is considered to have been successful thus far. Government funds have been available, not only to kill snails, but also to enable farmers to raise their living standards so that they can cope with the problem on their own (van der Schalie, 1972).

Iran.--The history of schistosomiasis control in Iran comes closest, perhaps, in analogy to the story thus far in the Lower Mekong Basin. The disease (urinary form) has existed since ancient times but is confined to isolated foci in Khuzesstan. A control project, assisted by WHO, was established in the Dez-Shahpur-Karkheh River basin in order to study snail ecology in relation to water management (construction of the Dez dam), train staff, and develop a program for prevention and control of schistosomiasis on an inter-departmental basis. The project included three phases: I, Fact-finding, 1959-1961; II, Experimental Control Studies, 1963-1965; and III, Operational and Evaluation Phase, 1965 to date.

Eight isolated foci, including an estimated infected population of 50,000 were the target of the WHO-assisted project.

In Phase I, base-line data on prevalence and on ecology of the transmitting snail (*Bulinus truncatus*) were gathered. In Phase II, molluscicides were field-tested, engineering methods of control of snail habitats (draining, filling, drying) were instituted, sanitation was introduced in some of the villages, and field trials of drugs were carried out. In Phase III, evaluation indicated that transmission had been blocked or was now unstable and the number of active cases had dropped from 50,000 to 8,000. The control activities are now continuing under the auspices of the Iranian government (Farooq, 1973).

Transmission by amphibious snails.--In Asia, *Schistosoma japonicum* is transmitted by geographic subspecies of *Oncomelania hupensis*. The disease has been most widespread in China and the Philippines, with important foci in Japan, a zoophilic form in Taiwan, and an isolated focus in Sulawesi, Indonesia.

Japan.--A combination of cementing irrigation ditches (to prevent the amphibious snails from finding breeding sites) and efficient mollusciciding with sodium pentachlorophenate, has virtually wiped out schistosomiasis from the five endemic areas in which it had been established (Yamanashi, Hiroshima, Saga, Fukuoka, and Chiba Prefectures). The disease is no longer a major public health problem, although occasionally small numbers of cases (and snails) reappear. It has been estimated on the basis of costs in Fukuoka Prefecture, that the cost *per capita* in endemic areas has been about US$42 over a 10-year period.

Philippines.--Effective use of engineering methods, including water management practices, drainage of swamplands, stream channeling, and pond construction, has demonstrated on the island of Leyte that schistosomiasis can be controlled. The Pilot Project in Palo, Leyte, was begun with WHO assistance, eventually received support from FAO and UNICEF, and is now the entire responsibility of the Philippine government. Molluscicides were considered only after land improvements were made. The Pilot Project, begun in 1953, is still in operation, is considered to be one of the most successful schistosomiasis control schemes in existence, and has demonstrated the economic feasibility of a national control program in the Philippines.

People's Republic of China.--According to Cheng (1971), great progress has been made in China toward the control of schistosomiasis. Before 1949, about 10.5 million persons were infected and 100 million were exposed. Also, 1.5 million cattle were infected. National campaigns utilizing manpower to destroy snails by burying them, cleaning irrigation ditches, and reclaiming land by earth fills, have been reported to be successful. Preventive measures included storage of human wastes to kill eggs. Treatment of cases relied upon tartar emetic or traditional Chinese herb medicines. The national program was in disarray during the Cultural Revolution (mid-1960s) but was reactivated in 1970. There are no reliable numerical data on the present status of schistosomiasis control in China, but the evidence is that control measures are based primarily on changing the ecological conditions that hitherto favored the snails.

APPENDIX VI

PROVISIONAL DIRECT COSTS FOR A SCHISTOSOMIASIS CONTROL PROGRAM AT KHONG ISLAND, LAOS

PROVISIONAL COSTS FOR INITIATING A SCHISTOSOMIASIS CONTROL PROGRAM AT KHONG ISLAND, LAOS

The following activities have been mentioned in the text as contributing to control of schistosomiasis elsewhere in the tropical world: Snail Control, Sanitation, Health Education, and Drug Treatment. The figures that follow are based on comparable costs for such programs in Africa, the Near East and the Americas, with adjustments for Southeast Asia where possible.

The initial activity, Training, is essential to the remainder of the program and should be started in advance of the implementation of other measures. Ability to assess the success of any of the measures proposed will depend on the availability of trained technicians in the laboratory and hospital on Khong Island and, eventually, on the presence of suitably prepared professional supervisors recruited from the region.

The phase of Implementation can be considered in two categories: (1) Control measures that can be applied now using existing resources (including Phase II, 3 & 4, snail control by habitat alteration and installation of a water supply and sanitation facilities on Khong Island) and (2) Control measures that will require the collection of additional basic data before they can be adopted (including Phase II, 1 & 2, determination of the molluscicide sensitivity of the target snails and the drug sensitivity of the parasites.

The first group of control measures constitute capital improvements. Estimated times for completion do not exceed three years. The second group of measures, requiring additional research, have been proposed below as a three-year program. However, experience in other countries indicates that schistosomiasis control projects tend to lengthen out in terms of time.

On Khong Island, as in other countries, a pilot control program will require an integrated approach, using all of the measures mentioned above. Ultimately, the validity of these measures will have to be assessed and effective co-ordination of all activities must be assured. In the cost estimates listed below, the coordinator's role has been combined with that of Senior Scientist in Phase II: 1. It is proposed that the Senior Scientist direct the studies on molluscicides and methods of application, a central activity whose importance cannot be overemphasized.

Provisional Costs for Initiating a Schistosomiasis
Control Program at Khong Island, Laos
(Figures in US Dollars)

A. PHASE I: TRAINING[1]	Estimated Costs			
	First Year	Second Year	Third Year	Total
Trainees				
Professional, 2 @ 9000/year[2]	18,000	19,000	20,000	57,000
Non-professional, Khong Island residents, 8 @ 400/year	3,200	3,200[3]	3,200[3]	9,600
Faculty from US Institution				
2 Professionals @ 1200/m x 3 (summers only)	7,200	7,200	7,200	21,600
Faculty from Mahidol Univ., Bangkok				
2 @ 800/m x 3	4,800	4,800	4,800	14,400
Subtotal Personnel	33,200	34,200	35,200	102,600
Transportation & Travel				
International air fares, 6 @ 1900	11,400	11,400	11,400	34,200
Regional air fares, 8 @ 90	720	720	720	2,160
Total Costs	45,320	46,320	47,320	138,960

Footnotes: Phase I, Training

[1]Direct costs only. Indirect costs may vary from 20 to 80% of labor costs or total direct costs. Assumes availability of facilities at Faculty of Tropical Medicine, Bangkok for teaching purposes.

[2] Figure provided by International Institute of Education Office, Bangkok, representing estimated cost of maintaining one Asian professional for one year in an advanced degree program in a U.S. institution of learning.

[3] Assumes that trainees (supervisory assistants, technicians, scientific assistants) will complete training and will then be employed on full-time basis on an eventual schistosomiasis control program. Work to be centered in the government hospital, Khong Island.

B. PHASE II: IMPLEMENTATION

	Estimated Costs			
	First Year	Second Year	Third Year	Total
1. SNAIL CONTROL[1]				
Personnel Compensation: U.S. Citizens				
Senior Scientist/ Project Coordinator	25,800	26,700	27,500	80,000
Secretary (Administrative)	8,000	8,600	9,200	25,800
Allowances & Contingencies	13,000	13,000	13,000	39,000
Personnel Compensation: Cooperating Country & Third Country Nationals				
Salary for professional associates, 3 @ 1333/yr	4,000	4,400	4,800	13,200
Regional professional personnel, 2 @ 2000, 1 @ 3600	7,600	8,600	9,600	25,800
Administrative Assistant	3,000	3,400	3,800	10,200
Secretary	2,000	2,200	2,400	6,600
Field Technicians, 4 @ 1700/yr	6,800	7,800	8,800	23,400
Drivers, 2 @ 1700/yr	3,400	4,400	5,400	13,200
Subtotal, Personnel Compensation	73,600	79,100	84,500	237,200

	Estimated Costs First Year	Second Year	Third Year	Total
Travel & Transportation of Persons				
1. Travel in USA (air fare, per diem, taxis, etc.)	500	500	500	1,500
2. Overseas Travel				
Senior scientist & family, 4 @ 950	3,800	-	3,800	7,600
Local travel, trains, taxis, etc.	1,000	1,000	1,000	3,000
Field trips: Laos, 100 x 20/day; Thailand upcountry, 100 x 16/day; airplane, Bangkok to Khong, 8 trips @ 800/trip	10,000	12,560	13,262	35,822
Subtotal, Travel	15,300	14,060	18,562	47,922
Transportation of Things				
Storage, packing, shipment of household goods, USA-Bangkok-USA	5,000	-	5,000	10,000
Packing & shipment of specimens, supplies, etc.	2,000	2,000	2,000	6,000
Subtotal, Transportation of Things	7,000	2,000	7,000	16,000
Equipment				
Field Vehicle	9,000	-	-	9,000
Microscopes: 2 @ 2500; 4 @ 1600	6,600	-	-	6,600
Subtotal, Equipment	15,600	-	-	15,600
Supplies & Materials				
Assorted glassware, chemicals, field equipment, maps, expendable items	6,000	6,000	6,000	18,000
Experimental animals, food, cages, aquaria, etc.	7,000	9,000	9,000	25,000
Subtotal, Supplies & Materials	13,000	15,000	15,000	43,000

	Estimated Costs			
	First Year	Second Year	Third Year	Total
Other Services:				
Consultants, 55 days @ 100/day	5,500	5,500	5,500	16,500
Collaborative studies with SE Asian and American institutions (subcontract funds)	25,000	25,000	25,000	75,000
Maintenance of vehicles, gas, oil, insurance, boat rentals, etc.	5,000	6,000	7,000	18,000
Subtotal, Other Services	35,500	36,500	37,500	109,500
Total Costs, Snail Control	160,000	146,660	162,562	469,222

Footnote: Phase II, Snail Control

[1]An initial three-year activity is envisaged to be devoted to base-line data collection and field application. Approaches include evaluation of laboratory and field response to conventional and novel molluscicides, development of data on population dynamics of snails, and basic studies of usefulness of biological control of snails in the Mekong River. Host institution in SE Asia would be the Faculty of Tropical Medicine, Bangkok.

2. CLINICAL STUDIES[1]

	First Year	Second Year	Third Year	Total
Personnel Compensation: U.S. Citizens				
Junior Scientist	17,497	18,080	18,663	54,240
Allowances & Contingencies	8,000	8,000	8,000	24,000
Personnel Compensation: Cooperating Country & Third Country Nationals				
Regional professional personnel, 2 @ 2000/yr	4,000	4,000	4,000	12,000
Salary for associated professional personnel, 3 @ 3000/yr	9,000	9,000	9,000	27,000

	Estimated Costs			
	First Year	Second Year	Third Year	Total
Laboratory technicians, 3 @ 1700/yr	5,100	5,100	5,100	15,300
Subtotal, Personnel Compensation	43,597	44,180	44,763	132,540
Travel & Transportation of Persons				
1. Travel in USA (air fare, per diem, taxis, etc.)	400	400	400	1,200
2. Overseas Travel				
Junior Scientist & family 3 @ 950	2,850	-	2,850	5,700
Field trips: Laos, 100 x 20/day; upcountry Thailand, 150 x 16/day; airplane, Bangkok to Khong, 4 x 800	7,600	7,600	7,600	22,800
Subtotal Travel	10,850	8,000	10,850	29,700
Transportation of Things				
Storage, packing, shipment of household goods, USA-Bangkok-USA	4,000	-	4,000	8,000
Packing & shipment of specimens, supplies, etc.	5,000	3,000	5,000	13,000
Subtotal, Transportation of Things	9,000	3,000	9,000	21,000
Equipment				
Field Vehicle	9,000	-	-	9,000
Compound microscope with camera; 4 dissecting microscopes; water bath; rotor shaker; ovens; refrigerator	20,000	-	-	20,000
Subtotal, Equipment	29,000	-	-	29,000
Supplies & Materials:				
Test drugs, needles, syringes, glassware, etc.	12,000	6,000	6,000	24,000
Experimental animals (mice, dogs, hamsters, primates), food, cages	15,000	15,000	15,000	45,000

	Estimated Costs			
	First Year	Second Year	Third Year	Total
Subtotal, Supplies	27,000	21,000	21,000	69,000
Other Services:				
Consultants, 90 days @ 100/day	9,000	9,000	9,000	27,000
Vehicle maintenance, gas, oil, etc.	5,000	6,000	7,000	18,000
Subtotal, Other Services	14,000	15,000	16,000	45,000
Total Costs, Clinical Studies	133,447	91,180	101,613	326,240

Footnote: Phase II, Clinical Studies

[1]A three-year investigation of the epidemiology of the Mekong schistosome is planned. Activities include determination of the drug response of the parasite in animals and man, epidemiological surveys and case findings in man and reservoir animals in Southern Laos and Northeast Thailand, and an evaluation of the pathobiology of Mekong schistosomiasis. Host institution in SE Asia would be the Faculty of Tropical Medicien, Bangkok. Studies would be integrated with those listed under "Snail Control" wherever possible.

3. ENGINEERING IMPROVEMENTS[1]

	Estimated Costs	
Phase I--Preliminary Engineering Work (9 months)[2]		
Salaries (16.75 professional man-months @ 2000/man-month[3]	33,500	
Local travel	3,500	
Field assistants	2,000	
Laboratory work	11,500	
Report preparation	500	
Subtotal, Phase I		51,000

	Estimated Costs	
Phase II--Final Design (6 months)[4]		
Salaries (12.5 professional man-months @ 2000/man-month)[3]	25,000	
Local travel	3,000	
Field assistants	1,500	
Laboratory work	1,000	
Report preparation	500	
Subtotal, Phase II		31,000
Phase III--Implementation and Construction (24 months)[5]		
Removal of earth and rock, including dynamiting	20,000	
Sand and earth fill, including transport (10,000 m^3 @ $5/$m^3$)	50,000	
Miscellaneous earthwork (bulldozing, grading, etc.)	25,000	
Construction of concrete embankment along 2 km stretch of shore; 2800 m^3 @ $60/$m^3$	168,000	
Stairways, 6 locations including 3 at wats; 40 m^3/stairway @ $100/$m^3$, + railings & access	24,000	
Development of bathing sites, public latrines, including land preparation, piping of water, etc.; 6 sites @ $1500/site	9,000	
Subtotal		296,000
Contingencies (15%)	44,400	
Subtotal		340,400
Profit (10%)	34,000	
Subtotal, Phase III		374,400
Grand Total, Phase I, II, III		456,400

Footnotes: Engineering Improvements

[1]Figures supplied by Southeast Asia Technology Co., Ltd., Bangkok (SEATEC): Improvements, as noted in the text of the report, include (a) Physical alteration of the shoreline at the main transmission site at Khong Town, (b) Removal of rheophytic shrubs, (c) Removal of peninsula at the transmission site, (d) Filling with sand or concrete of stillwater and eddy areas in Khong Town vicinity, (e) Construction of a stone/concrete embankment, with stairways at intervals, along Khong Town shoreline, and (f) Management of bathing sites by putting down sand, removing bushes, and constructing public latrines at the shore. (See accompanying Figure A).

[2]Includes field surveys (topography, mapping, demographic analysis, river flow analysis), development and calibration of a hydraulic model to test the validity and usefulness of proposed physical changes at Khong, development of data to serve as a basis for design improvement, refinement of cost estimates, and study report preparation.

[3]Professional personnel include project director; sanitary, hydraulic, structural, geotechnical, and resident field engineers; cost estimator; and surveyors.

[4]Includes preparation of detailed design of plans, cost estimates, and preparation of specification documents, supplementary documents, report, and construction drawings.

[5]Assumes that construction will employ a maximum of local labor and resources and that expert supervision is furnished from Bangkok. Cost of engineering services during construction phase are not included in the cost estimate.

4. SANITATION IMPROVEMENTS[1]

	Estimated Costs	
Phase I--Preliminary Engineering Work (5 months)[2]		
Salaries (5.0 professional man-months @ 1500/man-month)[3]	7,500	
Local travel	1,500	
Field assistant	300	
Data analysis	200	
Report preparation	500	
Subtotal, Phase I		10,000

	Estimated Costs	
Phase II--Final Design (5 months)[4]		
Salaries (2.5 professional man-months @1500/man-month)[3]	3,800	
Local travel	500	
Field assistant	200	
Report preparation	500	
Subtotal, Phase II		5,000
Phase III--Implementation and Construction (24 months)[5]		
Water distribution system for Khong Town for estimated 6,000 population @ $10/capita[6]	60,000	
Subtotal		60,000
Contingencies (15%)	9,000	
Subtotal		69,000
Profit (10%)	6,900	
Subtotal, Phase III		75,900
Grand Total, Phase I, II, III		90,900

Footnotes: Sanitation Improvements

[1]Figures supplied by Southeast Asia Technology Co., Ltd., Bangkok (SEATEC): Improvements include building river intake and pumping plants, treatment plant, and distribution system to public fountains and possibly to public laundries and selected public buildings.

[2]Preliminary engineering work includes site investigations to determine water supply needs, intake and treatment plant locations, layout of distribution system and number and location of public hydrants, and preparation of study report.

[3]Professional personnel include project director; sanitary, hydraulic, structural, and resident field engineers; cost estimator; surveyor.

[4]Final design work includes field checking of detail map, detailed engineering design, refined cost estimate, and preparation and printing of documents and drawings.

[5]Assumes that construction will employ a maximum of local labor and resources and that expert supervision is furnished from Bangkok. Cost of engineering services during construction is not included in Phase III cost estimate.

[6]Figure based on water supply costs of approximately US$6-10/per capita (capital investment) in Thailand (Ministry of Public Health, community Potable Water Project).

5. HEALTH EDUCATION[1]

	Estimated Costs[2] 1975	
Personnel Compensation: U.S. Citizen		
Professional educational consultant, 6 man-months @ 300/man-month (personal services contract)[3]	19,800	
Personnel Compensation: Regional Personnel		
Professional educator (12 man-months @ 700/man-month)	8,400	
Administrative-clerical (12 man-months @ 400/man-month)	4,800	
Driver (12 man-months @ 200/man-month)	2,400	
Subtotal, Personnel Compensation		35,400
Travel and Transportation		
International travel (one round-trip, SE Asia-USA)	1,900	
Local travel	700	
Supplies & Equipment		
Posters, leaflets, plasticized photographs, flip-charts	1,000	
Total Costs		39,000