RECENT RESEARCHES ON SCHISTOSOMIASIS

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PREVALENCE AND DISTRIBUTION OF SCHISTOSOMIASIS IN THE PHILIPPINES: A REVIEW

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Oncomelania quadrasi was first described by Möllendorff (1888) on specimens from Surigao Province in Mindanao and first recognized as the intermediate host of *Schistosoma japonicum* by Tubangui on the basis of observations made at Palo, Leyte in 1932. The subsequent history of information concerning its range is completely bound up with that of the parasite. It seems that the distribution of the snails and the annual rainfall pattern are closely correlated. As early as 1941, Tubangui and Pasco and later Pesigan (1948 a, b and 1950) pointed out that the snail is confined to areas with no annual dry season. The most interesting confirmation of the theory that the pattern of rainfall limits the distribution of *O. quadrasi* is the case of the north coast of Mindanao where a large area including the entire province of Misamis Oriental has a marked dry season and is conspicuously lacking in *O. quadrasi* although the snail is found adjacent to this area in every direction.

Local Distribution of the Snail Host

Within the endemic islands, the general distribution of the snail is related to local topography. Although most often found near sea level, the snail is known to occur at elevations up to nearly 900 metres in Bukidnon Province in Mindanao. At whatever elevation, the outstanding characteristic of snail-inhabited regions is their flatness. This feature makes for the retention of water, a point of obvious importance to an animal with an aquatic stage in its life-history. This point is well illustrated by descriptions of two areas where the details of snail distribution are known best—Leyte and Sorsogon. In Leyte *O. quadrasi* is confined to approximately the northeastern fourth of the island. The southwestern half cuts across rugged mountainous terrain and these mountains extend virtually to the sea, leaving no coastal plain at all. Northeast of the mountains, the land is so flat that the difference in elevation is less than 30 metres in more than 19 kilometres. Snails are found throughout this flat northeastern plain (Fig. 1).

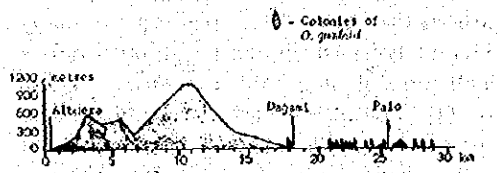

Fig. 1—Profile of Leyte showing relation between topography and distribution of *Oncomelania quadrasi*.

Sorsogon Province, at the extreme southeastern tip of Luzon, provides a somewhat different picture. The province consists mainly of a peninsula connected with the rest of the island by a narrow strip of land. The peninsula which is less than 20 km across is mountainous, with peaks up to 1,650 metres in elevation. But the central part is typically flat and low and is drained by the northward flowing Caloscos River. In the municipality of Irosin, the valley of this river is approximately 3 km wide, but as it leaves Irosin and flows through the adjacent municipality of Juban, the mountains encroach and the valley narrows to less than one km in width. All of the snail colonies are located in this valley, most of them where it is widest and flattest. A profile across the province (Fig. 2) going along a NE-SW line through Irosin

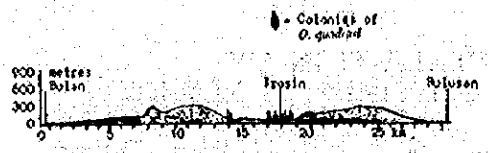

Fig. 2—Profile of Sorsogon province showing relation between topography and distribution of *Oncomelania quadrasi*.

shows the situation graphically. The concentration of the snail colonies at the edges of the valley may be due to the large number of springs and seepage areas at the foot of the mountains which have not been intensively farmed in contrast to the intensive rice farming that is done on the valley floor. A number of descriptions of snail habitats appear in the literature but none of them describes the habitats found in Sorsogon.

It is the wet places, of course, that actually harbour the snails. These places are of many different types which can be grouped into a small number of general categories. These are: (1) flood-plain forest and swamps which represent the most extensive original habitat of the snail; (2) ricefields where primitive agriculture is being practiced; (3) streams which are meandering and sluggish; (4) small swamps which can be called pockets located at the foot of rather high and steep banks and the sources of their water are seepages and springs emerging below the banks; and (5) road ditches and borrow-pits.

Not every snail colony seen in the Philippines can be fitted into the above categories, as many of them are really a combination of types.

In short, the known endemic areas in the Philippines are the provinces of Mindoro Oriental and Sorsogon in Southern Luzon; the provinces of North, East and Western Samar; Leyte; and Bohol in Eastern Visayas, and all the provinces of Mindanao with the exception of Misamis Occidental, Davao Oriental and Maguindanao. The known snail areas in the 22 affected provinces is estimated at 162,851 hectares. These endemic areas lie between 6° and 14° North latitude and, as mentioned, in all these endemic areas there is no definite dry season. The Weather Bureau classifies four types of climate in the Philippines. It is of interest to note that all endemic areas fall under the type II and IV climate, both of which are characterized by the absence of a dry season. Type II has a pronounced maximum rain period from November to January while Type IV has no very pronounced maximum rain period. On the other hand, Types I and III have distinct dry seasons.

Estimate of Cases

During the period from July 1949 to June 1950, under the auspices of the Schistosomiasis Research Programme established in the Department of Health of the Philippines, concerted efforts were made to survey the different endemic areas. Of the 35,509 individuals examined by direct faecal smear, 4,302 were found positive, giving a prevalence rate of 12.1 % in the 10 provinces of Mindanao, Leyte, Mindoro and Samar (Pesigan, 1950).

Following this survey, the importance of the problem of schistosomiasis came to be recognized, and the Philippine Government created the Division of Schistosomiasis in the Department of Health in 1951. At the request of the Government, the WHO sent a team of consultants to the Philippines in 1952. An estimate of 100,000 to 200,000 cases in the 12 endemic areas of the Philippine Islands was made; this figure was, however, revised to 200,000 - 300,000 in 1954 (McMullen *et al.*, 1954).

With the introduction of the concentration methods of stool examination and judging from the results of previous and recent surveys, it is now estimated that there are about 655,124 cases distributed throughout the

Table 1

Estimated number of cases in the Philippines as of 1975.

Place	No. of endemic Towns	Population exposed	Estimated No. of cases
Island of Luzon			
East Mindoro	4	120,300	9,560
Sorsogon	4	85,100	6,161
Sub-total	8	205,500	15,721
Visayan Islands			
Bohol	2	52,200	20,175
Leyte	24	522,200	133,487
North Samar	11	224,300	30,617
East Samar	9	162,500	21,082
West Samar	12	337,300	43,492
Sub-total	58	1,298,500	248,853
Islands of Mindanao			
South Zamboanga	6	133,000	32,240
North Zamboanga	1	53,400	5,270
West Misamis	2	55,700	5,646
North Lanao	2	30,200	9,890
South Lanao	2	25,500	7,082
North Agusan	4	238,600	25,161
South Agusan	11	196,300	35,842
Bukidnon	4	214,300	39,757
North Surigao	3	38,800	9,690
South Surigao	3	86,800	28,615
North Cotabato	5	246,600	35,580
Sultan Kudarat	2	88,500	28,100
South Cotabato	1	16,900	1,688
North Davao	14	421,200	75,046
South Davao	3	603,300	41,937
Sub-total	63	2,457,100	390,550
Total	129	3,961,000	655,124

endemic places out of a total of 3,961,000 exposed population or an estimated prevalence of 16.5% (Table 1). Most of the cases can be found in Leyte, Samar in the Visayas and Davao del Norte and Agusan in the Island of Mindanao.

The disease is rural in distribution and affects mostly farmers and their families. The infection during childhood and adolescence builds up rapidly until it reaches a peak in the 20 to 24 age group. There are significant sex differences between the age groups

past childhood, with the prevalence rates being higher for males than for females. Next to farmers, fresh-water fishermen and unskilled laborers have high infection rates among occupational groups.

Due to the magnitude of the disease problem, a National Schistosomiasis Control Commission was established to cope with this problem with various local and international agencies lending assistance to the control program.

SUMMARY

The known endemic areas in the Philippines are the provinces of Mindoro Oriental and Sorsogon in Southern Luzon; the provinces of North, East and Western Samar; Leyte; and Bohol in Eastern Visayas, and all the provinces of Mindanao with the exception of Misamis Oriental, Davao Oriental and Maguindanao. The total snail area in 22 affected provinces is estimated at 162,851 hectares. These endemic areas lie between 6° and 14° North latitude.

There are 655,124 estimated cases out of 3,961,000 exposed population or a prevalence of 16.5% based on the results of stool surveys conducted by Regional Schistosomiasis Advisory Teams and projected to the total population in the endemic areas.

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REVIEW OF SCHISTOSOMIASIS CONTROL IN THE PHILIPPINES

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INTRODUCTION

Schistosomiasis japonica is considered as one of the important public health problems in the Philippines. The disease is endemic in 129 towns of 22 provinces found mostly in the southern part of the islands which lie approximately between 6° and 14°N. On the basis of surveys conducted by field units, it is estimated that more than half a million people are infected with schistosomiasis. In view of this and the fact that the disease is highly prevalent in rural areas affecting mostly the agricultural sector of the population, it is considered a health problem with serious socio-economic ramifications. It is conservatively estimated that loss from morbidity, cost of treatment and death from schistosomiasis comes up to about $ 14.5 million per annum.

Principles of Control

Through the recommendations of a WHO Schistosomiasis expert team, the Schistosomiasis Control Pilot Project (SCPP) was established in Leyte, the main objectives of which were to determine the most effective and economical means of controlling schistosomiasis in a pilot area and if successful to prepare a national control program. Out of the investigations on the various aspects of the disease, a mass of information regarding its control has been accumulated. New knowledge on the biology of the snail formed the basis for the ecological or agro-engineering measures which were subsequently demonstrated to be effective in the control or eradication of the intermediate host. These methods involved drainage, ponding, filling and scientific agriculture. Application of molluscicides followed confinement of snails in small foci mostly in drainage ditches after the agro-engineering measures. In this regard, it was the experience that snails could be eradicated when complete control of water was achieved and that measures that fell short of this only gave varying degrees of success. Evaluation of these measures in the Pilot Area showed a 99% reduction of the snail population and a decrease in prevalence of infection among children 9 years and below from 12.4 to 3.3% within a period of 6 years from the time the snail control measures were instituted. Moreover, it was gratifying to note that these ecological or engineering methods led in most instances to reclamation of otherwise unproductive, waterlogged and swampy areas and made possible their utilization for agricultural pursuits.

Aside from snail control, it was also shown that transmission of the parasite to man could be reduced through environmental sanitation, health education and case detection and treatment. Environmental sanitation through proper waste disposal, provision of safe water for domestic use and consumption, control of stray animals, and construction of footbridges across snail-infested streams, is an important preventive measure. Likewise, health education through a better understanding of how the disease could be acquired and prevented could not be over-emphasized, although these have to cope with established human habits and cultural patterns and would require much time and effort before any substantial effect could be expected. Mass treatment is another important measure in a control program but the absence thus far of a

truly effective and non-toxic drug would limit the usefulness of this approach. These considerations, therefore, in effect explain why the Pilot Project concentrated more of its efforts onsnail control which offered the most effective means of controlling this disease within the shortest period of time.

From the standpoint of cost, however, ecological methods are very expensive if the expense is charged to public health alone. But, fortunately, there is a striking similarity between the measures that are desirable for snail control and those that are necessary from the standpoint of modern agricultural practices. These include not only the proper management of the fields themselves, but also water conservation and disposal and land reclamation. It would appear, then, that the agricultural benefits might be great enough to offset completely the cost of measures that otherwise would be prohibitively expensive.

The Control Program

It is evident that the control of schistosomiasis cannot be achieved by the Department of Health alone as this would necessitate enormous resources and expertise of other disciplines. In order to involve those other government agencies whose normal activities are related to the control of schistosomiasis and coordinate their activities, a National Schistosomiasis Control Commission with the Secretary of Health as Chairman and the heads of several executive departments of the government (Public Works & Communications, National Irrigation Administration, Agriculture and Natural Resources, Presidential Assistant on Community Development, Social Welfare Administration, Education, National Economic Council, National Waterworks & Sewerage Authority, etc.) as members was created. Its functions are described as follows:

1. Formulate and carry out a schistosomiasis control program.
2. Foster effective exchange of information and coordination of projects, programs and activities of various agencies connected with schistosomiasis control.
3. Establish effective liasion with assisting international agencies.
4. Submit recommendations to the President on administrative or legislative measures that will effectively carry out a national control program for the disease.

Initially, the Commission undertook control projects on a modest scale and at the same time tried to solve various problems related to program implementation. To offset recurrent lack of funds, assistance was sought from various international agencies. The US AID Food for Peace Program and, later, the UN/FAO World Food Program (WFP) provided food commodities for use as incentives to volunteer workers. The WFP asistance is a 5-year program amounting to $537,550. Under this program 1,000 hectares of snail infested areas have been cleared, 616,000 cubic meters of earth excavated in the construction of drainage ditches and 2,000 kg of molluscicide applied. This project, now in its fourth year, has made use of 385,000 man-days and 895 metric tons of food commodities.

Preliminary evaluation of these snail control measures showed a reduction in the snail population of 85%. So far, no effects on interruption of transmission in man have been demonstrated by comparison of prevalence surveys made before the program and those made three years after.

Other assistance to the program is from:

1. MISEREOR (an association of West German Catholic Bishops) which provided drugs for the treatment project in Leyte;
2. Japan International Cooperation Agency (JICA). JICA's assistance which covers a three-year period from 1972 to 1975 and

a possible extension for another three years has provided supplies, equipment, consultants and fellowships for collaborative studies on schistosomiasis research and control; and

3. World Bank which is providing a $25 million loan for an intergrated development project in Mindoro, which has a schistosomiasis control component.

Realizing, however, that the schistosomiasis problem, with its extent and magnitude, cannot be solved by measures short of an all-out effort, the Commission opted for a more comprehensive program of control. This will necessitate a revision of the charter of the Commission to make it more effective in carrying out such a program.

Among the salient points in this proposed revision are the following:

1. Limitation of membership to agencies that can truly contribute to the control effort;
2. Assignment of a full-time technical staff for the Commission;
3. Provision of adequate funds for control in the budget of each member-agency; and
4. A clear cut definition of responsibilities of the member-agencies as follows:
   i) Department of Health takes responsibility for gathering information and other data concerning the extent and magnitude of the schistosomiasis problem, case finding and treatment, health education, sanitation activities, research studies and mollusciciding;
   ii) Department of Public Works, Transportation and Communications undertakes drainage and flood control. Through its Water Resources Division in the Bureau of Public Works, it likewise provides safe water supply;
   iii) Department of Local Governments and Community Development supports "Self-help projects" through voluntary labour and provides grants-in-aid for small scale control projects such as footbridges and spring development;
   iv) Department of Agriculture promotes scientific farming practices and better land use of reclaimed and/or snail infested areas;
   v) Department of Education and Culture disseminates fundamental knowledge about the disease, its prevention and control and participates in the environmental sanitation campaign;
   vi) National Irrigation Administration takes charge of irrigation projects to ensure proper and full control of water for better farming practices.

A ten-year comprehensive control program is scheduled to start this year with the phasing of activities and projects closely linked with the infrastructure program of the National Irrigation Administration and the flood control and drainage program of the Department of Public Works, as schistosomiasis control is essentially one of control of water and land management supplemented by other measures. Such a scheme appears meritorious from the operational, logistic and financial standpoint.

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Evaluation of a Technique of Circumoval Precipitin Test Using Blood Taken on Filter Paper and a Microtiter Technique of Complement Fixation Test of *Schistosoma japonicum*

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Summary: For the circumoval precipitin test (COPT) blood was taken on quantitative blood sampling filter paper by finger prick from outpatients at the Schistosomiasis Control Pilot Project, Palo, Leyte, Philippines. The volume of serum available per strip of filter paper was 0.04 ml and this was extracted at 1:3, 1:5 and 1:8 dilutions. Lyophilized eggs of *Schistosoma japonicum* were mixed with the diluted serum on a microscope glass slide and incubated at 37°C for 2 days. The reaction was read following the criterion made by Yokogawa *et al.* [11]. The serum at 1:8 was too dilute to make correct diagnosis; serum at 1:3 dilution contained too much hemoglobin which made microscopic observation difficult and the extract at 1:5 was found to be appropriate. There was no remarkable difference in antigenicity among 3 preparations of lyophilized eggs from Kofu strain, Japan, and those of new and old preparations from Philippine strain. Under the best condition, false negative results appeared in 15.3% of 152 outpatients in Leyte and false positives in 2% of 50 human sera collected in Tokyo. This method of COPT is not satisfactory for the diagnosis of individual cases but is useful in the epidemiological assessment of *Schistosoma* infections because of the simplicity of blood sampling from dwellers of infested areas and also because it shows nearly the same sensitivity as that of a single fecal examination by the MIFC method.

A microtiter technique of complement fixation test (CFT) was also studied. This method, however, was less sensitive than the COPT or a single fecal examination as to give 23.7% false negatives. Frequency distributions of CF and COP titers were analysed among egg positive, egg negative and treated groups. The results showed that treatment with stibophen had little influence in lowering the serum response, especially in COPT.

Introduction

The methods for the immunologic diagnosis of schistosomiasis were reviewed by Kagan and Pellegrino [6] and the comparative studies on the sensitivity and specificity of these methods were discussed by Kagan [5]. In these studies, complement fixation (CFT) and circumoval precipitin tests (COPT) were evaluated as the methods of choice. The other techniques were not applicable to the field survey because of the complicated technical procedures involved.

The technique of COPT was successfully simplified by Yogore *et al.* [10] and Lewert and Yogore [7]. Cabrera *et al.* [1] recently made further study on the blood sampling method using filter paper. As blood obtained by finger prick was absorbed on the filter paper in their method, blood sampling from

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dwellers in the infested areas became much easier than obtaining blood from vein.

The present study deals with the evaluation of COPT method with serum extracted from the filter paper as well as a microtiter method of CFT using serum collected by a hematocrit tube. For this comparative study outpatients in the Schistosomiasis Control Pilot Project, Department of Health, Philippines were examined and conditions to increase the reliability of this COPT method were determined.

Materials and Methods

Sample collection: Outpatients visiting the Schistosomiasis Control Pilot Project (SCPP), at Palo, Leyte were examined during the period from October to December 1972. The number of patients examined was 209. These patients came to the SCPP laboratory with their stool samples to be examined for parasite eggs and while waiting for the results they were persuaded to submit blood specimens also. Because of the difficulty of collecting blood by venipuncture, blood samples were taken by finger prick. Blood was absorbed on a strip of quantitative blood collection filter paper (Toyo Roshi Type 1, 5×30 mm) which was kept in a refrigerator for drying for at least 1 day. This filter paper was then soaked in phosphate buffered saline (PBS) at pH 7.2 to extract serum for COPT. The volume of serum taken in this strip (5×30 mm) is 0.04 ml. In the routine technique for COPT, a strip of the filter paper was cut into 6 parts and soaked in 0.2 ml of PBS for about 1 hour at 30°C to extract the serum at 1:5 dilution. On a few occasions, serum was extracted at 1:3 or 1:8 by adjusting the volume of PBS.

Aside from the blood collected on the filter paper, blood was also taken at the same time in a capillary tube for hematocrit. The volume of blood in a hematocrit tube was about 40 µl. One end of the tube was sealed by fusing with a small flame and was spinned in a refrigerated centrifuge to separate the serum. The tube was then cut at a point between the serum and the sediment and the serum was blown into a small test tube. An equal amount of physiological saline was added into the same tube to make 1:2 dilution to be used for CFT. The diluted serum was inactivated by incubating at 56°C for 30 minutes before use.

Fecal examination: Fecal materials submitted by almost all outpatients were examined with the direct smear method at first. Those found negative for *Schistosoma* eggs by this method were further examined by the MIFC method. About 1 g of feces was emulsified in about 10 ml of MIF solution in a vial container and strained through a sheet of gauze. The emulsion was then mixed with ethyl ether and the sediment after centrifugation was examined microscopically.

Circumoval precipitin test: Lyophilized eggs of *Schistosoma japonicum* were used as antigen for COPT. Three preparations of *S. japonicum* eggs were used, one from Kofu strain and two from Philippine strain which were a new and an old preparation respectively. About 200 eggs were placed on a glass slide to which serum extracted from the filter paper was added and mixed. The mixture was covered with a microscope cover slip (24×24 mm) and the slip was sealed with vaseline. The glass slides were kept in a moist chamber for 2 days in an incubator at 37°C. The degree of precipitate formation was read microscopically and the serum showing type 1 or stronger precipitation following the classification of Yokogawa *et al.* [11] in one egg or more out of 50 mature eggs was regarded as positive.

Complement fixation test: A microtiter method modified from the principle of Kolmer's method by Ito [4] was used. The antigen was the extract of delipided adult *Schistosoma japonicum* in veronal buffered saline with sodium bicarbonate prepared by the method of Chaffee *et al.* [2]. The sheep cells were sensitized with an equal volume of 2 full hemolytic units of hemolysin.

The test serum was diluted with a calibrated diluter in serial 2 fold dilution from 1:4 to 1:128. One drop of antigen at an appropriate

concentration determined by block titration and 2 drops of diluted complement at 2 full hemolytic units were further added to each well and the microtiter plates were kept in a moist chamber in a refrigerator overnight. The following morning 2 drops of sensitized sheep cells were added to each well and the plates were sealed with a cellophane tape and kept in an incubator at 37°C for 1 hour to allow for the hemolytic reaction to tkae place. The plates were then placed on a table for 3 hours and the patterns of blood sediment were read. These patterns were classified into 5 categories and the well showing a large blood disc surrounded with faint yellowish coloring was determined as an endpoint of reaction. The serum having the titer at 1 : 4 or more was regarded as positive.

Results

Circumoval precipitin test.

The sensitivity of the COPT was compared among different dilutions of serum which were extracted from the filter paper. A strip of filter paper with blood was cut into halves and serum was extracted at 1 : 5 from one piece and at 1 : 8 from the other in 29 samples. The same comparison was made between dilutions at 1 : 3 and 1 : 5 in the other 27 samples. Positive COP reaction was found to be higher at 1 : 5 dilution than at 1 : 8 in one group and also at 1 : 3 than at 1 : 5 in the other group. The differences, however, were not statistically significant by chi square test (Table 1).

The differences in positive COPT reactions observed between egg positives and negatives in three different serum dilutions were next compared. The COPT positive reaction was significantly greater in egg positives at 1 : 5 and 1 : 3 serum dilutions. The difference was, however, not significantly larger by chi square test at 1 : 8 dilution (Table 2). This indicates that serum extracted at 1 : 8 or more is too dilute to make correct diagnosis. The extract of serum at 1 : 3 from filter paper contained much hemoglobin which interferes with the microscopic observation of precipitates. The appropriate extraction of the serum was observed to be in 1 : 5 dilution.

The antigenicity of three preparations of lyophilized eggs was compared. These are eggs from Kofu strain recently prepared in Japan, one freshly prepared at Leyte from Philippine strain and an old product from Philippine strain having been kept in a freezer for 3 years. Thirty two serum samples extracted from filter paper at 1 : 5 were tested with the 3 different egg preparations. The prevalence of positive COP reaction appears to be highest in stored eggs from Philippine strain followed by those freshly prepared in Leyte and those from Kofu strain. These differences, however, were not found to be statistically significant by chi square test

Table 1 Comparison of sensitivity of COP reaction among different dilutions of serum extracted from filter paper.

	serum dilution			
COP reaction	1 : 5 no. (%)	1 : 8 no. (%)	total	
+	22 (75.9)	19 (65.5)	41	$0.3 < Pr.\ (\chi^2 = 0.75) < 0.5$
−	7 (24.1)	10 (34.5)	17	Non-significant
total	29 (100)	29 (100)	58	
	serum dilution			
COP reaction	1 : 3 no. (%)	1 : 5 no. (%)	total	
+	24 (88.9)	22 (81.5)	46	$Pr. = 0.35$
−	3 (11.1)	5 (18.5)	8	Non-significant
total	27 (100)	27 (100)	54	

TABLE 2 Comparison of COP reactions between egg positives and egg negatives at different serum dilutions.

serum dilution		1:5		1:8		
COP reaction		+ no. %	− no. %	+ no. %	− no. %	total no. %
egg	+	22 (75.9)	7 (24.1)	19 (65.5)	10 (34.5)	29 (100)
	−	8 (42.1)	11 (57.9)	7 (36.8)	12 (63.2)	19 (100)
total		30	18	26	22	48
		$0.01<Pr.\ (\chi^2 = 5.58)<0.02$ Significant		$0.05<Pr.\ (\chi^2 = 3.80)<0.1$ Non-significant		
serum dilution		1:3		1:5		
COP reaction		+ no. %	− no. %	+ no. %	− no. %	total no. %
egg	+	24 (88.9)	3 (11.1)	22 (81.5)	5 (18.5)	27 (100)
	−	5 (41.7)	7 (58.3)	5 (41.7)	7 (58.3)	12 (100)
total		29	10	27	12	39
		Pr. = 0.004 Significant		Pr. = 0.019 Significant		

TABLE 3 Comparison of antigenicity for COPT among 3 products of lyophilized *Schistosoma* eggs.

COP reaction	Different products J no. (%)	P-new no. (%)	P-old no. (%)	total
+	28(87.5)	29(90.6)	30(93.8)	87
−	4(12.5)	3(9.4)	2(6.2)	9
total	32(100)	32(100)	32(100)	96

$0.8<Pr.\ (\chi^2 = 0.74)<0.7$ df = 2 Non-significant

J; Kofu strain of *Schistosoma*.
P; Philippine strain, new and old preparations.

TABLE 4 Comparison of COP reactions between egg positives and egg negatives by a single fecal examination with MIFC method in 152 outpatients.

COP reaction		+ no. (%)	− no. (%)	total no. (%)
egg	+	101 (84.9)	18 (15.1)	119 (100)
	−	18 (54.5)	15 (45.5)	33 (100)
total		119 (78.3)	33 (21.7)	152 (100)

$Pr.\ (\chi^2 = 13.3)<0.001$ Significant

(TABLE 3).

Cases examined by both fecal examination and COPT except for egg negatives of treated cases were classified into 4 groups and the results were compared (TABLE 4). The above exception was made for the purpose of evaluating the COPT correctly because immunologic reactions persisted in patients long after the eggs have disappeared from the feces following stibophen [3, 9]. COP positives in egg positives were significantly higher than those in the egg negative group as seen in TABLE 4. The percent positive by COPT was 78.3% or 119/152 which is the same as that obtained by a single fecal examination. The egg negatives who showed positive reaction by COPT and/or CFT were examined twice more for eggs. As some egg negatives turned out to be positives, the classification of the same population changed as shown in TABLE 5. The failure of detection by COPT in egg positives (false negative) was 19 of 124 or 15.3%. It can be seen from this result, that the efficiency of this COPT method was nearly the same as that of a single fecal examination by the MIFC method. Serum samples from 50 cases in Tokyo were examined by the same method and false positive was 2%.

This method of COPT is considered to be useful in the epidemiological assessment of schistosomiasis but is not satisfactory for individual diagnosis.

TABLE 5 COP reactions in the same population as in TABLE 4 after egg negatives were examined for eggs twice more.

COP reaction	+ no. %	− no. %	total no. %
egg +	105 (84.7)	19 (15.3)	124 (100)
egg −	14 (50.0)	14 (50.0)	28 (100)
total	119 (78.3)	33 (21.7)	152 (100)

Pr. ($\chi^2 = 16.2$) < 0.001 Significant

TABLE 6 Comparison of CF reactions between egg positives and negatives.

CF reaction	+ no. %	− no. %	total no. %
egg +	58 (76.3)	18 (23.7)	76 (100)
egg −	12 (42.9)	16 (57.1)	28 (100)
total	70 (67.3)	34 (32.7)	104 (100)

0.001 < Pr. ($\chi^2 = 8.9$) < 0.01 Significant

TABLE 7 Frequency distribution of titer of COP reaction presented by percentage of reacting eggs among egg positive, egg negative and treated groups.

COP + (%)	egg +	egg −	treated
0	15	17	13
2	4	3	3
4	2	4	1
6	3	3	2
8	6	1	2
10—14	15	1	8
15—19	7	3	7
20—24	15	2	9
25—29	8	1	5
30—34	7	0	5
35—39	2	0	1
40—	1	0	0
total	85	35	56

Complement fixation test.

Cases examined by both complement fixation test and MIFC method for eggs in feces, except for egg negatives of treated cases, were compared and results are shown in TABLE 6. The prevalence of CF positives in egg positives was significantly higher than in egg negatives but CF negatives in egg positives (False negative) was 23.7%. This was much higher than the 15.3% false negative in COPT

Fig. 1. Cumulative % of frequency distribution of COP positive egg per cent in egg positive, egg negative and treated groups on probability paper.

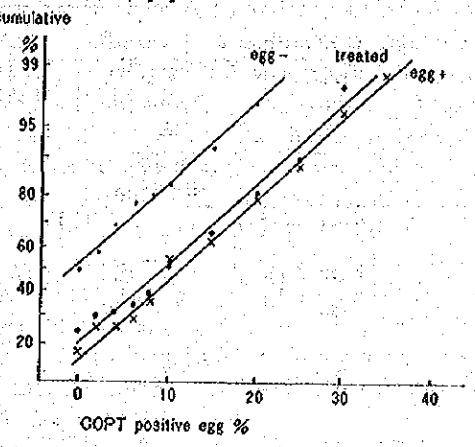


TABLE 8 Frequency distributions of CF titers in egg positive, egg negative and treated groups.

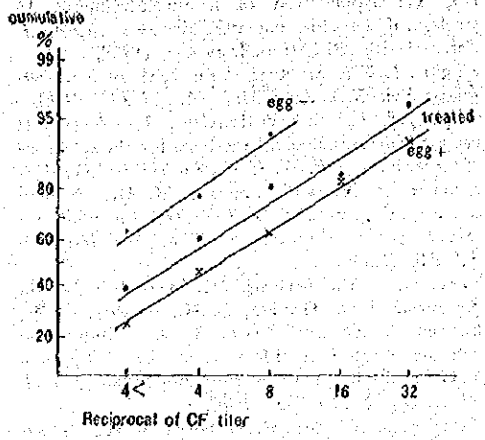
CF titer	egg +	egg −	treated
1: 4<	14	16	11
4	13	4	7
8	11	4	6
16	11	2	1
32	6	2	4
64	5	0	1
total	60	28	30

(TABLE 5). As the sensitivity of this microtiter method of CFT is lower than that of COPT and the method of blood sampling is more complicated than that for COPT, there is no advantage in applying CFT method to the field survey. There was no difference of antigenicity in CFT between Japanese and Philippine strains of *Schistosoma*.

Immunologic reactions after treatment.

Of 52 cases treated with stibophen in the past 4 years including incompletely treated cases and tested by both COPT and fecal examination, egg positives were 32 or 61.5% and COP positives were 40 or 76.9%. The frequency distribution of COP titer among egg positive, egg negative and treated cases are shown in TABLE 7 and cumulative percen-

Fig. 2. Cumulative % of frequency distributions of CF titers in egg positive, egg negative and treated groups on probability log paper.



tages of these distributions are illustrated on probability paper in Fig. 1. In this graphic comparison, the distribution of COP titer among treated cases is close to that of the egg positive group. The same analysis was made for CF reactions as shown in TABLE 8 and Fig. 2. The result was nearly the same as in COP. This result shows that immunologic reactions measured by COPT or CFT were not remarkably lowered by treatment with stibophen.

DISCUSSION

Many immunologic methods for the diagnosis of human schistosomiasis have been studied [5, 6] and techniques have been developed and are being simplified, especially in COPT [7, 10]. Most techniques, however, can be performed only in well equipped laboratories, except for the field COPT by Yogore *et al.* [10].

In the endemic areas, collection of blood samples by venipuncture on a large scale is usually difficult. In a recent study, Cabrera *et al.* [1] collected blood on filter paper by finger prick and serum extracted at 1:8 dilution from the filter paper was examined by the COPT. This method of COPT was further studied and evaluated in the present paper. The highest concentration of serum available from the filter paper was 1:3 dilution, but this contained dense hemoglobin which made the microscopic observation of precipitate difficult. Serum had to be extracted at 1:5 dilution in the present study and false negative in COPT occurred in 15.1%. The sensitivity of this method was found to be lower than that of the field COPT of Lewert & Yogore [7] who used undiluted serum and a different criterion in reading reaction. With diluted sera from non-schistosomiasis cases collected in Tokyo, false positive was 2%. From these results it is obvious that this method of COPT can not be applied for the diagnosis of individual human cases but it is a simple and practical method to assess human *Schistosoma* infections rapidly in the infested areas.

Among many modifications of the CFT, a simple technique was evaluated in the same population. The method was, however, less sensitive than COPT and no advantage was seen in its application to the field survey.

Antigenicity of the Japanese strain of *Schistosoma* did not differ from that of the Philippine strain to the serum from Layte in both COP and CF tests.

As was discussed above, the COPT is useful as a method of field survey. However, efficiency of fecal examination by the MIFC method can not be overlooked in this area. Pesigan *et al.* [8] reported that efficiency of a single fecal examination was 89.6% in the same area. In the present study it was also about 95% by the different estimating method. This high efficiency is not always expected because it depends on the average parasite load in an examined population. For instance at Kofu, Japan where the parasite loads are very small, several examinations had to be done and new cases appeared at each examination. The efficiency of a single examination by the MIFC was only 20.0% in this area. In the present study, outpatients in the SCPP in Leyte were examined and the prevalence of positive cases was estimated to be about 72% which might be extraordinarily higher than in

any of the infested areas in the Philippines. The efficiency of the MIFC method is considered still high in Leyte because the prevalence of schistosomiasis is around 30 to 40% in many villages.

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Diagnostic Methods for Important Helminthiasis and Amoebiasis in Southeast Asia and the Far East

CCB/SEAMEO-TROPMED (1975)

A TECHNIQUE OF CIRCUMOVAL PRECIPITIN TEST USING BLOOD TAKEN ON FILTER PAPER: ITS EVALUATION AND APPLICATION TO FIELD SURVEY

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For circumoval precipitin test (COPT), blood was taken on quantitative blood sampling filter paper (5 mm × 30 mm, Toyo Roshi Type 1) by finger prick from outpatients at the Schistosomiasis Control Pilot Project, Palo, Leyte, Philippines. The volume of serum available per strip of filter paper was 0.04 ml and this was extracted at 1 : 3, 1 : 5 and 1 : 8 dilutions. Lyophilized eggs of *Schistosoma japonicum* were mixed with diluted serum on a microscope glass slide and incubated at 37°C for 2 days. The reaction was read following the criterion made by Yokogawa *et al.*, (1967). The serum at 1 : 8 was too diluted to make correct diagnosis; serum at 1 : 3 dilution contained too much haemoglobin which made microscopic observation difficult and the extraction at 1 : 5 was found to be appropriate. There was no remarkable difference in antigenicity among 3 preparations of lyophilized eggs from Kofu strain, Japan, and those of new and old preparations from Philippine strain. Under the best condition, false negative results appeared in 15.3% of 152 outpatients in Leyte and false positives in 2% of 50 human sera collected in Tokyo. People in two villages near Palo, Leyte were examined by both faecal examination and COPT, and 576 blood samples and 442 faecal materials were submitted. Collection of blood samples by filter paper was much easier than that of faecal materials from dwellers. Epidemiological aspects of *Schistosoma* infection obtained by COPT were nearly the same as those by faecal examination except for the higher positive prevalence in COPT than that in faecal examination. In age distribution, the highest prevalence was seen at 10 to 19 age group. There was no difference in prevalence between both sexes and between two villages. The *Schistosoma* infection was found to distribute evenly among whole house-holds by the test of family accumulation. It can be said that this method of COPT is not satisfactory for the diagnosis of individual cases but is applicable to the epidemiological assessment of *Schistosoma* infections. The results of the study are presented in Tables 1 to 8.

Table 1

Comparison of sensitivity of COP reaction among different dilution of serum extracted from filter paper.

	serum dilution					
COP	1 : 5		1 : 8			
Reaction	no.	(%)	no.	(%)	Total	
+	22	(75.9)	19	(65.5)	41	$0.3 < Pr. (\chi^2 = 0.75) < 0.5$
–	7	(24.1)	10	(34.5)	17	Non-significant
Total	29	(100)	29	(100)	58	

	serum dilution					
COP	1 : 3		1 : 5			
Reaction	no.	(%)	no.	(%)	Total	
+	24	(88.9)	22	(81.5)	46	Pr. = 0.35
–	3	(11.1)	5	(18.5)	8	Non-significant
Total	27	(100)	27	(100)	54	

Table 2

Comparison of COP reactions between egg positives and egg negatives at different serum dilutions.

Serum dilution		1 : 5				1 : 8				Total	
COP reaction		Pos.		Neg.		Pos.		Neg.			
		no.	%	no.	%	no.	%	no.	%	no.	%
Egg	+	22	(75.9)	7	(24.1)	19	(65.5)	10	(34.5)	29	(100)
	–	8	(42.1)	11	(57.9)	7	(36.8)	12	(63.2)	19	(100)
Total		30		18		26		22		48	
		$0.01 < Pr. (\chi^2 = 5.58) < 0.02$				$0.05 < Pr. (\chi^2 = 3.80) < 0.1$					
		Significant				Non-significant					

Table 2 (Cont'd)

Serum dilution		1 : 3				1 : 5				Total	
		Pos.		Neg.		Pos.		Neg.			
COP reaction		no.	%	no.	%	no.	%	no.	%	no.	%
Egg	+	24	(88.9)	3	(11.1)	22	(81.5)	5	(18.5)	27	(100)
	–	5	(41.7)	7	(58.3)	5	(41.7)	7	(58.3)	12	(100)
Total		29		10		27		12		39	
		Pr. = 0.004				Pr. = 0.019					
		Significant				Significant					

Table 3

Comparison of antigenicity for COPT among 3 products of lyophilized *Schistosoma* eggs.

COP	Different products						Total
Reaction	J.		P-new		P-old		
	no.	(%)	no.	(%)	no.	(%)	
+	28	(87.5)	29	(90.6)	30	(93.8)	87
–	4	(12.5)	3	(9.4)	2	(6.2)	9
Total	32	(100)	32	(100)	32	(100)	96
	$0.8 > Pr.\ (\chi^2 = 0.74) > 0.7$				df = 2		
	Non-significant						

J = Kofu strain of *Schistosoma*; P = Philippine strain, new and old preparations.

Table 4

Comparison of COP reactions between egg positives and egg negatives by a single faecal examination with MIFC method in 152 outpatients.

COP		Positive		Negative		Total	
Reaction		no.	(%)	no.	(%)	no.	(%)
Egg	+	101	(84.9)	18	(15.1)	119	(100)
	–	18	(54.5)	15	(45.5)	33	(100)
Total		119	(78.3)	33	(21.7)	152	(100)
		$Pr.\ (\chi^2 = 13.3) < 0.001$		Significant			

Table 5

COP reactions in the same population as in Table 4 after egg negatives were examined for eggs twice more.

COP Reaction		Positive		Negative		Total	
		no.	%	no.	%	no.	%
Egg	+	105	(84.7)	19	(15.3)	124	(100)
	−	14	(50.0)	14	(50.0)	28	(100)
Total		119	(78.3)	33	(21.7)	152	(100)
		Pr. (χ^2 = 16.2) < 0.001		Significant			

Table 6

Comparison of CF reactions between egg positives and negatives.

CF Reaction		Positive		Negative		Total	
		no.	%	no.	%	no.	%
Egg	+	58	(76.3)	18	(23.7)	76	(100)
	−	12	(42.9)	16	(57.1)	28	(100)
Total		70	(67.3)	34	(32.7)	104	(100)
		0.001 < Pr. (χ^2 = 8.9) < 0.01		Significant			

Table 7

Frequency distribution of titer of COP reaction presented by percentage of reacting eggs among egg positive, egg negative and treated groups.

COP + %	egg +	egg −	Treated
0	15	17	13
2	4	3	3
4	2	4	1
6	3	3	2
8	6	1	2
10 - 14	15	1	8
15 - 19	7	3	7

Table 7 (Cont'd)

COP + %	egg +	egg −	Treated
20 - 24	15	2	9
25 - 29	8	1	5
30 - 34	7	0	5
35 - 39	2	0	1
40 -	1	0	0
Total	85	35	56

Table 8

Frequency distributions of CF titers in egg positive, egg negative and treated groups.

CF titer	egg +	egg −	Treated
1 : 4>	14	16	11
4	13	4	7
8	11	4	6
16	11	2	1
32	6	2	4
64	5	0	1
Total	60	28	30

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DISCUSSION

C.P. Ramachandran : When do clinical practitioners usually use immunological tests for schistosomiasis?

B.D. Cabrera : In the Philippines we use the COPT. When the eggs get into the mucosa we do not expect eggs to come out into the stool. With COPT using only dried blood filter paper we miss about 15-16% than with the stool.

Chamlong : What is the standard method for schistosomiasis in this area?

T. Ishizaki : In a heavy endemic area, we use the C.F. test.

H. Tanaka : COPT by blood on filter paper is used in a large population. This test is for screening giving 15% of false negatives.

Chamlong : So it varies with the population.

Sri Oemijati: Schistosomiasis has been reported in Sulawesi in the Lake Lindu Valley area and recently another foci in the Napu Valley. A dam will be constructed to irrigate the lowlands and also communications now has been increasing, to the valleys. So with the advent of better roads and

irrigation, the possibility of introducting the molluscan host and oriental schistosomiasis into the fertile lowland rice growing areas of other Palu and Poso valleys will greatly increase. NAMRU-2 have surveyed the Lindu Valley, and in this area of 1,500 people we found over 20% positive in one stool examination. In the Napu Valley with 1,000 people more than 50% positive. More studies are being carried out. Positive cases are brought to the hospital for clinical investigation.

B.D. Cabrera : Since most of the cases are in acute stage of infection COPT is done in addition to stool examination.

Savanat : Can COPT be used as a test for cure?

M. Yokogawa : I don't think COPT can be used as a test of negativity for at least one year.

H. Tanaka : As regards C.F. test in cases infected with schistosomiasis and treated with Stibophen, C.F. titer is not decreased after one course of treatment. It is quite a different data from that of Oliver Gonzales. In rabbits with *S. japonicum* and treated with niridazole at dose of 80 mg/kg given for 8 days, the titer of C.F. test will suddenly be decreased but remains positive for a year similar to that of COPT.

Savanat : What would be the test that you recommend for assessment for therapeutic success.

Chamlong : It would be very difficult to assess which test can be used for assessment of cure since treatment of schistosomiasis is not easy especially *S. japonicum*, at present there is no drug for cure.

V. Zaman : Do you routinely do rectal biopsy?

Chamlong : For rectal biopsy we need cooperation from the people. We do not have manpower or technicians. We have to be extremely careful not to cause haemorrhage and damage to the mucosa.

Savanat : How reliable are the immunological tests like the intradermal test C.F.T. and COPT for schistosomiasis in an area where there is *Schistosoma spindale*, the non-pathogenic schistosome.

J.H. Cross : We tried the intradermal test in an endemic area, it showed positive but when we did the stool examination, all were negative. For example, in Taiwan we made a survey, we tried intradermal test 10-15% were positive but cases had stool negative.

Savanat : In that case the intradermal test should not be used in the area where you find *S. spindale*. How about the C.F. test, IHA test and COPT. Are they specific?

I. Ishizaki : Some have sensitization because of penetration of cercaria in the skin. We should use schistosoma antigen and we must make sure the antigen is safe.

Chamlong : When we use purified antigen it works. Progress for purification of antigen requires time, expenses in the field to assess the efficacy of the test.

Savanat : Purifying antigen may not work for reasons that when a person is being infected by parasites the antibody response is not confined just to any one complement of the antigenic materials. In a given individual the hypersensitivity response may not be reactive against one kind of antigen but may be strongly reactive against another antigen which is used for skin testing. It would be recommended, to partially purify the crude antigen to get rid of the component which we know are non antigenic eg. in *E. histolytica* the last peak extract has no antigenic activity at all.

Chamlong : There was a case report of schistosomiasis in an aborigine in Malaysia. Now with the construction of dams in Malaysia, in the future there might be endemic areas of schistosomiasis. So how would you control this?

V. Zaman : We will conduct our epidemiological survey. In hospitals we can examine stools, COPT tests or do rectal biopsy.

Chamlong : Only a few months ago we had proven cases admitted to our hospital from the south of Thailand, one was a child. It means that there is active transmission. We still have to look for endemic foci but it is very difficult for surveys to be undertaken. How do you control schistosomiasis in Japan?

M. Yokogawa : The snail host was eradicated.

Savanat : I would suggest prevention of the disease. When schistosome has entered the body, the disease produced is due to delayed hypersensitivity to eggs antigen. On a child, we ought to find an immunological method to prevent the development of a delayed immune response, so the child will not get the disease. At the same time, we treat the patient with the drug. So I would like to hear your opinion.

H. Tanaka : I was interested in this in a low endemic area, If we have a specific technique which depends on the endemicity of the area and also on the worm load in the population, this worm load is important in determining to start the treatment.

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COMPLEMENT FIXATION AND CIRCUMOVAL PRECIPITIN REACTIONS FOR DIAGNOSIS AND AS TESTS OF CURE IN SCHISTOSOMIASIS

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Many immunologic tests have been so far examined for the diagnosis of human schistosomiasis. These test methods and their reliability were well reviewed and criticized by Kagan and Pellergrino (1961). Several immunologic techniques of diagnosis were compared by Kagan (1968) and two of these, the complement fixation (CF) and circumoval precipitin (COP) tests, were recommended as techniques of choice. These two techniques as well as the gel diffusion test are being studied for diagnosis in the schistosomiasis-infested area in Leyte, Philippines, as a part of the Philippine-Japan Cooperative Project. A technique of the COP test for field surveys was established. The present paper deals with the CF and COP methods as used in diagnosis and for evaluation of cure. The latter was studied in human cases of *Schistosoma mansoni* infection treated with stibophen in New York and in rabbits infected with *S. mansoni* or *S. japonicum* and treated with niridazole. Besides the CF and COP tests, the gel diffusion test was found to be a technique of choice for diagnosis in the study in Leyte. However, its sensitivity was a little lower than that of the COP test.

CF diagnostic test

Among many modifications of CF methods, the technique used for the present study was one utilizing the endpoint of 50% haemolysis in test tubes, each of which contained 1 ml total volume of reagents involving 3 CH_{50} complement. Antigen was an extract of lyophilized and delipidized adult worms in Coca's solution prepared by the method of Chaffee *et al.*, (1954). The sera of 6 baboons infected with *S. mansoni*, 5 baboons with *S. haematobium*, 3 rabbits with *S. mansoni* and 5 rabbits with *S. japonicum* were all negative before infection and all became positive (serum antibody titer 1 : 10 or greater) after infection. In the 5 baboons infected with *S. haematobium*, serum antibody titers were elevated several weeks prior to detection of ova in faeces or urine. Five mice infected only with *Trichinella spiralis* were negative at different periods after infection.

Of 48 human cases of *S. mansoni* infection, 46 were positive. Only 1 of 31 non-infected humans had a positive titer (1 : 20). This false positive case involved a technician who, for years, had been maintaining *S. mansoni* in the laboratory but repeated faecal examinations were negative for eggs.

The above CF technique is sensitive and specific but cannot be used widely in field surveys because of the complexity of the procedure which requires a well-equipped laboratory and a qualified technician. A simplified CF technique using Kolmer's principle performed on microtiter plates was tested in out-patients in the Schistosomiasis Control Pilot Project, Philippines (Tanaka *et al.*, 1975). Positive CF reactions were observed in 58 (76.3%) of 76 egg-positive cases and in 12 (42.9%) of 28 egg-negative cases, some of whom might have been infected, as egg-negatives were determined by a single faecal examination by the MIFC

method. It can at least be said that the simplified microtiter method was not so sensitive as the COP test, mentioned below. The CF test is considered to be sensitive and specific as a test tube method utilizing the endpoint of 50% haemolysis. However, the complicated procedure limited its wide application in the field survey.

COP diagnostic test

There are different procedures in the COP test and different criteria for reading precipitin reactions coresponding to each technique. In the present study, a criterion of reading made by Yokogawa *et al.*, (1967) was used. Lyophilized eggs were used as antigen in the field study in Leyte. The blood samples were taken by finger prick in heparinized capillary tubes (Microcap) or quantitative blood sampling filter paper (Toyo Type I). Plasma was isolated from the capillary tubes and serum absorbed on the filter paper was extracted with PBS solution at a 1:5 dilution while the filter paper was soaked in PBS at room temperature at 30°C for 30 min. to 1 hour. About 200 mature lyophilized eggs were mixed with plasma or diluted serum on a microscope slide, covered with a cover slip and sealed. Such preparations were kept in an incubator at 37°C for 3 days and the precipitin reactions were then read. For the field survey, only the strongest precipitation was taken into consideration and only sera showing a type II or stronger reaction were regarded as positive. Cases showing a type I reaction were regarded as doubtful and were further examined by the other diagnostic methods for individual clinical diagnosis.

In the test of plasma materials, positive COP reactions were observed in 144 (97.3%) of 148 egg-positives; all reactions were negative in 41 egg-negatives among inhabitants of a non-endemic area near Ormoc City, Leyte. Using the original criterion of Yokogawa *et al.*, (1967), results were 147 COP positives (99.3%) among the egg-positives and 4 positives (9.8%) among the egg-negatives, while by the criterion of Yogore *et al.*, (1968), there were 136 COP positives (91.9%) among the egg-positives and there were no positives among the egg-negatives.

In the test of extracted serum diluted at 1:5, there were 130 positive reactions (87.8%) among the egg-positives and no positive case was found among the egg-negatives by the present reading criterion. The results included 139 positives (93.9%) among the egg-positives and 3 positives (7.3%) among the egg-negatives using Yokogawa's (1967) criterion but only 78 (51.4%) were positive among the egg-positives and none was positive among the egg-negatives using the criterion of Yogore *et al.*, (1968).

The criterion of reading reactions used in the present study would be appropriate to the method of field examination of plasma or extracted serum. Serum titer in the blood absorbed on filter paper persisted for longer than 2 months if the samples were kept dried in a desiccator even at a room temperature of about 30°C. Blood samples taken on filter paper can be shipped to a laboratory at a long distance for examination. Since this COP technique is simple in procedure, specific, and more sensitive than a faecal examination, it can be used widely for field surveys.

CF test as a criterion of cure

In order to determine cure after treatment, a follow-up study by repeated faecal examinations is necessary during a period of at least 6 months. The discharge of eggs usually ceases soon after treatment and test methods other than faecal examination are needed to prove cure. For this purpose, the above two immunologic methods were examined.

Sera of 22 adult New York City residents known to have active schistosomiasis mansoni were studied by CF test by the test tube

method mentioned above before and after treatment. All but one patient had lived in Puerto Rico and completed at least a partial course of treatment with stibophen after the initial sera collection, and returned for follow-up studies 3 to 9 months post-treatment. Nineteen patients received at least 75 ml of the drug, 3 developed intolerance and received lower doses. All pre-treatment sera were positive in a range of 1 : 10 to 1 : 320. The titer of CF antibodies fell 2 or 3 tube dilutions in 10 patients(45%), remained essentially unchanged (no more than 1 tube dilution) in 10 patients (45%), and rose significantly in 1. Four patients (18%) became sero-negative. Three patients were found to have eggs of *S. mansoni* in post-treatment faecal specimens though antibody levels had fallen significantly in 2 of these (Dennis *et al.*, 1972). Thus, the reduction of CF antibody levels was not remarkable in humans treated with stibophen.

CF reactions were also examined in infected rabbits treated with niridazole. Two rabbits were injected subcutaneously with 12,000 cercariae of *S. mansoni*, and blood samples were taken monthly for a period of 9 to 12 months. The CF reactions, which were negative before infection, rose to high titers at the first bleeding 20 days after infection. Treatment was initiated 5 months after infection. Niridazole suspended in physiologic saline was given perorally at a dose of 75 mg/kg daily for 8 days. The CF titers of treated animals fell significantly during the first 20 weeks after treatment but remained positive at low titers at the end of this observation period (Tanaka *et al.*, 1972), whereas 4 baboons infected with *S. mansoni* without treatment did not show the abrupt fall of CF titers in a 2½ year period.

A similar experiment was performed in rabbits infected with *S. japonicum* (Matsuda *et al.*, 1972). CF reactions were observed in rabbits infected with 400 to 800 cercariae of *S. japonicum* and treated with niridazole at a daily dose of 80 mg/kg for 8 days. The CF reactions in 5 rabbits turned positive no later than 6 weeks after infection. Treatment was initiated when the titer of one rabbit reached 1 : 960, 7 weeks after infection, and those of the other two rose higher than 1 : 2,560, 12 weeks after infection. The CF titers in the treated rabbits fell rapidly 3 tube dilutions or more in serial 2-fold dilutions in the first 10 weeks, then fell slowly to about 1 : 40, 30 weeks after treatment. Two untreated rabbits did not show any reduction of CF titers.

The results differed much in humans treated with stibophen and in rabbits treated with niridazole. All treated rabbits were sacrificed later and complete cure was proven. It could be said that if a fall in antibody level was detected by the CF test, the animal had been completely cure by treatment.

COP test as a criterion of cure

In the COP test, a difficulty exists with regard to quantification of reactions. There are two parameters to show the strength of COP reactions; one is the percentage of reacting eggs and the other is the degree of strength of precipitation. A COP Index was established to include the above two parameters in one value. For this, strength of precipitation was classified into 4 grades, from 0 to 3, corresponding to negative, and Type I to Type III reactions by the criteria of Yokogawa *et al.*, (1967). The sum total of the grades of the observed eggs is divided by number of eggs observed and multiplied by a constant of 100/3 to convert the index to 100 when all eggs showed type III reaction as in the following formula:

$$\text{COP} = \frac{\text{Total of grades of precipitation} \times 100}{\text{No. of eggs observed} \times 3}$$

Sera from the rabbits infected with *S. japonicum* in the above experiment were examined

for COP reactions (Matsuda *et al.*, 1972). Serum reactions measured by the per cent of positive eggs reached 50 to 85% before treatment and fell gradually after treatment with much fluctuation to 10 to 15%, 30 weeks after treatment with niridazole. The COP Index ranged from 35 to 75 before treatment and regressed slowly after treatment with little fluctuation to about 5, 30 weeks after treatment. During the decline of the COP titer after treatment, the COP Index showed a smoother curve than the percentage of positive eggs.

For quantitative measurement of serum titer, the CF test was best followed by the COP Index and the per cent of reacting eggs. It was found that these immunologic tests can be used as criteria of cure in experimental animals in which complete cure can be obtained but not in human infections where complete cure cannot always be expected.

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Comparative Studies on Reading Criteria of Circumoval Precipitin Reaction of *Schistosoma japonicum* for Field Survey in Highly Endemic Area[1)]

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Summary: In the present study, an improved technical procedures of the circumoval precipitin (COP) test of *Schistosoma japonicum* are described. In the process of preparing egg antigen, the intestines of infected mice were homogenized and digested with trypsin. Eggs were then isolated by sedimentation and by repeated mild centrifugation at 500 rpm for 1 minute. After washing eggs with normal saline, the supernatant was taken out by an aspirator and replaced by cold distilled water. After adding a small amount of glycine, eggs were frozen in dry ice acetone and lyophilized. Lyophilized eggs were mixed with test serum on a microscope slide, covered with a cover slip which was sealed with vaseline and incubated at 37°C for 2 days in a moist chamber. The degree of precipitin formation was classified into types 1, 2 and 3, according to the criterion by Yokogawa *et al.* In this examination which was carried out in a highly endemic area, only types 2 and 3 were considered as positive reactions. Type 1 was regarded as doubtful and further examinations by the other detecting methods were used for individual diagnosis. By this criterion of reading, 144 (97.3 %) were positive for COP reaction using whole serum among 148 egg positives whose serum samples were taken in the Schistosomiasis Control and Research Project, Palo, Leyte, Philippines while 130 (87.8 %) had positive reaction using sera extracted from the filter paper at 1 : 5 dilution. When type 1 reaction was included in the positive reaction, the overall reaction positives were 99.3 %, and 93.9 % by whole and diluted sera, respectively. On the other hand, the false positives were 9.8 % and 7.3 % in corresponding serum samples. No false positive reaction was observed by the new criterion in 41 samples collected from hospital patients who were proven egg negatives in Ormoc City, Leyte where schistosomiasis is not endemic. This COP method was considered to be a highly sensitive tool in mass examination for schistosomiasis in the highly endemic area.

Introduction

The circumoval precipitin (COP) test is a sensitive and specific immunologic method for diagnosis of *Schistosoma* infections[4,6,7]. Since Oliver-Gonzales [6] tried this test for *S. mansoni*, many workers have been using it as a tool for immunologic diagnosis of schistosomiasis. [1,2,4,8,9,11,12,14–16]. There have been different procedures and different reading criteria by the workers depending upon the purposes of its application. In the previous papers

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by the present authors[10,11,14], a criterion proposed by Yokogawa *et al.* [16] which was derived from the original reading criterion made by Oliver-Gonzales [6] was used. Yogore *et al.* [15] established a different criterion for the purpose of avoiding false positives.

In both procedures of COP test, undiluted serum samples were used. In the field survey, however, serum obtained by venipuncture is not always available and blood is forced to be taken on filter paper by finger prick. Consequently diluted serum eluted from the filter paper has to be used. For the purpose of the field survey in the highly endemic area, a new procedure and a criterion of reading of COP test are necessary.

The present paper deals with improved procedures of preparation of egg antigen, a simplified procedure of COP test and comparative studies on criteria of reading reactions to find out an appropriate technique to detect the infected people efficiently in the highly endemic area of *Schistosoma japonicum* in Leyte, Philippines.

MATERIALS AND METHODS

Areas of serum sampling: Serum samples from the proven infected patients were collected at the Schistosomiasis Control and Research Project (SCRP), Palo, Leyte, Philippines. Blood was taken from the outpatients in the Project who were positive for *Schistosoma* egg by the MIFC method of fecal examination. The number of samples collected from the proven schistosomiasis cases was 148.

The samples of non-infected persons were collected from the hospitalized patients at the Ormoc Sugar Planters Association Hospital and at the Ormoc General Hospital in Ormoc City, Leyte which is located far from the *Schistosoma* infested area and where the infestation of the other helminths was almost the same as in *Schistosoma* infested areas on the same island. Serum samples collected from these patients were 41 in number.

Preparation of serum samples: About 10 ml of blood was taken from the hospitalized patients by venipuncture. As soon as the blood was taken in a syringe, a few drops of blood were applied on a strip of the quantitative blood sampling filter paper, 5 × 30 mm in area (Toyo-roshi Type 1) for the preparation of extracted serum. The serum was isolated from the remaining blood.

Blood samples from the outpatients at the SCRP was obtained by finger prick. Part of the blood was applied on similar filter paper and also taken into a heparinized capillary tube (Microcaps, Drummond) of about 100 μl in capacity. From the blood in the capillary tube, plasma was isolated by allowing the tube to stand vertically on a tube sealer holder (SEAL = EASE, Clay Adams) for several hours.

The blood samples on filter paper was kept in a refrigerator overnight for drying. The serum was extracted from the dried blood on filter paper by the procedure reported by Tanaka *et al.* [14] and Noseñas *et al.* [11]. The calibrated absorbing portion of filter paper was cut into 6 pieces and soaked in 0.2 ml phosphate buffered saline at pH 7.2 in a small test tube for 1 hour at 30°C to elute serum at 1 : 5 dilution.

Isolation of S. japonicum eggs: For harvesting a large number of eggs of *S. japonicum*, mice were infected heavily with cercariae. The infected *Oncomelania quadrasi* was crushed between two glass slides with a small amount of water and cercariae were collected in a small petri dish. Mice, 3 to 4 weeks old, were injected subcutaneously with 70 to 90 cercariae each. Since these heavily infected mice usually died about 8 weeks after infection, they were sacrificed a few days before death.

Eggs for the antigen were isolated from the intestine. The intestine was removed, cut longitudinally to open and washed in normal saline to remove the intestinal content. After removing water from the intestine using filter paper, the intestine was cut into pieces as fine as possible with scissors and was digested with trypsin. Trypsin (Merck, 2000E/G) was dissolved at 0.2 % in 1/15 M phosphate buffered saline at pH 8.0. The cut intestine was suspended in 5 fold volume of trypsin solution, emulsified in a metal blade homogenizer for 4

minutes and digestion was allowed to continue for 4 to 5 hours in a water bath at 37°C being shaken continuously.

After digestion, the suspension was again mixed in a metal homogenizer for 2 minutes, then strained through a sheet of gauze and again through two sheets of gauze.

The filtrate was centrifuged at 500 rpm for 1 minute and the supernatant which contained more tissue fragments and immature eggs was removed by an aspirator. The sediment was washed with normal saline by centrifugation at 500 rpm for 1 minute and the supernatant was removed. This procedure was repeated several times until the mature eggs were concentrated in the sediment.

In case tissue fragments still remained in the sediment after the above isolation process, a method of centrifugal fractionation on sucrose solution was used at the last step. In a centrifuge tube, 5 ml of 30 % sucrose solution was placed and an amount less than 1 ml of concentrated egg sediment was slowly mounted, centrifuged at 1,000 rpm for 2 minutes and the supernatant was removed. The sediment was washed with saline several times by centrifugation at 1,000 rpm for 1 minute at each time to remove the sucrose.

Lyophilization of eggs: The egg sediment in a centrifuge tube prepared by the above procedures was suspended in cold distilled water, centrifuged and supernatant was removed to replace the saline with cold distilled water. The sediment was then suspended in cold 1 % glycine solution and centrifuged. The supernatant was removed leaving the same amount of the sediment. The eggs were suspended in this remaining solution and were transferred into lyophilizing ampoules. During the above procedures, tubes containing eggs were placed in ice water to avoid egg hatching. The glycine solution made the eggs well dispersed when they were mixed with serum for COP reaction. Egg suspension in ampoules was immediately frozen in dry ice aceton and was lyophoilized. Lyophilized eggs could be kept in a small screw top tube in a desiccator in a refrigerator for several years without losing antigenicity.

Preparation of specimens for COP test: Dried eggs in an ampoule were transferred by a slender spatula to a microscope glass slide. For each serum sample, about 200 mature eggs were used and two samples were placed on a glass slide. One drop of a serum sample was added over eggs on a slide and covered with a cover slip, 18 × 18 mm in size the rims of which were previously applied with a small amount of white vaseline for sealing. These prepared specimens were kept in a wet chamber and incubated at 37°C for 2 days. Although precipitation could take place at room temperature in the tropic area, the incubation at 37°C was necessary to make the degree of reaction stable.

Criteria of reading reaction: In the previous papers by the present authors [10,11,14], the degree of precipitation was read following the classification made by Yokogawa *et al.* [16]. A difficulty arising from this criterion was high frequency of false positives in the highly infested area like in Leyte, Philippines. In the present study, 2 different reading criteria as described below were compared.

a. In the present study, a new reading method was used which was derived from the method by Yokogawa *et al.* [16]. In this method, all mature eggs, about 200 in number under a cover slip, were observed microscopically and only the strongest precipitin reaction was taken into consideration. Thus, only types 2 and 3 were regarded as positives, while type 1 was labeled doubtful and was regarded as negative for the purpose of mass examination in a field survey.

b. In the criterion by Yokogawa *et al.*, degree of precipitin formation was classified into 3 types, *i.e.* type 1 or tiny and small blebs, type 2 or medium size of blebs and type 3 or multiple and/or large blebs and septate precipitates. By observing 30 or 50 eggs, percentage of eggs with any types of precipitate was used as a parameter to show the strength of reaction of each serum sample.

Classification of degree of the reaction by Yokogawa *et al.* is shown in Fig. 1 by photographs.

RESULT

Whole sera and eluted sera from filter paper at 1 : 5 dilution were examined for COP test. The degree of precipitate formation was read after 2 days. The strongest reaction by each specimen was read and classified by the types defined by Yokogawa *et al.* The frequency distribution of strength of reactions of serum samples from 148 known schistosomiasis cases and 41 proven non-infected persons as well as percentage of each type to the total number examined is shown in TABLE 1.

The infected cases showed mostly the type 3 reaction with whole sera and type 2 or 3 with the diluted sera. The type 1 reactions were observed at several per cent among the non-infected cases in both whole and diluted sera.

All examined cases were classified into reaction positives and negatives according to the present criterion and is shown in TABLE 2. With whole serum 97.3 % of the proven infected cases were positive for reaction and none of the non-infected was reaction positive. The positive reaction was reduced to 87.8 % with the use of diluted sera. By this method, COP reaction positives could be diagnosed as *S. japonicum* infected.

The same cases examined were also classified by the reading criteria of Yokogawa *et al.* and the results are shown in TABLE 3. The method by Yokogawa *et al.* was more sensitive in which positive COP reactions were 99.3 % and 93.3 % by whole and diluted sera, respectively. On the other hand, false positive were as high as 9.8 % and 7.3 % by whole and diluted sera, respectively. By this method, nearly all the infected will be detected though doubtful cases will be involved in reaction positives. This method is useful to pick up all doubtful cases especially in the area of low endemicity.

DISCUSSION

For the purpose of using an immunologic technique for the diagnosis of schistosomiasis in field study, it is preferable that all procedures are simplified and the result is reproducible. In the present study, the eggs were successfully lyophilized, their antigenicity was well maintained after lyophilization and the reactions were made stable.

In the process of isolating eggs from tissue fragments, the most important technique was to centrifuge slowly in a short time. By this, mature eggs were sedimented in the centrifuge tube and most tissue fragments remained in the supernatant [13].

Replacement of saline with distilled water before lyophilizing eggs was essential to keep the antigenicity of the eggs stable. This process had to be performed in an ice cold water bath, otherwise the eggs would hatch.

Addition of a small amount of glycine prevented strong adhesion of eggs in the process of

TABLE 1 Frequency distribution of the degree of COP reactions* among 148 schistosomiasis patients and 41 proven non-infected persons using whole and diluted sera.

Schistosoma	No. examined (%)	Test serum	Types of COP reaction							
			No reaction		Type 1		Type 2		Type 3	
			No.	(%)	No.	(%)	No.	(%)	No.	(%)
Egg positives	148 (100)	Undiluted	1	(0.7)	3	(2.0)	8	(5.4)	136	(91.9)
		Diluted (1:5)	9	(6.1)	9	(6.1)	52	(35.1)	78	(52.7)
Proven non-infected	41 (100)	Undiluted	37	(90.2)	4	(9.8)	0		0	
		Diluted (1:5)	38	(92.7)	3	(7.3)	0		0	

* Only the strongest reaction in each specimen was taken into consideration and reactions were classified by types defined by Yokogawa *et al.*

TABLE 2 Result of COP reactions by whole sera and diluted sera among *Schistosoma* infected and non-infected persons by the present method.

Schistosoma	COP reaction by whole sera				COP reaction by diluted sera (1:5)				Total	
	no.+	(% +)	no.−	(% −)	no.+	(% +)	no.−	(% −)	no.	(%)
infected	144	(97.3)	4	(2.7)	130	(87.8)	18	(12.2)	148	(100)
non-infected	0	(0)	41	(100)	0	(0)	41	(100)	41	(100)
Total	144		45		130		59		189	

TABLE 3 Result of COP reactions observed following the method of Yokogawa *et al.*

Schistosoma	COP reaction by whole sera				COP reaction by diluted sera (1:5)				Total	
	no.+	(% +)	no.−	(% −)	no.+	(% +)	no.−	(% −)	no.	(%)
infected	147	(99.3)	1	(0.7)	139	(93.9)	9	(6.1)	148	(100)
non-infected	4	(9.8)	37	(90.2)	3	(7.3)	38	(92.7)	41	(100)
Total	151		38		142		47		189	

lyophilization. Thus, prepared eggs were evenly dispersed in the serum and this made the microscopic observation of precipitin easy and satisfactory.

The reading criterion by Yokogawa *et al.* [16] is sensitive and nearly all the infected can be detected. This method can be useful as the 1 st step in the screening for cases in low endemic areas. By this method, doubtful cases are picked up from a large population living in an endemic area which will necessitate verification by other diagnostic methods.

In the highly endemic area, however, if this method of COP test is used as the 1 st screening step, nearly the same effort will be necessary to make a definite diagnosis by the other detection method. For this reason, the criterion of positive reaction was modified in the present study so that the reaction positives can be regarded as actually infected cases.

By the present criterion, the efficiency of detection was as high as 97.3 % with whole serum and reduced to 87.8 % with the serum eluted from filter paper at 1 : 5 dilution. This efficiency is considered to be much higher than that of the fecal examination by MIFC method.

For the diagnosis of individual outpatients, the type 1 reaction should not be overlooked and regarded as a doubtful case. The other detection techniques like the concentrated fecal examination or rectal biopsy are to be undertaken to make a definite diagnosis. Under these consideration, the present criterion of reading can be used for both mass examination and for the individual diagnosis.

On the other hand, the reaction positives by the criterion by Lewert and Yogore [9,15] can be diagnosed as infected patients. However, the sensitivity was not so high as the other two methods described in the present paper. The reaction with the serum eluted from the filter paper was of no value by this method as was reported by Lewert and Yogore [9].

In the field survey for schistosomiasis japonica, collection of fecal samples is sometimes difficult in the endemic area. Collection of blood samples by finger prick on the filter paper or in a capillary tube is easy. Even if the diluted serum from the filter paper is used, the efficiency of detection is considered to be higher than the fecal examination by existing methods. However, about 12 % of false negatives can not be eliminated when the diluted serum is used.

When the blood taken on filter paper is dried and kept in a firmly sealed container with silica gel, sample materials can be sent by mail to a distant well equipped laboratory without loosing the titer of antibody in the hot tropical con-

dition.

Somehow, sensitivity and specificity were more satisfactory with the use of plasma isolated from the blood taken in a heparinized capillary tube as well as with the use of whole serum. However, this sampling method could be used only at areas close to the laboratory where samples could be submitted on the day of samplings.

It can be said that the efficiency of case detection will be remarkably elevated if the present method of COP test is used combined with the fecal examination.

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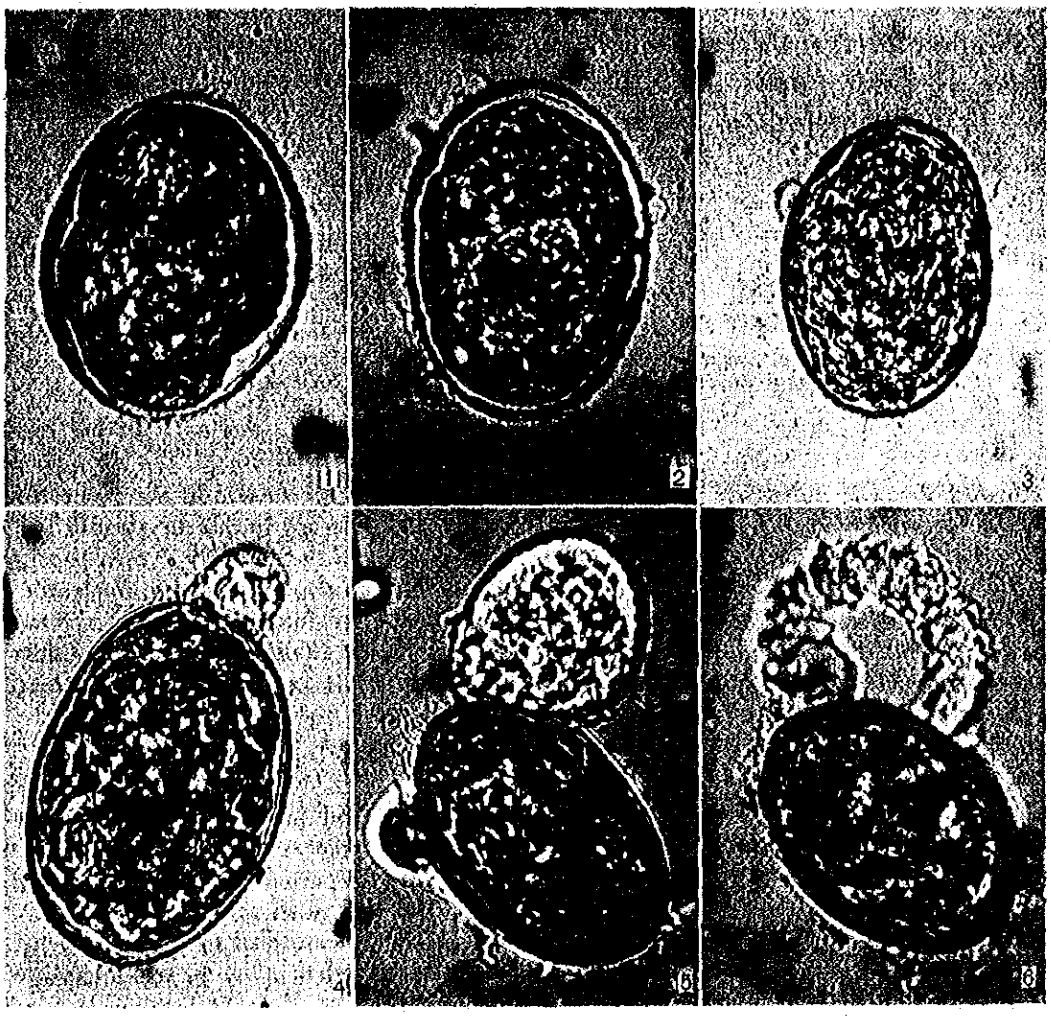

Fig. 1 Classification of COP reactions on lyophilized *S. japonicum* eggs following the criterion by Yokogawa *et al.*

1) Type 0: egg without any precipitate.
2-3) Type 1: tiny and small blebs.
4) Type 2: medium bleb.
5) Type 2 & 3: medium and large blebs.
6) Type 3: septate precipitate.

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Evaluation of the Circumoval Precipitin Test Using Dried Blood on Filter Paper as a Diagnostic Tool in Epidemiological Survey for Schistosomiasis

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Summary: The suitability of the circumoval precipitin (COP) test in a field survey for schistosomiasis in the rural area using dried blood samples on filter paper was evaluated with reference to the stool examination by the merthiolate-iodine-formaldehyde concentration (MIFC) technique. Both blood and stool samples were collected simultaneously among over 600 inhabitants of two schistosomiasis endemic communities (barrios) of Palo, Leyte, Philippines. The method of collecting blood samples on filter paper, the procedure for COP test and its sensitivity in detecting schistosomiasis cases are described. Blood sampling from finger prick was acceptable to both adults and children so that blood samples were readily obtained while the collection of stool samples required repeated visits to the homes. A significantly higher percentage of schistosomiasis positives were obtained by COP test than by stool examination. The COP test results were comparable to the stool examination results on the following aspects: 1) highest infection rate in the 10–19 age group and lowest infection rate in the 60 years old and above; 2) positive rates for schistosomiasis did not differ significantly between the two barrios and 3) no family accumulation of the disease in the two communities was observed in this survey in1972.

The stability of the antibody in filter paper, the reliability of the test and the simple and rapid procedures involved make this filter paper method of COP test very practical and suitable for schistosomiasis survey in rural areas.

Introduction

The circumoval precipitin test is now an established serological test for schistosomiasis. It was first reported by Oliver-Gonzales (1954) [1] and other investigations [2–13] followed which confirmed its high sensitivity and specificity, particularly for schistosomiasis japonica [14]. The use of lyophilized eggs for antigen [15] which could remain stable for as long as three years when stored in vials in a desicator and kept at 4°C has obviated the necessity of frequent antigen preparation [14]. The COP test can be conveniently performed in the field during surveys using whole serum obtained from finger prick if materials for this purpose are available [16]. A more convenient way of carrying a field survey for schistosomiasis with COP test is to use dried blood on filter paper. Blood sampling is possible even from distant places and sufficient time for the transport of samples to the laboratory is allowable since the antibody in filter paper could remain stable for as long as 70 days [17] when kept at room

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temparature. In the present study, an evaluation of the COP test using dried blood on filter paper in a field survey for schistosomiasis was attempted with respect to the merthiolate-iodine-formaldehyde concentration (MIFC) technique of stool examination described by Blagg *et al.* (1955) [18]. The MIFC technique of stool examination was adopted as the standard diagnostic procedure for schistosomiasis survey by the Schistosomiasis Control and Survey Teams of the Philippine Department of Health after Pesigan *et al.* (1958) [19] found it to be a rapid and reliable procedure for both qualitative and quantitative detection for *S. japonicum* eggs.

Materials and Methods

Two adjacent inland communities (Barrios Anahaway and Castilla) in Palo, Leyte, Philippines were selected as target areas in this study. The two barrios are situated about 5-1/2 and 7 kilometers, respectively, from the town proper (poblacion) of Palo. The two barrios are around 1-1/2 kilometers apart with intervening rice fields between them. The combined population is slightly over 600 and the two chief occupations of the people are rice farming and copra making.

A sketch map was prepared for each barrio to indicate the locations of houses and other public buildings. Each house was assigned a number and all household occupants who submitted samples were listed and identified by code numbers.

About 0.1 ml of blood from a finger prick was collected in filter paper strip (Toyo Roshi Type I) which measures 5 mm × 30 mm. This was kept in a refrigerator to dry for at least 24 hours. The serum available in the filter paper was approximately 0.04 ml.

To facilitate the collection of stool specimens, stool containers were distributed. They were collected as soon as filled and fecal samples were examined qualitatively by the MIFC technique.

The circumoval precipitin test was likewise performed at the SCPP laboratory. The dried blood on the filter paper was cut into four parts and soaked in 0.2 ml PBS (phosphate buffered saline) at pH 7.2 for an hour at 30°C to extract the serum at 1: 5 dilution. One drop of the serum extract was placed into about 200 lyophilized *S. japonicum* eggs on a glass slide. A cover slip (18 mm × 18 mm) with vaseline on the rims was placed over the mixture. The glass slide was placed inside a moist chamber and kept in an incubator at 37°C for two days. The reaction was read under a microscope and was regarded positive when at least one egg out of 30 mature eggs counted showed precipitate formation of at least Type I following the classification made by Yokogawa *et al.* (1967) [12].

Results and Discussion

A total of 577 serum samples and 443 fecal samples were collected from the inhabitants of the two barrios. The results of both the stool examination and the circumoval precipitin test are presented in Tables 1 and 2, respectively. Out of 173 stools examined in Anahaway, 85 or 49.1% were positive for *S. japonicum* eggs and out of 220 examined in Castilla, 92 or

Table 1 Difference in prevalence of egg positives between two barrios in Dec. 1972.

Barrio	No. examined	No. (+)	% (+)	No. (−)	% (−)
Anahaway	173	85	49.1	88	50.9
Castilla	220	92	41.8	128	58.2
Total	393	177	45.0	216	55.0

$0.25 > Pr(\chi^2 = 1.81) > 0.1$.
Difference is *not significant*.

Table 2 Difference in prevalence of COPT positives between two barrios in Dec. 1972.

Barrio	No. examined	No. (+)	% (+)	No. (−)	% (−)
Anahaway	309	141	65.9	73	34.1
Castilla	195	168	57.9	122	42.1
Total	504	309	61.3	195	38.7

$0.1 > Pr(\chi^2 = 2.959) > 0.05$.
Difference is *not significant*.

41.8% were positive for eggs. In the COP test, 141 or 65.9% had positive reactions out of 309 examined in Anahaway and 168 or 57.9% had positive reactions out of 195 examined in Castilla. Both diagnostic methods revealed no significant differences in the prevalence of schistosomiasis between the populations of the two barrios. The over-all results of the parasitological survey showed that 177 out of the 393* persons examined or 45% were positive for *S. japonicum* eggs by a single fecal examination. While 309 out of 504 persons examined or 61.3% had positive COP reactions. TABLE 3 shows the comparative sensitivity between the fecal examination by MIFC and the COP test by the filter paper method in detecting schistosomiasis cases. The 15.6% difference in positive rates in favor of the COP test was found to be significant by the chi-square test.

Family accumulation of schistosomiasis cases in the two barrios was analysed. The probabilities of positive cases (p) in all dwellers

TABLE 3 Comparison of the sensitivity of the COP test and the fecal examination in detecting schistosomiasis cases.

Method	No. examined	No. (+)	% (+)	No. (−)	% (−)
Fecal	393	177	45.0	216	55.0
COPT	393	238	60.6	155	39.4
Total	786	415	52.8	371	47.2

$0.001 > Pr(\chi^2 = 18.996)$.
Difference is *significant*.

TABLE 4 Distribution of *Schistosoma japonicum* egg positives and COPT positives among families in two barrios of Palo, Leyte with observed and (estimated) number of households.

A. *Fecal examination for eggs:*

No. of persons/household (n)	No. of household	Number of egg positives in a household (r)							
		0	1	2	3	4	5	6	7
1	60	36(33.7)	24(26.3)						
2	42	16(13.2)	15(20.7)	11(8.1)					
3	25	6(4.4)	9(10.4)	7(8.1)	3(2.1)				
4	14	2(1.4)	1(4.3)	8(5.1)	2(2.7)	1(0.1)			
5	11	0(0.6)	3(2.4)	4(3.7)	2(2.9)	2(1.1)	0(0.2)		
6	6	1(0.2)	0(0.9)	1(1.7)	3(1.8)	1(1.1)	0(0.3)	0(0.0)	
7	4	0(0.1)	0(0.4)	0(0.9)	3(1.2)	1(0.9)	0(0.4)	0(0.1)	0(0.0)
Total	162	61(53.5)	52(65.4)	31(27.7)	13(10.7)	5(3.6)	0(0.9)	0(0.1)	0(0.0)

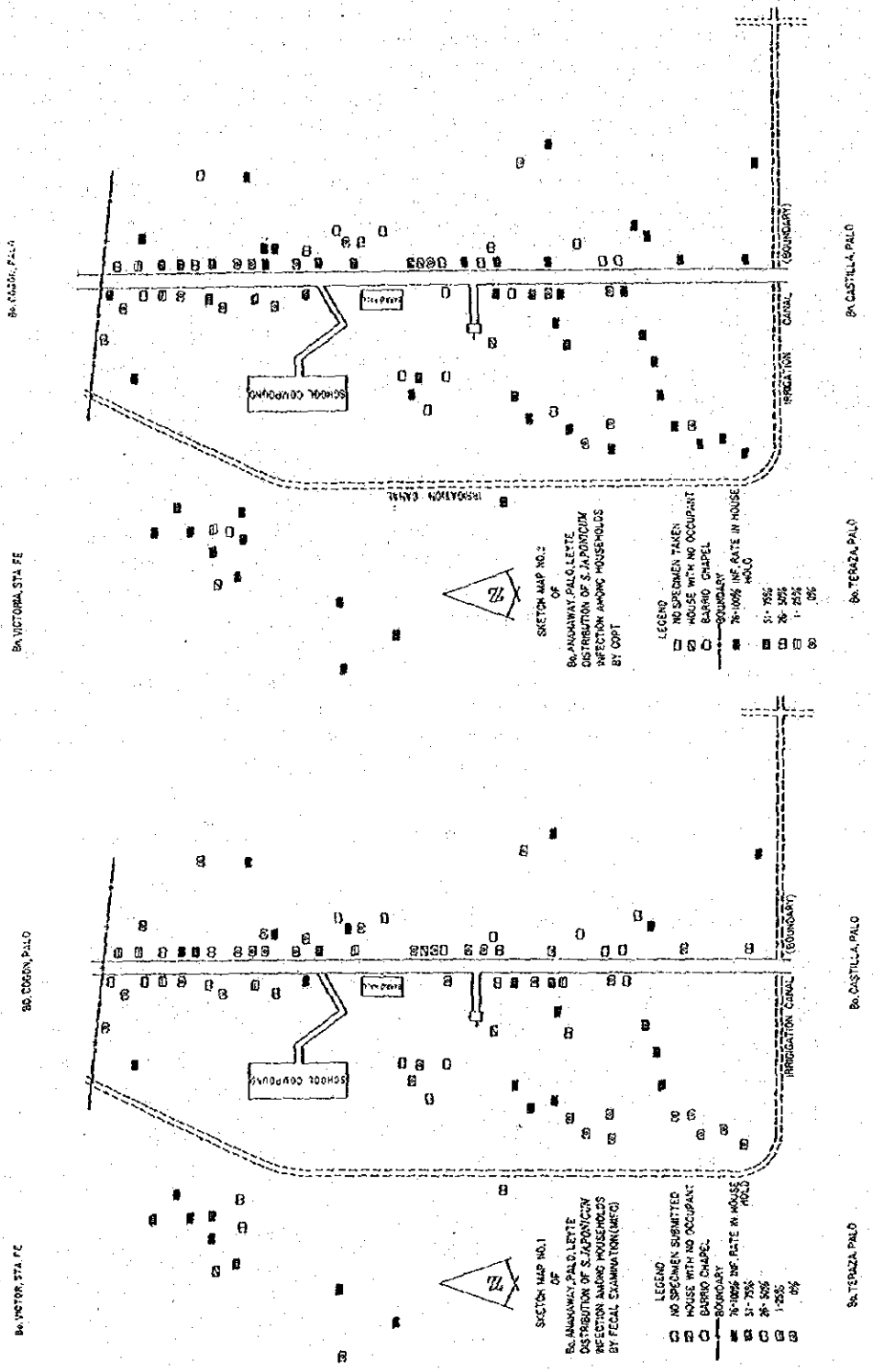
$P = 0.4391$ $DF = 3$
$\chi^2 = 4.7034$ $0.1 < Pr. < 0.2$ Not significant

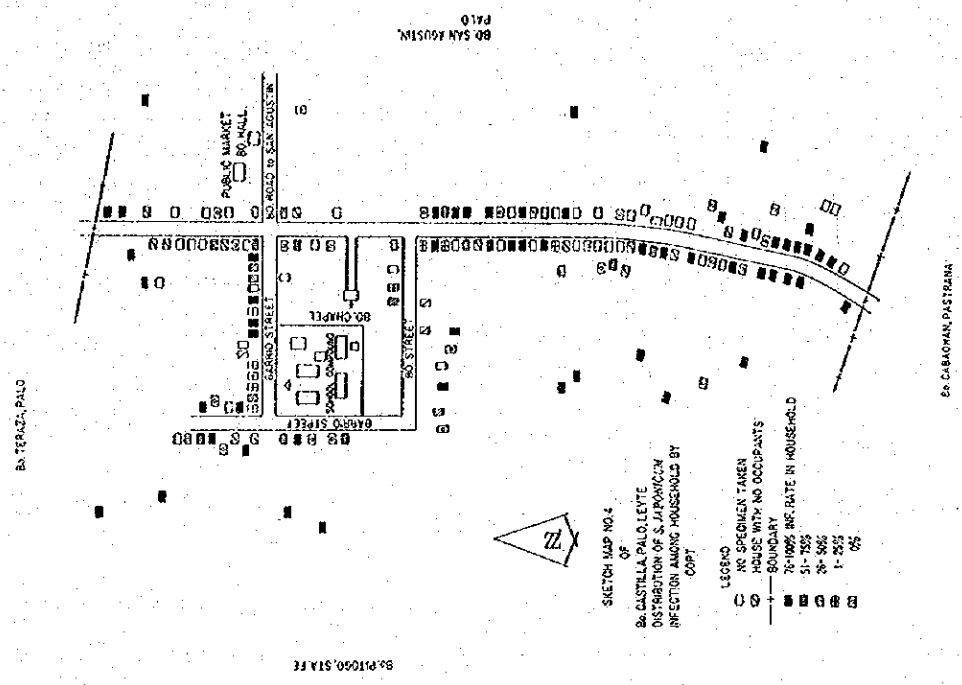
B. *COPT positives:*

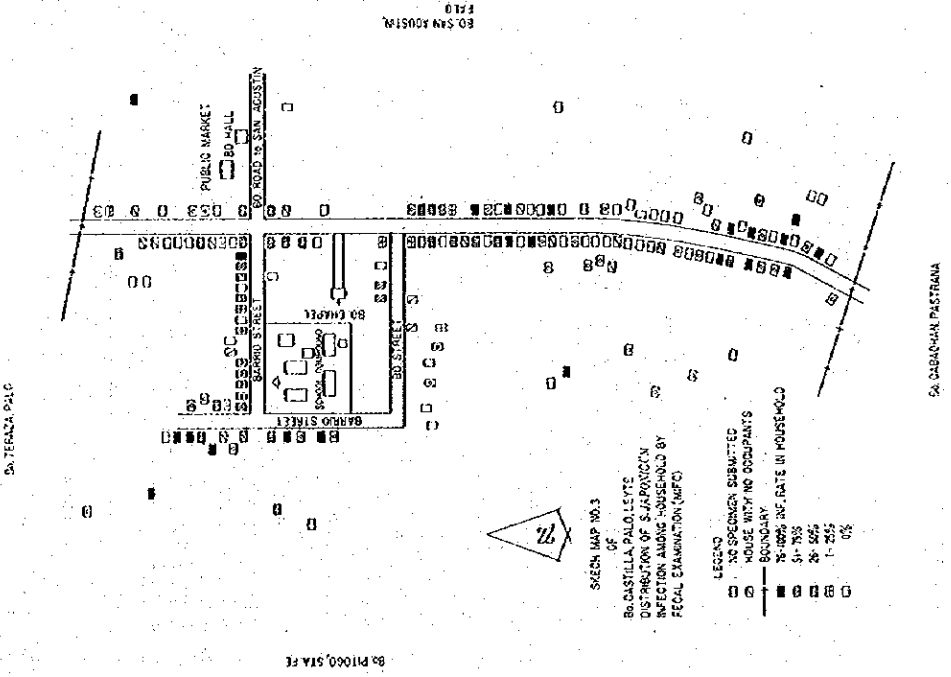
No. of persons/household (n)	No. of household	Number of COPT positives in a household (r)							
		0	1	2	3	4	5	6	7
1	62	26(23.9)	36(38.1)						
2	59	11(8.8)	22(27.9)	26(22.3)					
3	24	1(1.4)	4(6.5)	14(10.5)	5(5.6)				
4	21	1(0.5)	2(2.9)	5(7.1)	5(7.5)	8(3.0)			
5	14	0(0.1)	2(0.9)	3(3.0)	3(4.8)	4(3.9)	2(1.2)		
6	7	0(0.0)	1(0.2)	1(0.9)	1(1.9)	1(2.2)	3(1.4)	0(0.4)	
7	5	1(0.0)	1(0.1)	0(0.3)	1(0.9)	1(1.4)	1(1.4)	0(0.7)	0(0.2)
Total	192	40(34.6)	68(76.8)	49(44.1)	15(20.7)	14(10.5)	6(4.0)	0(1.1)	0(0.2)

$P = 0.6149$ $DF = 4$
$\chi^2 = 5.218$ $0.2 < Pr. < 0.3$ Not significant

* Negative cases with history of treatment were excluded in this count.


Bo. ANAHAWAY, PALO
Bo. CASTILLA, PALO
Bo. VICTORIA, STA. FE
Bo. TERAZA, PALO
IRRIGATION CANAL
(BOUNDARY)
SCHOOL COMPOUND
SKETCH MAP NO. 2
OF
Bo. ANAHAWAY, PALO, LEYTE
DISTRIBUTION OF S. JAPONICUM
INFECTION AMONG HOUSEHOLDS
BY COPT
LEGEND
NO SPECIMEN TAKEN
HOUSE WITH NO OCCUPANT
BARRIO CHAPEL
BOUNDARY
76-100% INF. RATE IN HOUSE HOLD
51 - 75%
26 - 50%
1 - 25%
0%
Bo. COGON, PALO
Bo. VICTOR, STA. FE
SKETCH MAP NO. 1
OF
Bo. ANAHAWAY, PALO, LEYTE
DISTRIBUTION OF S. JAPONICUM
INFECTION AMONG HOUSEHOLDS
BY FECAL EXAMINATION (MFC)


PUBLIC MARKET
BD. HALL
BD. ROAD to SAN AGUSTIN
BARRIO STREET
BD. CHAPEL
SCHOOL BUILDING
BD. STREET
Bo. TERAZA, PALO
Bo. CABANOAN, PASTRANA
Bo. PITOGO, STA. FE
SKETCH MAP NO. 4
OF
Bo. CASTILLA, PALO, LEYTE
DISTRIBUTION OF S. JAPONICUM
INFECTION AMONG HOUSEHOLD BY
COPT
LEGEND
NO SPECIMEN TAKEN
HOUSE WITH NO OCCUPANTS
BOUNDARY
76-100% INF. RATE IN HOUSEHOLD
51- 75%
26- 50%
1- 25%
0%


PUBLIC MARKET
BD. HALL
BD. ROAD to SAN AGUSTIN
BARRIO STREET
BD. CHAPEL
SCHOOL BUILDING
BD. STREET
Bo. TERAZA, PALO
Bo. CABANOAN, PASTRANA
Bo. PITOGO, STA. FE
SKETCH MAP NO. 3
OF
Bo. CASTILLA, PALO, LEYTE
DISTRIBUTION OF S. JAPONICUM
INFECTION AMONG HOUSEHOLD BY
FECAL EXAMINATION (MIFC)
LEGEND
NO SPECIMEN SUBMITTED
HOUSE WITH NO OCCUPANTS
BOUNDARY
76-100% INF. RATE IN HOUSEHOLD
51- 75%
26- 50%
1- 25%
0%

were 0.4391 by stool examination and 0.6149 by COP test. For obtaining the estimated frequencies of household when positive cases distribute evenly over all household, total number of household examined were classified into two dimension by the number of persons (n) and the number of positive cases (r) in a household.

The probability of the number of household in which r number of persons are positive of n number of persons when positive cases distribute evenly can be estimated by ${}_nC_r p^r q^{n-r}$ where p is the positive probability among all dwellers and q is the negative probability $(1-p)$. This can be calculated by expanding $F(p+q)^n$ in varying n from 1 to 7 in the present study where F is the total number of household. The observed and estimated frequencies of household in which r number of persons are positive, are obtained by adding the values along each column (TABLE 4).

In both diagnostic tests the observed and estimated number of household did not differ significantly (TABLE 4). This means that schistosomiasis cases distribute almost evenly among the families of the two communities as are illustrated in the sketch maps 1 to 4.

Aside from schistosomiasis, three intestinal helminths were harbored by the majority of the inhabitants as shown in TABLE 5. These are *Ascaris lumbricoides* (73.8%), *Trichuris trichiura* (94.8%) and hookworms (45.4%) The relationship of COP reactions to the four helminth infections was determined by correlating the numbers of COP test positives and negatives with the numbers of egg positives and negatives for each helminth on 393 persons examined. The statistical analysis of the data by chi square test revealed that the differences of prevalences of COP positives between egg positives and negatives were not significant in *Ascaris*, *Trichuris* and hookworm infections, but significant in *S. japonicum* infection (TABLE 6). This indicates that the COP reaction is dependent only on *S japonicum* infection.

It can be seen in TABLE 7 that the highest infection rate for schistosomiasis is in the age group 10 to 19 by both diagnostic methods and lowest in the 60 years old and above.

Fig. 1 shows the age and sex distribution of *S. japonicum* egg positives and COP test positives with 90% confidence levels of the means. The mean prevalences for COP test

TABLE 5 Differences in sex distribution of helminth infections by fecal examination and circumoval precipitin test.

A. *Fecal examination for eggs:*

	Schistosoma			*Ascaris*			*Trichuris*			Hookworm			
Egg	No. +	% +	No. −	No. +	% +	No. −	No. +	% +	No. −	No. +	% +	No. −	Total
Male	109	(49.8)	110	154	(74.9)	65	210	(95.9)	9	116	(53.0)	103	219
Female	90	(40.2)	134	173	(77.2)	51	210	(93.8)	14	85	(37.9)	139	224
Total	199	(44.9)	244	327	(73.8)	116	420	(94.8)	23	201	(45.4)	242	443
	$\chi^2 = 3.74$			$\chi^2 = 2.39$			$\chi^2 = 0.64$			$\chi^2 = 9.48$			
	$0.1 > Pr. > 0.05$			$0.2 > Pr. > 0.1$			$0.5 > Pr. > 0.3$			$Pr. < 0.01$			
Difference:	Not significant			Not significant			Not significant			Significant			

B. *COP test:*

Reaction	No. +	% +	No. −	Total
Male	165	(65.6)	97	262
Female	172	(58.3)	127	299
Total	337	(61.9)	224	561

$\chi^2 = 2.95$ $0.1 > Pr. > 0.05$

Difference is not significant

TABLE 6 Relationship of the circumoval precipitin test reactions to helminth infections

COPT reaction	*Schistosoma* egg			*Ascaris* egg			*Trichuris* egg			Hookworm egg			Total
	+	−	%	+	−	%	+	−	%	+	−	%	
+	139	99	(45.8)	180	58	(59.2)	225	13	(72.2)	112	126	(58.3)	238
−	38	117	(54.2)	115	40	(40.8)	150	5	(27.8)	65	90	(41.7)	155
Total	177	216	(100)	295	98	(100)	375	18	(100)	177	216	(100)	393
	$\chi^2 = 39.18$			$\chi^2 = 0.04$			$\chi^2 = 0.799$						
	Pr.<0.001			0.8<Pr.<0.9			Pr. = 0.217			0.3<Pr.<0.5			
Difference:	Significant			Not significant			Not significant			Not significant			

positives were higher than those for egg positives in all age groups of both sexes, except that for the age group 40–49 among males. The 90% confidence limits of the means were relatively narrower for COP test positives than for egg positives. In both sexes, the infection rate builds up rapidly during early childhood reaching its peak in the age group 10–19, after which it tapers down gradually as age increases. This is best illustrated by the COP test results among males and by the stool examination results among females. Although it appears in Fig. 1 that males have generally higher infection rates than females, the statistical analysis by chi-square test revealed that there is no significant sex difference in the infection rates by both diagnostic methods as shown in TABLE 5.

The circumoval precipitin test using dried blood on filter paper is less sensitive than whole serum [16] or dried serum on filter paper [20] due to its dilution with PBS during the process of extraction from the filter paper. It will be seen in TABLE 6 that this method of COP test failed to pick up 38 or 21.5% out of 177 confirmed schistosomiasis cases by stool examination.

Nevertheless, it has been proven in this field study that the COP test using dried blood on filter paper is significantly more sensitive than the stool examination by the MIFC technique in detecting schistosomiasis cases. This test was likewise found to be superior to the formalin-ether concentration technique by Cabrera *et al.* [21]. The sensitivity of this method of COP test was also examined quantitatively by the present authors [22].

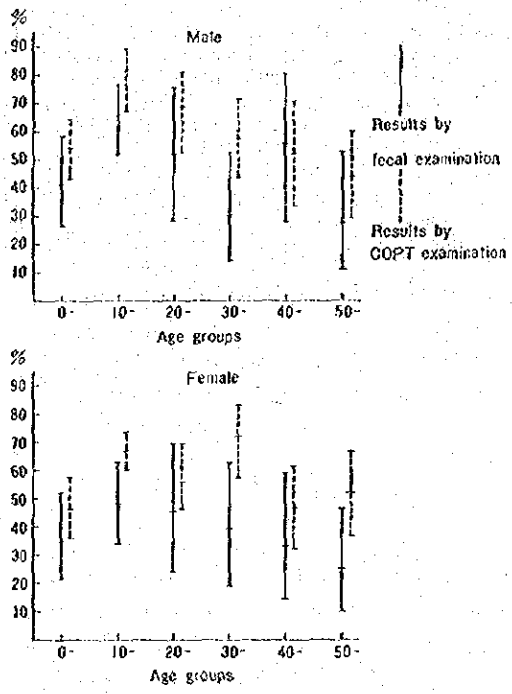
The collection of blood samples on filter paper from finger prick was generally easy and convenient for both the subject and the technician since the procedure could be performed anywhere contact was made with the subject. In contrast, some difficulties were encountered in the collection of stool specimens because of differences in the time and habits of defecation of the people.

In the light of all the preceding observations,

TABLE 7 Age distribution of *S. japonicum* and other helminth infections by COPT and fecal examination.

Age	COP (*S. japonicum*)			Fecal examination								
					S. japonicum		*A. lumbricoides*		*T. trichiura*		Hookworm	
	No. examined	No. +	% +	No. examined	+	%	+	%	+	%	+	%
0- 9	136	69	50.7	106	41	38.7	91	85.8	105	99.1	47	44.3
10-19	217	161	74.2	162	92	56.8	129	79.6	153	94.4	63	38.9
20-29	60	37	61.7	43	21	48.8	27	62.8	41	95.4	19	44.2
30-39	63	41	65.1	49	17	34.7	30	61.2	48	98.0	30	61.2
40-49	47	23	48.9	37	16	43.2	20	54.1	31	83.8	18	48.6
50-59	33	16	48.5	29	10	34.5	16	55.2	25	86.2	14	48.3
60 & up	21	10	47.6	17	2	11.8	14	82.4	17	100.0	10	58.8
Total	577	357	61.9	443	199	44.9	327	73.8	420	94.8	201	45.4

Fig. 1. Age distribution of prevalence of *Schistosoma japonicum* egg positives and COPT positives with 90% confidence level.



the authors consider that the COP test using dried blood on filter paper is adequately reliable and is a very practical diagnostic tool for schistosomiasis. Blood collection is rapid and can cover many subjects in a short period. It is well accepted by the people and the examination procedure is relatively simple. It is, therefore, recommended for use in field surveys for schistosomiasis, either in conjunction with or in place of stool survey.

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ESTIMATION OF ANNUAL INCIDENCE OF SCHISTOSOMIASIS JAPONICA AMONG SCHOOL CHILDREN IN THE PHILIPPINES

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INTRODUCTION

One of the parameters used for the evaluation of a control measure for schistosomiasis in a locality, is the change in human infection rates. For this purpose, the prevalences of infection in school children at grades I to III are being widely used in connection with the control programs in the Philippines. Although the incidence is considered to be a good parameter to show the intensity of infection directly, it is rarely used because of difficulty of observing it before control since it takes about 2 years to measure the incidence. In the present study, a trial was made to estimate the annual incidence indirectly from the age distribution of prevalences among school children at ages 7 to 10 so that the estimated incidence can be used as a base-line datum before schistosomiasis control.

MATERIALS AND METHODS

Survey Data: The data on the prevalence of schistosomiasis among school children at different ages appearing in the annual and quarterly reports of the National Schistosomiasis Control Commission, Department of Health, Republic of the Philippines were analysed. As the prevalences among school children at grades I to III are being used as base-line data of *Schistosoma* infection, children are examined periodically for *Schistosoma* eggs by faecal examination using the MIFC method. At grades I to III, children are mostly 7 to 9 years old and some are 10 years old or older. In most survey data in the 1969 to 1972 reports, classification of prevalences by ages was also added. The number of data showing the age difference of prevalences was about 20 during that period in various endemic areas in the Philippines.

Calculation of Incidence : When the prevalence of schistosomiasis at age t is P_t, and the probability of egg negatives at age t is Q_t or $1-P_t$, and if the annual incidence is a, the probability of negatives at age $t+i$, Q_{t+i}, can be estimated by the equation $Q_{t+i} = Q_t (1-a)^i$. If $b = 1-a$, this relation can be written as the following equations:

$$Q_{t+i} = b^i Q_t \text{ or } 1 - P_{t+i} = (1-a)^i (1-P_t)$$

The first equation can be modified as follows; by letting $t = 0$ and $i = x$, and converting to logarithm:

$$\log Q_x = X \log b + \log Q_o$$

Since age X and the probability of negatives at age X, (Q_x) are known from the observed data, $\log b$ and $\log Q_o$ can be calculated using the formula of the regression line between X and $\log Q_x$ or Y as $Y = AX + B$. As $A = \log b = \log (1-a)$, the annual incidence (a) can be obtained by the following formula:

$$a = 1 - \text{antilog } A$$

An example of the calculation is shown in Table 1. In this sample, the formula of regression line is $\log Q_x = -0.00949\ X + 0.05597$ and the annual incidence is 2.16 %.

This study was performed as a part of the RP-Japan Medical Cooperation Project supported by Japan International Cooperation Agency.

Table 1

Estimation of the annual incidence from the faecal examination for *Schistosoma* eggs in Dulag, Leyte in March 1970.

Age (X)	No. exam.	No. pos.	Prevalence +(P)	Prevalence −(Q)	log Q (Y)	Y. est.	Preval. est.
7	404	11	0.027	0.973	−0.01189	−0.01046	0.0238
8	462	19	0.041	0.959	−0.01818	−0.01995	0.0449
9	435	28	0.064	0.936	−0.02872	−0.02944	0.0655
10	250	22	0.088	0.912	−0.04001	−0.03893	0.0857
T	1551	80	0.0516				

$A = -0.00949$, $B = 0.05597$ in $Y = AX + B$, Annual incidence $a = 0.02161$.

Table 2

Chi square test between observed and estimated prevalences in percentage. The sample is the same as in Table 1.

Age	No. exam.	No. pos.	Prevalence observed %	Prevalence estimated %	χ^2
7	404	11	2.7	2.38	0.00044
8	462	19	4.1	4.49	0.00036
9	435	28	6.4	6.55	0.00002
10	250	22	8.8	8.57	0.00007
Total	1,551	80	5.16*		0.00089

$0.00089 \times 1551/4 = 0.345$, $df = 4 - 2 = 2$, $0.9 > Pr(\chi^2 = 0.345) > 0.8$, Non-significant.
* Mean prevalence.

Reliability of the Incidence Obtained : The prevalence of each age group can be estimated by using the obtained formula of regression line. The prevalence at age X is available by the formula $P_x = 1 - \text{antilog}\ (AX + B)$. An example of calculation is also shown in Table 1. If there is no significant difference between observed and calculated prevalences by chi square test the incidence thus obtained can be regarded as reliable.

Chi square test was performed following the method of Litchfield and Wilcoxon (1949) and of Swaroop (1966). This method is basically used for the chi square test for goodness of fit between observed and calculated percentages on a regression line. In this method chi square at each age group is calculated as follows:

Chi square for each difference =

$$\frac{(\text{observed} - \text{expected}\ \%)^2}{(\text{expected}\ \%) \times (100 - \text{expected}\ \%)}$$

The sum of the individual chi square values must be multiplied by the average number examined at each age group. Degree of freedom in this method is the number of age groups minus 2.

An example of chi square test by this method is shown in Table 2. The sample in Table 1 is also used and the sum of chi square is 0.00089 and the value multiplied by the average number examined is 0.345 of which

reliable level is at a probability between 0.9 and 0.8 at degree of freedom 2. In this example, estimated prevalence is not heterogenous to the observed prevalence. Therefore, the estimated incidence was regarded as reliable.

RESULTS

Following the above method, the incidence was calculated and its reliability was tested in all survey data. The incidences obtained were reliable in 16 data and were not in 5. The other 15 data not presented in Tables 1 and 2 are shown in Table 3 in the order of the reliability of the incidence. The calculated values of incidence *(a)*, constants *A* and *B* in the formula of regression line, the results of chi square and its reliable level are attached to each survey datum. The other data of which incidence was not reliable are also listed in Table 4.

The highest annual incidence was 8.71% and in 3 localities values were negative. Much higher incidences such as 13.6, 18.9 and 22.9% were found in Tabontabon, Gandara and Lala, respectively but these values were not reliable. The annual incidence of schistosomiasis in these age groups was less than 8.7% in survey data so far available in the Philippines. Reliability of the incidence obtained seems to increase with the increase of the total number examined. The minimum number to be examined, to obtain a reliable incidence appears to be about 200 in four age groups.

To examine the relationship between the annual incidence and mean prevalence in each locality, the results from all localities are presented in Table 5 in the order of high incidence. Between the incidence and mean prevalence, a close correlation showing a coefficient 0.8044 was found.

DISCUSSION

There are theoretical difficulties in obtaining the incidence from the observed prevalence. For this estimation, certain conditions have to be assumed; that the incidence has been constant for the last several years being equal over ages 7 to 10 and that no natural cure occurs. These conditions are hardly expected in any locality because the incidence may fluctuate yearly by the changes of natural environment and of the chances of exposure to infection among dwellers. This fluctuation of incidence might cause an abrupt big difference of prevalences between adjacent two age groups as seen in 5 localities in Table 4.

If the above assumed conditions were existing in a locality, the observed prevalence should follow the simple catalytic curve made by Muench (1959). In the present study, the prevalences estimated by the simple catalytic curve using the calculated incidence did not fit the observed data.

In 3 localities, the incidences were minus values. This may be due to a high apparent cure rate (or negative conversion rate of eggs) which was ignored in the present study and the actual incidence might be smaller than the apparent cure rate in these areas. The apparent cure rate can be estimated by the analysis of the two stage catalytic curve proposed by Muench (1959). In the present study, after obtaining the incidence, an attempt was made to estimate the apparent cure rate by using the formula of two stage catalytic curve by the trial and error method. The apparent cure rate, however, could not be obtained successfully. Most probably the incidence obtained by the present method is underestimated by ignoring the apparent cure.

Thus, the use of such a method in obtaining the incidence for one of the pre-control baseline data is not ascertained. This method should be further evaluated by comparing the

Table 3

Survey data on age difference of prevalence of schistosomiasis and estimated reliable incidence.

Locality and date	Age	No. exam.	No. pos.	Prevalence % Observed	Prevalence % Estimated	Results of calculation
Tolosa	7	217	3	1.4	1.41	a 2.57%
Leyte	8	182	8	4.4	3.94	A −0.0113
Dec. 1969	9	143	8	5.6	6.41	B 0.0731
	10	76	7	9.2	8.82	χ^2 0.2999
	T	618	26	4.21*		0.9>Pr>0.8
Kapatagan	7	42	12	28.6	25.74	a 2.60%
Lanao del Norte	8	79	19	24.1	27.67	A −0.0114
Mindanao	9	39	11	28.2	29.55	B −0.0492
Mar. 1970	10	21	7	33.3	31.38	χ^2 0.6204
	T	181	49	27.07*		0.8>Pr>0.7
4 schools	7	101	2	2.0	2.91	a 0.09%
Victoria	8	133	6	4.5	3.00	A −0.0004
Or. Mindoro	9	71	2	2.8	3.09	B −0.0100
Dec. 1970	10	35	1	2.8	3.18	χ^2 0.8843
	T	340	11	3.24*		0.7>Pr>0.5
5 villages	7	76	1	1.3	2.36	a 2.49%
Naujan	8	100	6	6.0	4.80	A −0.0109
Or. Mindoro	9	77	6	7.8	7.17	B 0.0664
Sep. 1969	10	23	2	8.7	9.48	χ^2 0.8462
	T	276	15	5.43*		0.7>Pr>0.5
5 villages	7	172	29	16.9	17.60	a 3.41%
Isulan	8	165	36	21.8	20.41	A −0.0150
Cotabato	9	107	24	22.4	23.13	B 0.0215
Mindanao	10	/	/			χ^2 0.2715
Sep. 1970	T	444	89	20.05*		0.7>Pr>0.5
Calbayog City	7	62	2	3.2	2.46	a −0.79%
Samar	8	63	0	0	1.69	A 0.0034
Sep. 1970	9	51	1	2.0	0.92	B −0.0347
	10	13	0	0	0.14	χ^2 1.5923
	T	189	3	1.59*		0.5>Pr>0.3
Gandara	7	96	15	15.6	14.09	a 3.23%
West. Samar	8	100	13	13.0	16.86	A −0.0143
	9	98	22	22.4	19.55	B 0.0340
	10	56	12	21.4	22.16	χ^2 1.7593
	T	350	62	17.71*		0.5>Pr>0.3

a = Annual incidence, A and B = Constants of regression line $\log Q_x = AX + B$.
χ^2 = Difference between observed and estimated prevalences.
* Mean prevalence.

Table 3 (Cont'd)

Locality and date	Age	No. exam.	No. pos.	Prevalence % Observed	Prevalence % Estimated	Results of calculation
Caibaan, Marasbaras & Sagkahan	7	79	1	1.3	0.66	a 0.97%
	8	54	0	0	1.62	A −0.0042
Tacloban	9	76	3	3.9	2.57	B 0.0267
Leyte	10	32	1	3.1	3.52	χ^2 1.8256
Jun. 1970	T	241	5	2.07*		0.5>Pr>0.3
Calbayog City	7	85	1	1.2	0.99	a −0.18%
Samar	8	55	0	0	0.81	A 0.0008
Jun. 1970	9	59	1	1.7	0.63	B 0.0099
	10	40	0	0	0.45	χ^2 1.8761
	T	239	2	0.84*		0.5>Pr>0.3
Alangalang	7	82	14	17.1	15.12	a 6.43%
Leyte	8	254	43	16.9	20.58	A −0.0289
Jun. 1970	9	195	53	27.2	25.69	B 0.1309
	10	157	48	30.6	30.47	χ^2 2.2873
	T	688	158	22.97*		0.5>Pr>0.3
Calbayog City	7	51	2	3.9	5.20	a 5.49%
Samar	8	70	6	8.6	10.40	A −0.0245
Dec. 1970	9	32	7	21.9	15.32	B 0.1486
	10	31	5	16.1	19.98	χ^2 2.0566
	T	184	20	10.87*		0.5>Pr>0.3
Sadaan	7	8	2	25.0	32.46	a 8.71%
Midsayap	8	22	11	50.0	38.34	A −0.0396
Cotabato	9	16	6	37.5	43.71	B 0.1067
Mindanao	10	/	/			χ^2 1.5413
Sep. 1970	T	46	19	41.30*		0.3>Pr>0.2
Pasi	7	28	1	3.5	4.48	a −0.28%
Socorro	8	41	1	2.4	4.21	A 0.0012
Or. Mindoro	9	20	2	10.0	3.94	B −0.0285
Sep. 1971	10	13	0	0	3.67	χ^2 3.7077
	T	102	4	3.92*		0.2>Pr>0.1
Palo	7	313	46	14.7	12.63	a 7.71%
Leyte	8	390	72	18.5	19.36	A −0.0348
Dec. 1969	9	321	70	21.8	25.58	B 0.1851
	10	148	50	33.8	31.31	χ^2 4.4030
	T	1,172	238	20.31*		0.2>Pr>0.1
Kapatagan	7	10	0	0	7.34	a 6.60%
Lanao del Norte	8	20	3	15.0	13.46	A −0.0297
Mindanao	9	24	8	33.3	19.17	B 0.1745
Jun. 1970	10	22	3	13.6	24.50	χ^2 5.2117
	T	76	14	18.42*		0.1>Pr>0.05

Table 4

Survey data of schistosomiasis showing unreliable incidence.

Locality and date	Age	No. exam.	No. pos.	Prevalence % Observed	Prevalence % Estimated	Results of calculation
Oquendo	7	76	9	11.8	8.99	a 8.46%
Calbayog City	8	85	8	9.4	16.69	A −0.0384
Samar	9	62	18	29.0	23.73	B 0.2277
Dec. 1970	10	73	21	28.8	30.18	χ^2 6.25
	T	296	56	18.92*		0.05>Pr>0.02
Lala	7	33	5	15.2	4.72	a 22.86%
Danao del Norte	8	37	9	24.3	26.5	A −0.1127
Mindanao	9	33	8	24.2	43.29	B 0.7679
Dec. 1970	10	14	9	64.3	56.25	χ^2 9.43
	T	117	31	26.50*		0.01>Pr
Gandara	7	73	14	19.2	7.58	a 18.91%
Samar	8	56	7	12.5	25.06	A −0.0910
Sep. 1969	9	39	13	33.3	39.23	B 0.6030
	10	25	14	56.0	50.72	χ^2 12.45
	T	193	43	24.87*		0.01>Pr
Tabontabon	7	85	8	9.4	3.96	a 13.62%
Leyte	8	98	6	6.1	17.05	A −0.0636
Mar. 1970	9	54	18	33.3	28.35	B 0.4276
	10	69	26	37.7	38.11	χ^2 19.42
	T	306	58	18.95*		0.001>Pr
Matulatula	7	26	0	0	−3.78	a 8.01%
and Pola	8	14	0	0	4.53	A −0.0363
Mindoro	9	19	2	10.5	12.18	B 0.2699
Sep. 1972	10	14	3	21.4	19.21	Impossible
	T	73	5	6.85*		

estimated incidence with the one obtained by the direct measurement in field surveys.

SUMMARY

An attempt was made to estimate the annual incidence of schistosomiasis from the prevalences at ages 7 to 10 in the Philippines. Assuming that the incidence a has been stable for the past several years, there would be no age difference of the incidence in these age groups and that natural cure would be negligible. If the probability of egg negatives at age X is Q_x and the probability remaining negative in one year is b where $b = 1 - a$, a relation among X, Q and b can be written by an equation $Q_x = b^x Q_o$. Since the prevalence at age X, (P_x) is $1 - Q_x$, the above

Table 5

Relationship between the annual incidence and mean prevalence in localities.

Locality	Incidence %	Mean prevalence %	No. exam.
Sadaan	8.71	41.30	46
Palo	7.71	20.31	1172
Kapatagan	6.60	18.42	76
Alangalang	6.43	22.97	688
Calbayog	5.49	10.87	184
Isulan	3.41	20.05	444
Gandara	3.23	17.71	350
Kapatagan	2.60	27.01	181
Tolosa	2.57	4.21	618
Naujan	2.49	5.43	276
Dulag	2.16	5.16	1551
Caibaan	0.97	2.07	241
Victoria	0.09	3.24	340
Calbayog	−0.18	0.84	239
Pasi	−0.28	3.92	102
Calbayog	−0.79	1.59	189

Correlation coefficient 0.8044.

equation can be written as $1 - P_x = (1 - a)^x \times (1 - P_o)$ which shows a relationship between the prevalence and incidence. The original formula can be converted to the logarithm as $\log Q_x = x \log (1 - a) + \log Q_o$. As this is the formula of a regression line between age X and $\log Q_x$, a can be estimated by the observed prevalences of four age groups. For the reliability test of the estimated incidence, the estimated prevalence at each age must be obtained using a value. If the observed and estimated prevalences are not significantly different, the obtained value of the incidence could be regarded as reliable. Reliable incidences could be obtained in 16 localities from survey data. The annual incidence ranged from 8.71 % to −0.79 %. In 3 localities, the incidences were minus values. In these areas, the apparent cure rate must be greater than the incidence. Incidences much higher than 8.71%, such as 22.86 % and 19.42 % were observed in some localities. However, these values were not reliable by chi square test. A correlation coefficient of 0.804 was found between the incidence and the mean prevalence of these age groups.

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Clinical Studies of the Cerebral Schistosomiasis Japonica in the Leyte Island, the Philippines — in Comparison with the Same Disease in the Kofu City Area —

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ABSTRACT

Schistosomiasis Japonica (SJ) is caused by infection with Schistosoma japonicum, and its lesion are located mainly in the portal system and liver which are parasitized by this worm. However, the socalled cerebral Schistosomiasis Japonica (CSJ) is sometimes seen which is complicated by neuropsychiatric symptoms due to an ectopic parasitism of the worm in the cerebral nervous system.

We examined the CSJ in Philippines which are one of the areas heavily infected with Schistosoma Japonicum. The present paper deals with the results of a preliminary survey with special reference to clinical symptoms and electroencephalographic (EEG) findings in comparison with the same disease in the Kofu City area which we have experienced so far.

Subjects and Methods: From the list of patients living in Palo Town, Leyte, Philippines which was prepared by the Schistosomiasis Control and Research Project (SCRP) of Palo Town, we selected 75 patients randomly from 300 and some patients with suspected CSJ who showed neurological symptoms such as convulsion, paroxysmal disturbance of consciousness, finger tremor, and ataxia. The ages of the subjects anged from 14 to 63 years, with a mean of 33. The ratio of males to females was 48 : 27. On these 75 subjects, we performed detailed interview physical examination, neurological examination, and EEG, taking about on month from February, 1975.

Results are as follows:

1) Of the 75 subjects, 71 (91 %) had paroxysmal disease, consisting of Jacksonian type in 33, psychomotor seizure in 24, grand mal in 13, and autonomic seizure in 1. The remaining 4 had

cerebellar ataxia, hepatosplenomegaly, dysthyroidism, or Korsakoff's syndrome.

2) Of the 71 with paroxysmal disease, 49 showed late onset seizure (onset after the age of 20), and 51 had frequent seizures (more than once monthly).

3) EEG was judged to be normal in 24 (32 %), borderline in 13 (17 %), and normal in 38 (51 %) . The abnormal and borderline EEGs were characterlized by asymmetrical findings in the bursts of slow waves and basic rhythms. There was no case with seizure discharge other than paroxysmal slow wave.

4) Discussion was made in reference to the strong suspicion that cerebral symptoms of the subjects, paroxysmal disease in particular, were a syndrome associated with Schistosoma Japonicum, and to the difference between CSJ in Japan and that in the Philippines.

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CURRENT CHEMOTHERAPY OF SCHISTOSOMIASIS JAPONICA IN THE PHILIPPINES

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Up to the present there has been no drug found suitable for the treatment of schistosomiasis japonica on a mass scale. While some of the drugs recently formulated have been moderately effective, the many side effects leave much to be desired. This has therefore limited the usefulness of mass treatment as one of the approaches to the control program in the Philippines. As a general rule, treatment is given to symptomatic cases among school children 10-14 years, as this is the segment of the population that contributes more to spread of the disease on account of its higher relative transmission index (Pesigan *et al.*, 1958) and possibly to prevent the more serious consequences of chronic schistosomiasis.

The only drug registered with the Philippine Food and Drug Administration for the treatment of schistosomiasis japonica at present is Stibophen. Other promising drugs which have been evaluated are pararosaniline pamoate, niridazole and sodium antimony dimethylcysteine tartrate.

Stibophen

Stibophen is a trivalent antimonial preparation containing 13.5% antimony and marketed as a 6.3% solution. It was first used on a widescale by Pesigan *et al.*, (1951) who reported that after a course of nine intramuscular injections, 78.2% out of 1,083 who were treated ceased to pass viable eggs.

Further evalution of this drug with a longer period of stool follow-up was conducted to study the efficacy of increased dosage (Santos *et al.*, 1970). A total of 151 patients who were divided into two groups comprised the subjects of this study. One group consisting of 72 patients received 10 intramuscular injections at one ml per 10 kilogram body weight per day. The initial 2 injections given on 2 consecutive days were sensitivity test doses and were approximately $\frac{1}{3}$ and $\frac{2}{3}$ respectively, of the regular daily dose which was administered on the third day and every other day thereafter. The total dose administered to an adult patient weighing no less than 50 kg was therefore 45 ml. This form of treatment schedule was usually the one followed by the Rural Health Units.

In the second group of patients consisting of 79 patients, the above schedule was extended to 15 injections or a total of 70 ml for an adult patient. Two stools were examined monthly for a period of 6 months after treatment. The method of stool examination was the Merthiolate-Iodine-Formaldehyde Concentration (MIFC) of Blagg *et al.*, (1955), using 1 ml by volume of the stool. Complaints were noted and recorded during treatment and the monthly follow-up for 6 months.

Patients who received only 10 intramuscular injections gave a stool negative conversion rate of 61.2% one month after treatment. It decreased to 38.3% months later. Decrease in egg counts for the corresponding periods were 96.5% and 82% respectively.

In the second group of 79 patients, the stool negative conversion rate one month after was 86.7% which decreased to 48.6% after six months. Decrease in egg counts for the corresponding periods were 99% and 96.2%, respectively.

The most frequent reactions observed were dizziness, body weakness, nausea and vomiting,

anorexia, and joint and muscular pains. Two had severe reactions lasting for 3 days, one after the fourth injection and the other after the sixth injection. Treatment in both cases was discontinued. Actually, the 151 patients represented only those who completed the required course of treatment. A dropout of 41.9% was observed, mostly due to the pain at the site of injection and reactions which were observed in 83.9% of the patients who started receiving the drug in this investigation.

Although it is apparent that the efficiency of Stibophen could be improved with increased dosage, there is also a concomittant rise in frequency of undesirable side effects.

Pararosaniline Pamoate

CI-403-A is the code name for pararosaniline pamoate, a complex azo dye derived from gentian violet which is chemically the pamoate salt of tris (p-aminophenyl) carbonium 4, 4' methylenibis (3 hydroxy-2-napthoate) and which was worked out by Elslager *et al.*,(1961).

In Leyte, this drug was evaluated using three dosage schedules (Pesigan *et al.*, 1967). The schedule which gave the best results was the one wherein a dose of 35 to 40 mg per kg per day was given for two weeks followed by a seven day rest period and then resumption of treatment for another two weeks. Treatment was continued thereafter once a week for 16 to 24 weeks.

Results of stool follow-up 4 months after treatment showed that only 37.7% became negative. Egg count however was reduced by 88.2%. It is interesting to note here that 12 months after treatment, an egg count reduction of 79.4% could still be observed.

Pararosaniline pamoate is apparently safe and non-toxic, but it has the disadvantage of being given over a very long period of 52 treatment days spread out over a total of 293 days.

Niridazole

Niridazole or Ambilhar has a chemical formulation of 1-(5-nitro-2-thiazolyl)-2-imidazolidinone and comes in 500 mg tablets. Oral doses of 20-30 kg daily for five to seven days in 14 patients with *S. hematobium* infection apparently cured all of them.

After some preliminary trials with lower dosages, niridazole was administered to two groups of 72 patients at a dose of 20-25 mg per kg per day (Santos *et al.*, 1971). One group received the drug daily for 14 days while the other received the drug for 10 days followed by 4 doses at weekly intervals. Since results in both groups did not differ significantly, the combined results of follow-up by faecal examination of both groups showed that 58.9% became stool negative while egg counts were reduced by 98.0%, 6 months after treatment.

The common reactions observed were dizziness, nausea, vomiting. These were generally mild in most of the cases except in three who had hallucinations, which however subsided after withdrawal of the drug.

Other treatment schemes are presently being tried in Leyte with special emphasis on the administration of repeated smaller dosage to determine its suppressive action.

Sodium Antimony Dimethylcysteine Tartrate

Sodium antimony dimethylcysteine tartrate is another antimonial preparation developed recently by A. H. Robins. It is a water soluble compound with an antimony content of 14.5%. The dimethylcysteine component detoxifies arsenicals and antimonials without affecting their antiparasitic action (Ercoli, 1967).

A preliminary trial on 20 patients who were given the recommended dosage of 400 mg per day intramuscularly for 5 days showed that the drug was very effective. All patients

were stool negative for six months after treatment (SCPP, 1970). The drug, however, was not well tolerated. In the first group of 10 hospitalized patients, there was evidence of myocardial injury in 9 from the EKG tracings. Common reactions observed were anorexia, malaise, vomiting, pain at site of injection, fine vesicular eruptions, fever and nausea.

Another trial was conducted using smaller doses based on mg per kg body weight to determine whether efficacy would be maintained and toxicity reduced (SCPP, 1973). Six groups of six patients each were given 3, 5, 6 and 7 mg per kg for 5 to 7 days. Only the group which received 5 mg per kg daily for 7 days showed good results with 100% being stool negative six months after treatment. The other groups gave stool negative conversion rates of 50 to 83.3%, efficacy being related to dosage. However, myocardial toxicity was still evident in 48.5% of all the patients treated and this too appeared to be dose related.

SUMMARY

For the past several decades, the drug being used for the treatment of schistosomiasis in the Philippines has been Stibophen. It is administered intramuscularly at a dose of 1 ml per 10 kg body weight with a maximum of 5 ml every other day after 2 initial daily smaller sensitivity doses at a total dose of 45 to 70 ml for adult patients.

In recent years, a number of drugs for the treatment of schistosomiasis have been developed. These were evaluated clinically either in the hospital or in field trials in Leyte. Unfortunately, none of these were found to be suitable for mass treatment on account of toxicity or prolonged course of treatment. In view of the pressing need for a safe and effective schistosomicidal agent, the search for a better drug is imperative.

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AMBILHAR IN THE TREATMENT OF *SCHISTOSOMA JAPONICUM* INFECTION AND AS AN EGG SUPPRESSANT FOR MASS TREATMENT

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INTRODUCTION

Ambilhar (niridazole) is a nitrothiazole compound in yellowish tablet form of 100 and 500 mg per tablet. An initial trial at the Schistosomiasis Control Pilot Project at Palo, Leyte with the recommended dose of 25 mg/kg body weight for 7 days was found ineffective against the disease. Longer period of treatment for 10 to 14 days at the same daily dose was therefore tried with impressive cure and egg reduction rates but with moderately severe side reactions such as headache, dizziness, restlessness, nausea, vomiting, body weakness, anorexia, insomnia, and skin eruptions (Santos *et al.*, 1971). The more alarming symptom was hallucination in 3 patients which however disappeared when the drug was discontinued. With this dosage for 14 days, stool negative conversion rates ranging from 43.9 to 84.8% and an egg reduction of over 90% during the 6 months of stool follow-up were obtained.

To reduce its toxicity, other treatment schedules were tried with the hope of using this drug for mass treatment.

Preliminary trials in Bo. Pawing, Palo, Leyte

In Bo. Pawing, 2 schedules of treatment were adopted: 1) Schedule I at 10 mg/kg body weight/day for 35 days; and, 2) Schedule II at 15 mg/kg/day for 24 days. It will be noted that the total amount of the drug received per kilogram body weight was approximately the same in both groups as in the 14-day treatment at 25 mg/kg/day, being 350 and 360 mg, respectively. The total daily dose was divided into 2 parts, one part given in the morning and the other part in the afternoon.

Altogether, 56 patients were treated under Schedule I and 45 patients under Schedule II. Both dosage schedules were well tolerated and the side reactions such as dizziness, nausea, vomiting, and body weakness were generally mild. However, in the group receiving the 10 mg dose, one 12-year old female patient developed dizziness and transient convulsive seizures on the 30th day of treatment. In the 15 mg group, a 7-year old boy also developed the same side reactions on the 9th day. Both were advised to discontinue treatment.

The results of the stool follow-ups using the modified MIFC technic (Santos *et al.*, 1968) and examining one gram of faeces by volume showed a stool negative conversion rate of 32.9% in the first month of stool follow-up for Schedule I which subsequently dropped to as low as 12.5% (Table 1). For Schedule II, the stool negative conversion rate in the first month was 57.2% lowering to 26.7% during the subsequent follow-ups. Egg reduction rates, on the other hand, were quite high in both groups ranging from 84.3% to 88.4% for the former and from 95.3% to 97.9% for the latter schedule. It is obvious from these results that the larger daily dose effected better stool negative conversion and egg reduction rates.

Localized Mass Treatment in Santa Fe

Encouraged by these preliminary trials, a localized mass treatment of cases was undertaken in 5 adjacent schistosomiasis endemic

Table 1

Efficacy of Ambilhar under 2 dosage schedules* among early schistosomiasis cases.

Particulars	Treatment Schedule	Pre-Treatment Egg Count	Stool follow-up		
			1-2 mo.	3-4 mo.	5-6 mo.
Average Egg Count/gram of faeces	I	237.0	28.7	27.2	35.8
	II	246.4	4.2	8.4	9.6
Percent Egg Reduction	I	-	87.5	88.4	84.3
	II	-	97.9	96.4	95.3
Stool negative conversion rate	I	-	32.9	12.5	19.5
	II	-	57.2	26.7	36.8

No. of patients treated: Schedule I (10 mg/kg body weight/day for 35 days) = 56.
Schedule II (15 mg/kg body weight/day for 24 days) = 45.

barrios in Santa Fe, Leyte with the cooperation of the Provincial Health Office and the Rural Health Unit. This time no egg quantitative determination was made. Cases with splenomegaly, haemoglobin of less than 10 gm% and those with a history of epileptic fits or psychotic tendencies were excluded from this limited mass treatment of cases. The dosage used was 15 mg/kg/day for 24 days.

Out of 451 positive cases, 264 qualified for treatment. A total of 134 or 50.8% dropped out or discontinued the treatment due to a variety of causes, the most common of which was the long duration of treatment. Of the 130 who completed the treatment course, stool negative conversion rates ranged from 96.6% for the first month to 58.3% at the end of the 6 month stool follow-up (Table 2).

Since infected individuals with very low egg counts were included in this group of patients, a higher stool negative conversion rate can be expected. It can be mentioned further that this dosage schedule was generally well tolerated by the patients. Of the 129 dropouts, over 70% had no side reactions whatsoever. The long course of treatment, therefore, appears to be discouraging to quite a number of them as shown by this number of dropouts.

Table 2

Stool negative conversion rate with Ambilhar among 130 schistosomiasis patients treated at a dose of 15 mg/body weight/day for 24 days.

Post-treatment monthly stool follow-up	No. negative/ No. exam.	Stool negative conversion rate
1	115/119	96.6
2	95/113	84.1
3	98/120	81.7
4	96/111	86.5
5	65/99	65.7
6	60/103	58.3
6 monthly average		78.8

Suppressive Course of Treatment

With this experience in the preliminary trials, most especially on the large number of dropouts arising from the relatively long course of treatment, a suppressive management was thought of and tried in some patients. Instead of treating the patients for 24 days, the duration of treatment was shortened to 10 days.

Of the 35 patients treated, 28.6% of the patients became negative one month after treatment. This decreased to zero in the 6th month of stool follow-up. Egg reduction was 93.5% during the first month of stool follow-up. Subsequent examinations showed a range of 69.8% to 88.7% (Table 3).

Table 3

Suppressive dose of Ambilhar at 15 mg/kg body weight daily for 10 days among 35 early schistosomiasis cases.

Duration of stool follow-up	% Egg Reduction	% becoming negative
1 month	93.5	28.6
2 months	88.7	8.6
3 months	85.2	5.9
4 monhts	69.8	2.9
5 months	82.6	2.9
6 months	87.9	0
Average	84.6	

With this finding, trials on a relatively bigger number of patients will be carried out using this 10-day treatment to be repeated every 3 to 6 months or until subjects cease to pass viable eggs. This is intended to be carried out especially among school children who will be followed up periodically.

SUMMARY

Ambilhar or niridazole at a dose of 25 mg per kg body weight for 7 days was found ineffective against *S. japonicum* infection. Longer period of treatment for 10 to 14 days gave impressive stool negative conversion and egg reduction rates but with moderately severe reactions, the most alarming of which was hallucination. To minimize toxicity, the daily dose was reduced but given for a longer duration so that the total amount of the drug given per kilogram body weight was approximately the same as the 25 mg per kg per day for 10 to 14 days.

Of the two treatment schedules tried, the 15 mg per kg per day for 24 days was found relatively effective. Although the drug with this treatment regimen was well tolerated, a drop-out of 50.8% was observed. Ambilhar was therefore tried as an egg suppressant. With a 10-day treatment, all patients were again positive after 6 months. Egg reduction rates during the 6 months stool follow-up ranged from 69.8 to 93.5%. Further trials using this dose to be repeated every 3 to 6 months is contemplated.

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STUDIES ON THE LABORATORY BREEDING AND INFECTIVITY OF *ONCOMELANIA HUPENSIS QUADRASI*[*,**]

By

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ABSTRACT

The results of the studies on the laboratory breeding of *O.h. quadrasi*, diatom culture used for snail food, rate of growth and reproduction and infection rate using different number of miracidia for snail infection are summarized as follow;

1). For diatom culture the best culture solution found is the medium to which 40 mg of Ca $(NO_3)_2 \cdot 4H_2O$ and 20 mg of Na_2SiO_3 were added to the soil-filtrated solution of Tamiya et al.

2). The growth of snails using diatom for food was from 1.80-2.10 mm to 3.57-4.47 mm in inner soil-filter circulating tank in 90 days.

3). The use of L-tryptophane and Dextrose as snail food resulted were 10.8 and 12.4 youngs per female.

4). For a good percentage of snail infection, it is suggested that each snail be infected with 3-5 miracidia.

INTRODUCTION

In the immuno-serological studies of helminthic diseases, it is necessary that a large amount of antigen must be available. To achieve this the life cycle of *Schistosoma japonicum* in the laboratory should be established for the immunological study of schistosomiasis. In this connection, however, one crucial problem is the breeding of snails, although successful breeding of *O.h. quadrasi* has been reported in clay pot (Wagner & Wong, 1956) and petri dishes (Davis & Iwamoto, 1969), it maybe mentioned that the breeding of snails, therefore, is the subject of these investigations with the main object of determining the effects of diatom diets and other substances in the breeding of snails in the aquarium. Similarly, the infectivity of *O.h. quadrasi* was also studied.

A. DIATOM CULTURE FOR SNAIL FOOD

As a preliminary experiment, the use of Bacillariophyta (diatoms) for snail food was

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tested using the method of Tamiya et al (1965) and its modifications was enumerated below:

1. Method of diatom collection

A plankton net was set 1-2 meters deep in a river for 1-2 minutes. The net was then pulled up slowly and the collected water poured into a glass bottle. The procedure was repeated until 200-300ml of water was collected. The collected sample was then brought to the laboratory and 2 volumes of Bacillariophyta culture solution was added, and allowed to stand for 1-2 weeks under direct sunlight. The diatom (*Fragilaria* spp. and *Melosira* spp.) grew inside the glass bottle.

2. Culture media for diatoms

Diatom culture media were prepared using the Tamiya medium and its modifications as follows;

Medium 1. : The culture medium of Tamiya et al[a]

Medium 2. : Medium 1 diluted 10 times with distilled de-ionized water.

Medium 3. : Same as medium 1 but instead of distilled de-ionized water, soil-filtrated solution[b] was used.

Medium 4. : Same as medium 2 but diluent used was soil-filtrated solution.

Medium 5. : Medium 1 plus 40mg of Ca $(NO_3)_2 \cdot 4H_2O$ and 20mg of Na_2SiO_3.

Medium 6. : Same as medium 2 plus 40mg of $Ca(NO_3)_2 \cdot 4H_2O$ and 20mg of Na_2SiO_3.

Medium 7. : Same as medium 5 but soil-filtrated solution was used as diluent instead of distilled de-ionized water.

Medium 8. : Same as medium 6 but instead of distilled de-ionized water, soil-filtrated solution was used.

*(a) The formula of Tamiya et al culture medium.

$Ca(NO_3)_2 \cdot 4H_2O$	40mg
K_2HPO_4	10mg
$MgSO_4 \cdot 7H_2O$	25mg
Na_2SiO_3	20mg
Na_2CO_4	20mg
Fe-citrate	3mg
Distilled de-ionized water	1000ml

*(b) Preparation of soil-filtrated solution.

Mix 1 kg of soil with 1000ml of distilled de-ionized water, boil for 1 to 1½ hours and allow to stand in a dark room for 2 days. Filter twice and boil filtrate for 30 minutes and then add an equal volume of distilled de-ionized water to the filtrate.

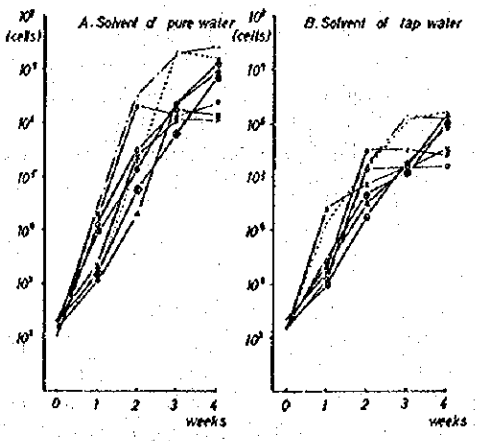
3. Methods of diatom cultivation

Several trials were made in cultivating diatom. For each trial, either distilled de-ionized water or tap water as diluent was used.

These trials were made under 3 different conditions namely: (1) under direct sunlight for 7 days and then in the shade by the window; (2) in the shade by the window throughout the period of observation; and (3) under direct sunlight. The observation period for all these trials was for 28 days.

4. Resuts and Discussion

The best medium for diatom culture among those trials was medium 7. This is a modified Tamiya's culture medium made by the addition of 40mg of $Ca(NO_3)_2 \cdot 4H_2O$ and 20mg of Na_2SiO_3 in soil-filtrated solution instead of distilled de-ionized water. The culture was incubated in direct sunlight for 7 days and placed in the shade for 21 days. In this medium the diatoms multiplied from 1.4×10^2 to $3.7 \times$



1·····The cultivated solution of TAMIYA et al.
2·····One-tenth diluted solution of 1.
3·····The solution to which we add the soil-filtrated solution with 1.
4·····The solution to which we add the soil-filtrated solution with 2.
5·····The solution to which we add 40mg of $Ca(NO_3)_2 \cdot 4H_2O$ and 20mg of Na_2SiO_3 with 1.
6·····The solution to which we add 40mg of $Ca(NO_3)_2 \cdot 4H_2O$ and 20mg of Na_2SiO_3 with 2.
7·····The solution to which we add the soil-filtrated solution with 5.
8·····The solution to which we add the soil-filtrated solution with 6.

Fig. 1. Comparison of the rate of diatom multiplication using Tamiya medium and its modifications.

1) Set the diatoms in direct sunlight for 7 days. after exposure, set them in a shaded place by the window.

10^7 cells over the 4 weeks observation period. Two other culture media gave a diatom multiplication above 10^7 cells in 4 weeks. These were culture media numbers 8 and 2 with distilled de-ionized water as diluent (*Fig.* 1.)

It was also observed that culture medium number 2 using distilled de-ionized water as diluent and cultivated in the shade was as good as the Tamiya medium (No 1.). Both media effected the multiplication of the diatoms only up to 10^4 cells. The difference of diatom multiplication in medium 2 & 1 was found to be not significient (*Fig.* 2.). However when cultured under direct sunlight, culture medium number 2 was found better than the Tamiya medium (*Fig.* 3.)

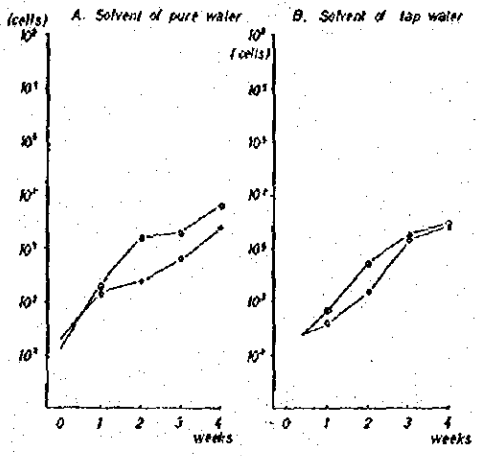


Fig. 2. Comparison of the rate of diatom multiplication using Tamiya medium and its modifications.
2) Set the diatoms in a shaded place by the window only

In Japan, the cultivation of *Melosira* spp. and *Fragilaria* spp. under direct sunlight was found by Iwanaga & Tsuji in 1972 to give a diatom multiplication of from 1.5×10^3 cells at the start of cultivation to 7.1×10^9 cells in two weeks when then they used a modified Tamiya et al culture medium using soil-filtrated solution instead of distilled de-ionized water as diluent.

The rate of multiplication was found to be more rapid than that of the present Philippine study. This difference may possibly be

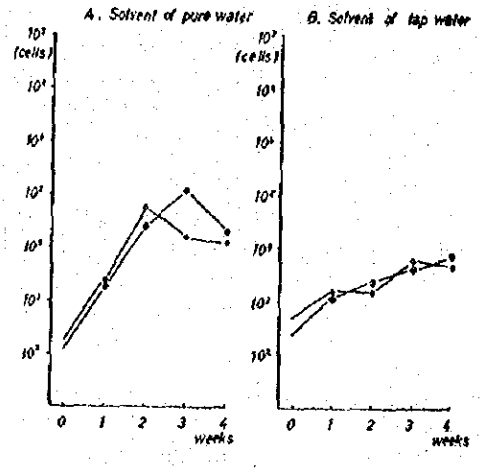


Fig. 3. Comparison of the rate of diatom multiplication using Tamiya medium and its modifications.
3) Set the diatoms in direct sun light only

explained by the difference in temperature of the diatom culture and possibly the use of not so sterile bottles in the Philippine study.

B. Growth and breeding of snails

1. Materials and methods

An inner soil-filter circulating tank measuring $35\times22\times18$ cm was used in this study as shown in *Fig.* 4. The soil-filter was prepared using a 2 cm bottom layer of stones of about 3 to 6 mm in diameter; 3 cm of sand layer using about 1 to 2 mm diameter obtained from a fresh water source; and a soil layer of about 4 cm in maximun depth so set-up to form a slope. The soil used was taken from a snail habitat. Water was added to a depth of about 5 cm from the surface of the sand layer.

The aquarium was made to function without any snails for 20 to 30 days to allow the formation of the biochemical layer. With this set up it is not necessary to change the water in the aquarium for at least one year. However, if some protozoa or free-living nematoda grow in it, the water must be changed immediately.

200-300 young snails were measured and placed in the aquarium. For the growth in size, samples of the snails were measured every month for 3 months.

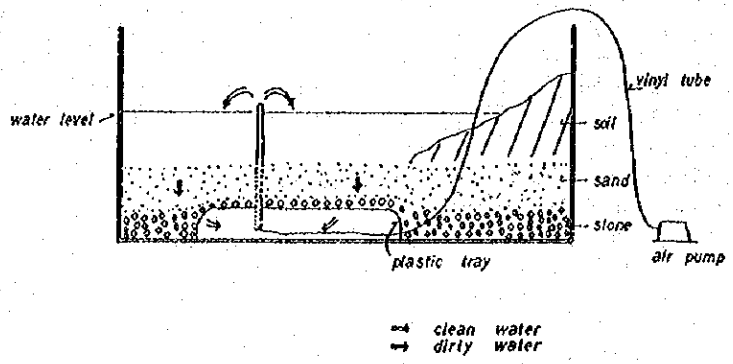


Fig. 4. Aquarium (inner soil-filter circulating tank)

The effect of L-tryptophane and Dextrose on the reproduction of snails was likewise studied. 50 male and 50 female snails were placed in the aquarium as in Fig. 4. L-tryptophane or Dextrose was poured into the aquarium every 2 weeks. The L-tryptophane given at one time was computed to be 0.03g/100ml and Dextrose, 0.05/100ml. After 2 months, the adult snails were remove from the aquarium and on the third month, the young snails were picked up one by one and counted.

For snail diet, diatoms of the *Fragilaria* spp. and *Melosira* spp. of the fresh water type of phyto-plankton were used. The *Fragilaria* spp. is beltshaped, 40-60μ in width and yellowish or light-brown in color. The *Melosira* spp. is thread-shaped, 5-30μ in width and lightbrown in color. The snails were fed in the morning every 2 days with 10^6-10^7 cells per snail.

For further care and maintenance of the aquarium, the snails that crawled out of the water were brought back to the water using brush or forcep and to maintain the moisture of the aquarium wall and soil, water is sprayed every 2 or 3 days.

2. Results & Discussion

The increase in size of young adult snails 1.80-2.10 mm long, average 1.98mm, was to 3.57-4.47 mm, average 3.95 mm in the 90 day observation period as shown in *Table* 1. The average rate of growth during the 90 days period was 0.52, 0.71 and 0.74 mm noted on day 30, 60 and 90 respectively. Thuus, the rate of growth was slowest during the first month and more rapid on the third month.

The use of L-tryptophane and Dextrose for snail food showed that snails given Dextrose bred better than those given L-tryptophane (*Table* 2). Only very few snails died using both solutions.

Table 1. The growth of snail with diatom-diet

Measurement	Day of observation			
	0	30	60	90
Length (mm)	2.10-1.80	2.60-2.31	3.95-2.80	4.47-3.57
Average length(mm)	1.98	2.50	3.21	3.95
Growth	*(0)	(0.52)	(1.23)	(1.97)

( ): The growth of snail from the beginning (mm)

Table 2. The number of the young snails produced using L-tryptophane and Dextrose sol.

Solution	No. of dead adult snails	No. of young snails	No. of young/female
L-trypto-phane	2	539	10.8
Dextrose	3	619	12.4

In the laboratory, Chi and Wagner (1957) reported that the rate of growth in clay pot of *O.h. quadrasi* was 0.2 mm per week up to the 12 weeks and after this time it was about 0.1 mm per week. Mc Mullen (1947) on the other hand, observed that under field conditions, the rate of growth was more than twice

the observation of Chi and Wagner. This present report, however, it was observed that the rate of growth was slower (0.15mm/week) than those reported by the above mentioned investgators. This may be due to the difference in the diet since there was less calcium and other components of diatom used in the present study that were essential for the growth of snails. This was based on the findings of Iwanaga and Tsuji (1972) that with diatoms the rearing of *O.h. nosophora* in the laboratory was possible. Chi and Wagner (1957) and Wagner and Wong (1956) also reported that the fecundity of adult snails is controlled by temperature and foods. Likewise, Moose et al (1962) and Davis and Wenner (1970) also reported similar findings. This far no reports has been made on the effects of the chemical factors on snail breeding. There are few reports on marine molluscs (Bivalve spp., for example, *Pinctada martensii* (miyauchi, 1966)), in gastropod((*Macroschisma dilatata*, *Penepatella stellaeformis* (Yoshida, 1975)) and in *O.h. nosophora* (Iwanage, 1975) on the effect of simple and complex amino acid on the fecundity of adult snails. Iwanaga (1975) reported that both L-tryptophane and Dextrose increases the fecundity of *O.h. nosophora* and the youngs produced were 42.8 and 45.4 per female respectively.

C. Experimental infection *O.h. quadrasi* with *S. japonicum* miracidia, Philippine strain.

1. Materials and methods

O.h. quadrasi were collected in Cogon Palawan, Leyte island. A total of 876 snails collected from this area were crushed and examined for sporocyst or cercaria of *S. japonicum*. All were found negative. Because of the negative findings, the remaining snails were used for the infection with miracidia of *S. japonicum* hatched from eggs obtained from infected mice intestine. Two methods of snail infection were done; the first was individual infection of the snail with 1 to 10 miracidia placed in a test-tube with about 2 ml of water and the second was a mass infection where numerous miracidia were placed in a small beaker containing about 50 snails. The snails were exposed overnight and the forllowing day, rinsed with de-ionized water and placed in prepared aquarium (*Fig.* 4).

The snails were crushed and examined for sporocysts or cercaria 8-10 weeks after experimental infection.

2. Results and Discussion

The highest rate of infection obtained in this experiment was in the group of snails given 3 miracidia each. This was 58.1%. The mortality of snails in this group was 6.7 % (*Table* 3). The group given 5 miracidia each and that with mass infection gave lower infection rate and higher mortality rate especially the group that received mass infection.

Table 3. Infection rate and mortality of *O.h. quadrasi* infected with *S. japonicum* LEYTE strain

Shell length (mm)	No. of snails examined	No. of miracidia /snail	Infected snails*	Dead snails
5<	50	1	11 (22.0)	1 (2.0)
	105	3	61 (58.1)	7 (6.7)
	163	5	70 (42.9)	15 (9.2)
	53	10	20 (37.8)	14 (26.4)
	243	(mass)	99 (40.7)	125 (51.4)

( ): Infection rate and mortality (in %)
* : Immature cercaria (sporocyst)

It may be mentioned that all the snails infected and examined later for *S.japonicum* infection showed immature cercaria (sporocyst) only.

In comparison other investigators gave varying results. Hunter (1946) reported that when snails were individually exposed to 5 miracidia the infection rate was 33.3%, but the mortality rate was high, while all snails that survived the mass infection were found positive but the mortality rate was extremely high (in about 90%)

On the other hand, Pesigan et al (1958) found 44% of snails positive that were infected with 1 miracidium each and 75% for snails given 5 miracidia each.

Our results of an increase of the mortality rate among the infected snails when infected with 10 miracidia or more as also observed by

Okamoto (1963) working with *O.h. nosophora*. Based on our results the optimum number of miracidia to be used for infection is from 3 to 5 per snail.

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LABORATORY BREEDING OF *ONCOMELANIA NOSOPHORA* IN JAPAN

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For the artificial breeding of *Oncomelania nosophora* in the laboratory, snail diet is an important culture condition to establish. The authors tried out some phytoplankton (algae) as diets for *Oncomelania* snails, and also evaluated the effects of certain chemical and physical factors on the fecundity of adult snails.

Snail growth

For the snail growth experiments, 50 *Oncomelania nosophora* of the Yamanashi strain (2-3 mm in length) were used. As food, *Melosira* sp., *Navicula* sp. and *Achnanthes* sp. (Bacillariophyta), *Senedesmus* sp., and *Spirogyra* sp. (Chlorophyta) and *Oscillatoria* sp. (Cyanophyta) were examined.

Inner soil filter aquariums at 20 ± 2°C were used as culture chambers. The culture chamber measured 20 × 26 × 20 cm. Water from a filter circulated at a rate of 80 - 100 ml per minute. *Melosira* sp., *Navicula* sp., *Achnanthes* sp. and *Senedesmus* sp. were added in the form of 10-30 million cells per snail every day. The others were added in equivalent amounts under the same conditions.

Components of the Bacillariophyta solution were : 1 liter of pure water with 40 mg of Ca $(NO_3)_2 \cdot 4H_2O$, 10 mg of K_2HPO_4, 25 mg of $MgSO_4 \cdot 7H_2O$, 20 mg of Na_2SiO_3, 20 mg of Na_2CO_3, 3 mg of Fe-citrate and 50 ml of filtrated soil solution.

The best growth was obtained with *Melosira* sp. in Bacillariophyta solution; snails grew an average 2.56 mm (from 4.37 to 6.93 mm) after 90 days cultivation. The next best growth was with *Melosira* sp. in pure water (3.93 mm in growth length), *Navicula* sp. in Bacillariophyta solution (3.12 mm), and *Achnanthes* sp. in Bacillariophyta solution (3.09 mm), as shown in Table 1.

Bacillariophyta are better food than Chlorophyta or Cyanophyta for snail growth, and Bacillariophyta solution is good as culture medium.

The fecundity of adult snails

For the experiments on the fecundity of adult snails, 25 each of male and female *Oncomelania nosophora* of the Yamanashi strain (6-8 mm in length) were used. These adult snails were bred about 2 months in the inner soil filter aquarium at 20 ± 2°C in 3-3.5 liters of water, and then the adult snails were removed to another aquarium. One month later, young snails were collected (with filter-net, 42 meshes), and counted.

Observations were made on the effects of the following 24 chemical and physical factors: (a) pure water only; (b) Bacillariophyta solution only; (c) light change (light to darkness every 5 days) in Bacillariophyta solution; (d) temperature change (20 ± 2°C to 10 ± 2°C every other week) in Bacillariophyta solution; (e) addition of 1.4 gm L-Tryptophane; (f) 1.4 gm of L-Tyrosine; (g) 0.01 gm of Vitamin B_{12} every 2 weeks in Bacillariophyta solution; (h) 0.001 mg of $KMnO_4$ every 5 days in Bacillariophyta solution; (i) 3 gm of Dextrose; (j) 3 gm of L-Tryptophane; (k) 0.3 gm of L-Tryptophane; (l) 0.15 gm each of L-Tryptophane and Dextrose; (m) 0.15 gm each of L-Tryptophane and L-Tyrosine; (n) 0.15 gm each of L-Tryptophane and Vitamin B_{12};

Table 1

Snail growth with several species of phytoplankton.

Aquaria	Diets	Periods of breeding				Dead snails
		0	30	60	90 (days)	
Pure water	*Melosira* sp.	2.59 -	4.23 (1.64)	4.86 (2.23)	6.52 (3.93)	0
	Navicula sp.	2.47 -	3.01 (0.54)	4.20 (1.73)	5.05 (2.58)	0
	Achnanthes sp.	2.39 -	3.11 (0.72)	4.01 (1.62)	4.96 (2.57)	0
	Senedesmus sp.	2.80 -	2.99 (0.19)	3.11 (0.31)	3.48 (0.68)	2
	Spirogyra sp.	2.60 -	2.90 (0.30)	3.50 (0.90)	3.80 (1.20)	0
Bacillariophyta sol.	*Melosira* sp.	2.56 -	4.26 (1.70)	4.91 (2.35)	6.93 (4.37)	0
	Navicula sp.	2.48 -	3.27 (0.79)	4.19 (1.71)	5.60 (3.12)	0
	Achnanthes sp.	2.01 -	3.25 (1.24)	4.25 (2.24)	5.10 (3.09)	0
	Senedesmus sp.	2.99 -	3.36 (0.37)	3.65 (0.66)	3.85 (0.81)	5
	Spirogyra sp.	2.40 -	2.90 (0.50)	3.50 (1.10)	3.70 (1.30)	0
	Oscillatoria sp.	2.40 -	3.10 (0.70)	3.60 (1.20)	4.00 (1.60)	0

( ) = The growing size from the beginning (mm).

(o) 0.15 gm each of L-Tryptophane and L-Methionine; (p) 3 gm of L-Methionine; (q) 0.15 gm each of L-Methionine and L-Tyrosine; (r) 0.15 gm each of L-Methionine and Dextrose; (s) 0.15 gm each of L-Tyrosine and Dextrose; (t) 0.15 gm each of L-Tyrosine and Vitamin B_{12} every 2 weeks in pure water; (u) water level changing; (v) high water level in light to low water level in darkness; (w) low water level in light to high water level in darkness every 5 days; (x) alternating light and darkness every 5 days for 30 days and after this, water level changing every 5 days for the next 30 days continuously.

The best results were represented by 1,135 young snails (survival rate: 91.9%) in dextrose in pure water (i), followed by 1,120 young snails, (survival rate: 89.1%) in L-Tryptophane and Dextrose (l); 1,071 young snails, (survival rate: 90.8%) in 0.3 gm of L-Tryptophane (k), 1,062 young snails, (survival rate: 64.8%) with light change (c), as shown in Table 2.

CONCLUSION

Diets of Bacillariophyta are better than Chlorophyta or Cyanophyta for snail growth.

Bacillariophyta solution as a culture medium promotes snail growth. Dextrose and L-Tryptophane were better than L-Tyrosine, L-Methionine, Vitamin B_{12} and potassium permanganate for the fecundity of adult snails.

Of 3 kinds of amino acids, the best results were obtained with L-Tryptophane, followed by L-Tyrosine, and the least with L-Methionine.

Vitamin B_{12} and potassium permanganate reduced fecundity and the survival rate of adult snails. The effects of temperature,

Table 2

Influences on the fecundity of adult snails.

	Environment conditions	No. of adults survived (per 25 each) ♂	♀	Young snail: No. of total	No. of survived	No. of dead	Survived rate (%)
a	Pure water (control)	25	25	652	431	221	66.1
b	Bacillariophyta sol. (Tamiya *et al.*, 1965)	25	25	640	420	220	65.6
c	Bacillariophyta sol. + (light ⇄ dark)	24	25	1062	688	374	64.8
d	,, + (20 ± 2 ⇄ 10 ± 2°C)	25	23	824	756	68	91.7
e	,, + L-Tyrosine	23	24	871	605	266	69.5
f	,, + L-Tryptophane	22	21	907	644	263	71.0
g	,, + Vitamin B_{12}	22	20	653	224	429	34.3
h	,, + $KMnO_4$	20	21	354	225	129	63.6
i	Pure water + Dextrose	23	24	1135	1043	92	91.9
j	,, + L-Tryptophane	23	20	508	347	161	68.3
k	,, + L-Tryptophane	25	25	1071	973	98	90.8
l	,, + L-Tryptophane + Dextrose	23	23	1120	999	121	89.1
m	,, + ,, + L-Tyrosine	20	23	826	725	101	87.8
n	,, + ,, + Vitamin B_{12}	22	22	702	500	202	71.2
o	,, + ,, + Methionine	23	24	824	626	198	76.0
p	,, + L-Methionine	22	23	498	367	131	73.7
q	,, + ,, + L-Tyrosine	22	24	539	411	128	76.3
r	,, + ,, + Dextrose	24	24	825	625	200	75.8
s	,, + L-Tyrosine + Dextrose	23	22	906	801	105	88.4
t	,, + ,, + Vitamin B_{12}	20	20	620	456	164	75.3
u	,, + (low water ⇄ high water)	20	20	554	415	139	74.9
v	,, + (light high water ⇄ dark low water)	20	20	547	412	135	75.3
w	,, + (light low water ⇄ dark high water)	23	24	961	861	100	89.6
x	,, + (light ⇄ dark) ⇄ (low. w ⇄ high. w)	20	20	778	638	140	82.0

light-low water and darkness- high water were observed. Light-high water reduced the fecundity of adult snails.

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THE DETERMINATION OF THE ONCOMELANIA HUPENSIS QUADRASI POPULATION DENSITY USING THE BANANA LEAF METHOD IN FOUR MUNICIPALITIES OF EASTERN LEYTE, PHILIPPINES*, **)

By

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ABSTRACT

Banana leaves was used in the snail density determination in 4 municipalities of Leyte. The highest was observed in Palo and the lowest was Tanauan. There appeared to be no correlation between the distribution and the infection rate of snails. The infection rate was highest in the rain months of November-December.

INTRODUCTION

The determination of the population density of *Oncomelania hupensis quadrasi* using the ring and tube methods were studied and established in 1958 by Pesigan and co-workers. An equally good method using filter paper was introduced by Tanaka and co-workers (1975). These workers also proved that the banana leaves was as good a method as the filter paper in snail collection which served as the basis for this study.

Thus, in the present study, the value of fallen dried banana leaves was assessed in the determination of the snail population and incidentally, for the collection of snails to determine the snail infection rate in the survey of the different endemic areas in Easern Leyte, Philippines.

MATERIALS AND METHODS

The banana leaves were cut into appropriate sizes of 20×20 cm and 10 pieces of these were placed along the snail colonies at an interval of 2 m. The following day, they were collected in polyethlene bags and brought to the laboratory, where they were washed and transferred to a white enamel pan and the snails were individually picked-up with forceps.

The number of snails collected were enumerated, measured and were accordingly classified into large, moderate and small. The population density of *O. h. quadrasi* was studied in the 4 different snail colonies in the municipalities of Palo, Santa-Fe, Pastrana and Tanauan of the Province of Leyte, Philippines using fallen dried banana leaves (*Fig.*

*) 岩永譲, M. J. サントス, B. L. ブラス：レイテ島に於ける O. h. quadrasi の分布状況

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2, 3, 4, 5).

RESULTS

In Palo, the lowest number of snails collected was observed in Gacao oxbow (3 snails) and the highest was Tibak depression (331) (*Table* 1). The lowest and highest snail densities found in the other municipalities were found in San Juan ricefield (9) and Maslog Lumbia (271) in Santa-Fe (*Table* 2); So-ong spring (6) and Cancarohas creek (436) in Pastrana (*Table* 3); and Baliong creek (8) and Batang stream (222) in Tanauan (*Table* 4).

The snail infection rate was similarly investigated in the different snail colonies in Palo, Santa-Fe, Pastrana and Tanauan as shown in *Tables* 5, 6, 7 *and* 8. The data gathered showed that the heavily infected snail colonies were Naliwatan stream and Villaco creek, both in Palo (*Table* 5) and Batang stream in Tanauan (*Table* 8).

The infection rate of snails with *S. japonicum* was observed twice a month for 5 months from November' 74 to March' 75 in Naliwatan stream, in Palo. The results showed that the highest infection rate was at the beginning of December (*Table* 9).

DISCUSSION

Pesigan T.P and co-workers introduced in 1958 the use of the ring and tube methods for snail density determination. In Palo, they

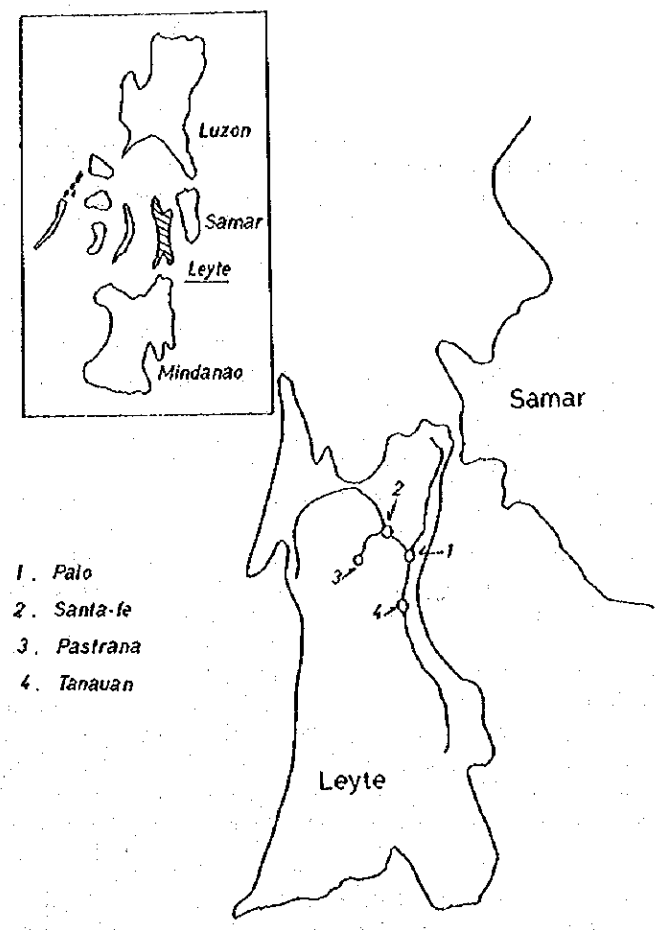

Fig. 1. Map of Leyte island, showing the municipalities of Palo, Santa-fe, Pastrana and Tanauan.

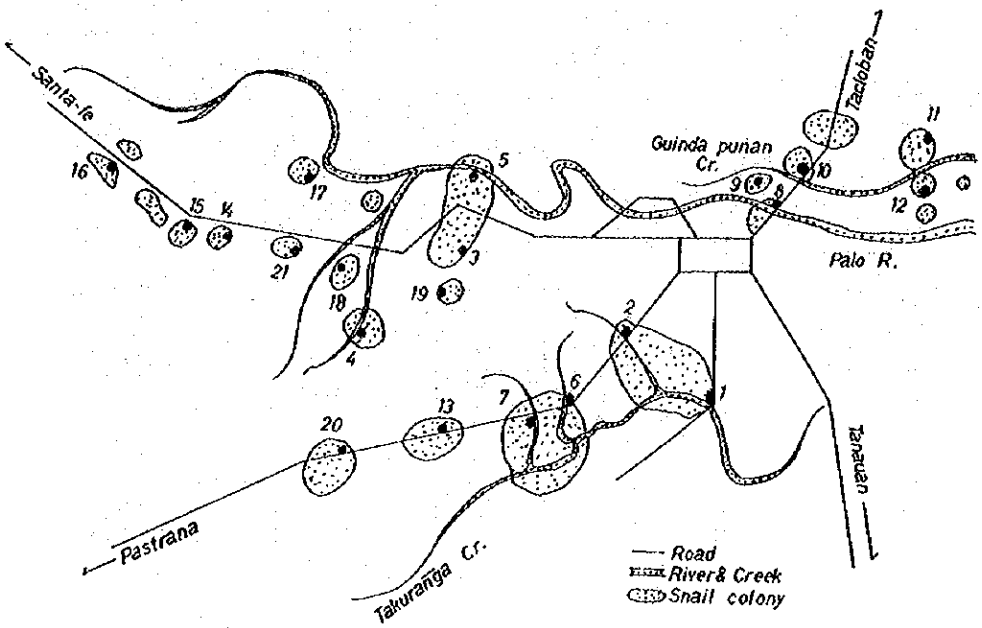

Fig. 2. Palo

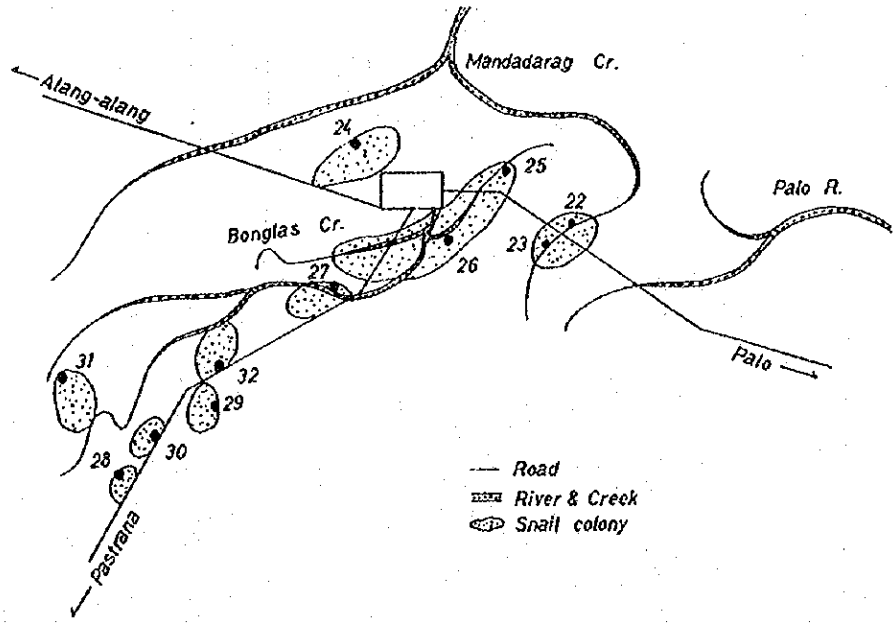

Fig. 3. Santa-fe

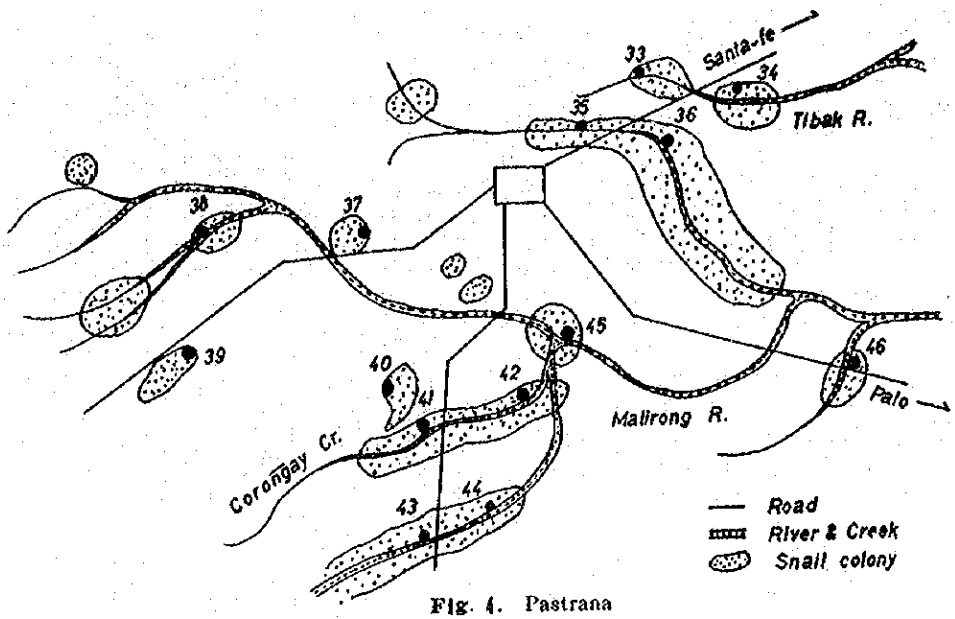

Fig. 4. Pastrana

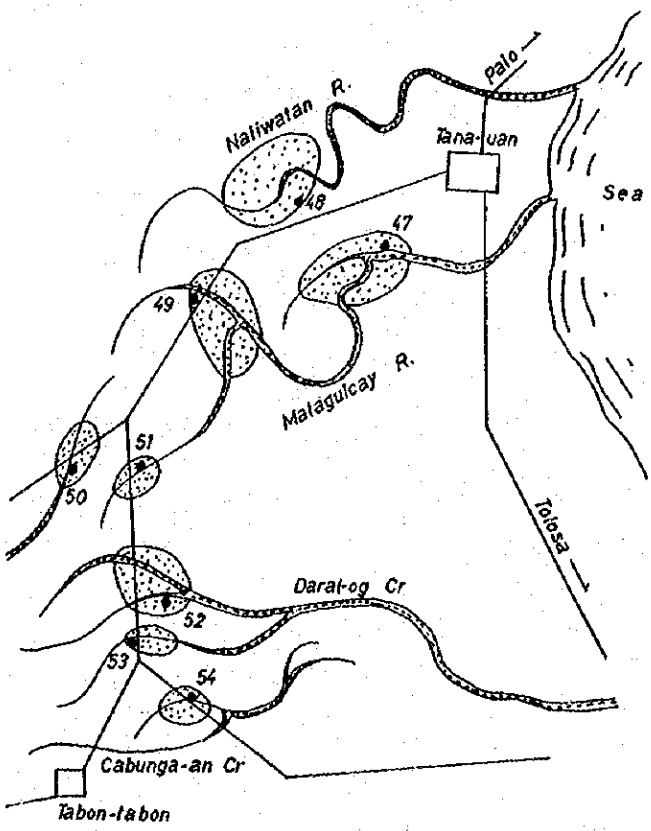

Fig. 5. Tanauan

Table 1 The distribution of *O.h. quadrasi* in Palo, Leyte.

Station number	Colony	No. of snails			Total collected
		L	M	S	
1	Lower Hubang Str.	10	6	5	21
2	Upper Hubang Str.	41	38	47	126
3	Vicob-Malaigang Cr.	58	89	122	269
4	Naliwatan Str.	3	6	11	20
5	Agoong Cr.	0	0	7	7
6	Binog Str.	18	15	23	56
7	Gacao Oxbow	1	2	0	3
8	Guindapunan Str.	25	17	10	52
9	Mirador Swp.	18	2	8	28
10	Sambulawan Swp.	5	9	12	26
11	Bantiles Sts.	7	4	15	26
12	Bakhao Pal. Swp.	2	5	3	10
13	Gacao Road ditch	16	20	11	47
14	Tibak Road ditch	23	65	0	88
15	Tibak Depression	160	171	0	331
16	Bulod Str.	4	13	10	27
17	Nalicaban Cr.	16	21	105	142
18	Malirong River poc. No.2	34	24	58	116
19	Naliwatan Str.	15	2	16	33
20	Alcaraz Farm	19	20	15	54
21	Villaco Cr.	7	3	0	10

L : 4mm< M : 3.9–3.0mm S : 2.9mm>

Table 2 The distribution of *O.h. quadrasi* in Santa Fe, Leyte

Station number	Colony	No of snails			Total collected
		L	M	S	
22	Dimabaha pal. Swp.	69	99	0	168
23	Dimabaha Str.	13	24	0	37
24	Tayong Str.	24	29	0	53
25	Maslog Lumbia	46	205	20	271
26	Maslog Pal. Swp.	40	64	5	105
27	San Roque Pal.	5	5	0	10
28	San Juan Spr.	8	13	0	21
29	San Juan Road Ditch	13	50	0	63
30	Casilion Str.	19	65	75	159
31	Maslog Str	16	40	0	56
32	San Juan Rice-Field	2	5	2	9

L : 4mm< M : 3.9–3.0mm S : 2.9mm>

Table 3 The distribution of *O.h. quadrasi* in Pastrana, Leyte

Station number	Colony	No of snails			Total collected
		L	M	S	
33	Upper Socsocon Str.	11	15	63	89
34	Lower Socsocon Str.	5	4	17	26
35	Upper Odlon Str.	55	34	183	272
36	Lower Odlon Str.	30	24	51	105
37	Cancarohas Cr.	25	24	0	49
38	Cancarohas Cr. (Leoncio-Jenilla)	90	109	237	436
39	So-ong Spr.	6	0	0	6
40	Corongay Cr. (Trib)	28	10	0	38
41	Upper Corongay Cr.	12	9	32	53
42	Lower Corongay Cr.	26	38	86	150
43	Upper Mongabonga Str.	45	40	96	181
44	Lower Mongabonga Str.	38	19	22	79
45	Lusok Depression	29	31	78	138
46	Cabaohan Cr.	24	37	58	119

L : 4mm< M : 3.9—3.0mm S : 2.9mm>

Table 4 The distribution of *O.h. quadrasi* in Tanauan, Leyte

Station number	Colony	No. of snails			Total collected
		L	M	S	
47	Morepes Str.	3	4	3	10
48	Naliwatan Str.	4	5	10	19
49	Pafawan Swp.	7	8	1	16
50	Belisong Str.	18	40	48	106
51	Batang Str.	81	51	90	222
52	Bangon Str.	34	7	8	49
53	Amasihan Str.	19	20	108	147
54	Baliong Cr.	1	1	6	8

L : 4mm< M : 3.9—3.0mm S : 2.9mm>

surveyed Vicob-Malaigaug, pocket No. 2, Nalicaban creek and found that Vicob-Malaigang had the highest snail density while the other two areas have almost the same snail density. With the banana leaf method, our results tend to show the same results but there was no correlation found between the distribution and infection rate of snails.

In addition to the number of infected snails with *S. japonicum* in Naliwatan stream, the snail infection rate appeared to be closely related the rainfall. This collaborates McMullen's (1947) observation that the snail infection rate decreased during the dry season.

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Table 5 The rate of infected snail with *S. japonicum* in Palo, Leyte

Station num	Colony	Snail sizes						Total snails exam.	No. infect.	% infect.
		4.0mm			3.9—3.0mm					
		No. exam.	No. infect.	% infect.	No. exam.	No. infect.	% infect.			
1	Lower Hubang Str.	414	11	7.7	57	0	0	471	11	2.3
2	Upper Hubang Str.	278	67	24.1	235	5	2.1	515	72	14.0
3	Vicob-Malaigang Cr.	471	42	8.9	191	11	5.8	662	53	8.1
4	Naliwatan Str.	190	43	22.6	25	2	8.0	215	45	20.9
5	Agoong Cr.	315	25	7.9	199	0	0	514	25	4.9
6	Binog Str.	198	21	10.6	40	0	0	238	21	8.8
7	Gacao Oxbow	46	6	13.0	15	0	0	61	6	8.8
8	Guindapunan Str.	123	1	0.8	45	0	0	168	1	0.6
9	Mirador Swp.	104	5	4.8	13	0	0	117	5	4.3
10	Sambulawan Swp.	90	3	3.3	48	1	2.1	138	4	2.9
11	Bantiles Str.	57	7	12.3	33	0	0	90	7	7.8
12	Bakhao Pal. Swp.	42	2	4.8	44	0	0	86	2	2.3
13	Gacao Road Ditch	591	13	2.2	335	1	0.3	926	14	1.5
14	Tibak Road Ditch	156	0	0	148	0	0	304	0	0
15	Tibak Depression	525	3	0.6	586	0	0	1111	3	0.3
16	Bulod Str.	26	1	4.0	29	0	0	55	1	1.8
17	Nalicaban Cr.	334	17	5.1	176	6	3.4	510	23	4.5
18	Malirong River poc. No. 2	304	34	11.2	201	29	14.4	505	63	12.5
19	Naliwatan Str.	120	10	8.3	91	4	4.4	211	14	6.6
20	Alcaraz Farm	216	1	0.5	200	1	0.5	416	2	0.5
21	Villaco Cr.	26	6	23.1	6	0	0	32	6	18.8

Table 6 The rate of infected snail with *S. japonicum* in Santa Fe, Leyte

Station num	Colony	Snail sizes						Total snails exam.	No. infect.	% infect.
		4.0mm			3.9-3.0mm					
		No. exam.	No. infect.	% infect.	No. exam.	No. infect.	% infect.			
22	Dimabaha Pal. Swp.	62	2	2.9	99	0	0	168	2	1.2
23	Dimabaha Str.	111	0	0	171	2	1.2	282	2	0.7
24	Tayong Str.	33	3	10.0	30	0	0	63	3	4.8
25	Maslog Lumbia	46	1	2.2	205	0	0	251	1	0.4
26	Masloga Pal. Swp.	40	1	2.5	64	1	1.6	104	2	1.9
27	San Roque Pal.	25	0	0	41	0	0	66	0	0
28	San Juan Spr.	19	0	0	21	0	0	40	0	0
29	San Juan Road Ditch	16	0	0	60	0	0	76	0	0
30	Casilion Str.	93	0	0	229	0	0	322	0	0
31	Maslog Sr.	16	0	0	40	0	0	56	0	0
32	San Juan Rice-Field	11	0	0	10	0	0	21	0	0

Table 7 The rate of infected snail wih *S. japonicum* in Pastrana, Leyte

Station num	Colony	Snail sizes						Total Snails exam.	No. infect.	% infect.
		4.0mm			3.9-3.0mm					
		No. exam.	No. infect.	% infect.	No. exam.	No. infect.	% infect.			
33	Upper Socsocon Str.	56	3	5.4	215	9	4.2	271	12	4.4
34	Lower Socsocon Str.	51	0	0	59	0	0	110	0	0
35	Upper Odlon Str.	938	7	0.7	591	4	0.7	1529	11	0.7
36	Lower Odlon Str.	265	5	1.9	436	2	0.5	701	9	1.3
37	Cancarohas Cr.	196	4	2.1	89	0	0	281	4	1.4
38	Cancarohas Cr. (Leoncio-Jenilla)	200	0	0	204	0	0	404	0	0
39	So-ong Spr.	11	0	0	0	0	0	11	0	0
40	Corongay Cr. (Trib)	59	1	1.6	18	0	0	77	1	1.3
41	Upper Corongay Cr.	86	0	0	111	0	0	197	0	0
42	Lower Corongay Cr.	35	0	0	52	0	0	87	0	0
43	Upper Monga-bonga Str.	171	6	3.5	226	2	0.9	397	8	2.0
44	Lower Monga-bonga Str.	100	5	5.0	111	2	1.8	211	7	3.3
45	Lusok Depression	118	6	5.1	107	2	1.9	225	8	3.6
46	Cabaohan Cr.	111	5	4.5	227	2	0.9	338	7	2.1

Table 8 The rate of infected snail with *S. japonicum* in Tanauan, Leyte

Station num.	Colony	Snail sizes						Total snails exam.	No. infect.	% infect.
		4.0mm			3.9-3.0mm					
		No. exam.	No. infect.	% infect.	No. exam.	No. infect.	% infect.			
47	Morepes Str.	16	0	0	14	0	0	30	0	0
48	Naliwatan Str.	149	11	7.3	117	3	2.6	266	14	5.3
49	Palawan Swp.	124	1	0.8	87	1	1.1	211	2	0.9
50	Belisong Str.	103	4	3.9	152	5	3.2	255	9	3.5
51	Batang Str.	351	35	10.0	267	24	9.0	618	59	9.5
52	Bangon Str.	138	8	5.8	115	4	3.5	253	12	4.7
53	Amasihan Str.	156	0	0	186	0	0	342	0	0
54	Baliong Cr.	1	0	0	1	0	0	2	0	0

Table 9 The transition of the infection rate of the snail with *S. japonicum* in Naliwatan Str. (Palo)

Month		Snail size						Totel snails exam.	No. infect.	% infect.
		Large			Moderate					
		No. exam.	No. infect.	% infect.	No. exam.	No. infect.	% infect.			
Nov.	b	35	10	28.6	92	18	19.6	127	28	22.1
Dec.	a	119	43	36.1	82	17	20.7	201	60	30.0
	b	187	45	24.1	19	4	21.1	206	49	23.8
Jan.	a	51	13	25.5	70	8	11.4	121	21	17.4
	b	75	21	28.0	98	12	12.2	173	33	19.1
Feb.	a	16	4	25.0	27	2	7.4	43	6	14.0
	b	135	34	25.2	78	4	5.1	213	38	17.8
Mar.	a	105	18	17.1	49	2	4.1	154	20	13.0
	b	153	27	17.6	162	15	9.3	315	42	13.3

a : Beginning of the month.
b : Middle of the month.

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IMMUNOLOGICAL DIFFERENCES OF *ONCOMELANIA*

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Three species of *Oncomelania* were compared using immunoelectrophoretic techniques. Antigenic communities between *Oncomelania* and *Schistosoma japonicum* were also studied by immunoelectrophoresis.

Comparisons of three species of *Oncomelania*

The extract antigens of *Oncomelania hupensis nosophora* collected from Yamanashi in Japan, *Oncomelania hupensis quadrasi* from Leyte in the Philippines and *Oncomelania hupensis formosana* from Changhua in Taiwan, and antisera from rabbits immunized with these snail antigens were used for this experiment.

The serum from rabbits immunized with *O. h. nosophora* showed 20 bands with *O. h. nosophora* antigen, 15 bands with *O. h. quadrasi* and 10 bands with *O. h. formosana* antigen. In the case of anti-*O. h. quadrasi* serum, it was possible to demonstrate 24 bands with *O. h. quadrasi* antigen, 15 bands with *O. h. nosophora* antigen and 8 bands with *O. h. formosana* antigen. The anti-*O. h. formosana* serum showed 17 bands with *O. h. formosana* antigen, 15 bands each with *O. h. nosophora* and *O. h. quadrasi* antigens, as shown in Table 1. The strongest reactions were observed in the homologous antigen-antibody systems in the cross reactions among three species of *Oncomelania*, and common precipitations also exist in the case of heterologous reactions.

Absorption procedures were performed to compare antigenicity among *Oncomelania* species. Two bands were recognized as the residual reaction of anti-*O. h. formosana* serum absorbed with *O. h. nosophora* antigen, as shown in Fig. 1. In the anti-*O. h. formosana* serum, one band was recognized after absorption with *O. h. quadrasi* antigen, as shown in Fig. 2, and this residual band would be specific for *O. h. formosana*. The immunoelectrophoregram in Fig. 3 showed residual bands of anti-*O. h. nosophora* serum absorbed with

Table 1

Cross reactions among three species of *Oncomelania*.

		Immunized rabbit sera		
		O.h. nosophora	*O.h. quadrasi*	*O.h. formosana*
Antigens	*O.h. nosophora*	20	15	15
	O.h. quadrasi	15	24	15
	O.h. formosana	10	8	17

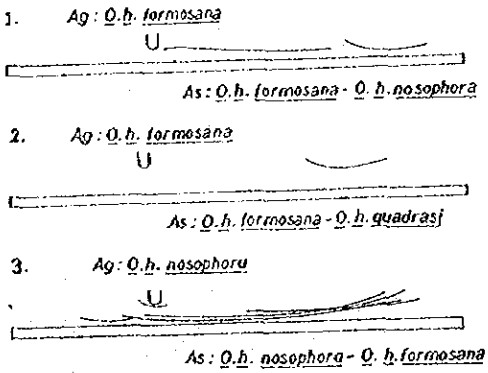

Fig. 1—Immunoelectrophoregram between *Oncomelania hupensis formosana* and *O. h. nosophora* using absorption technique.

Fig. 2—Between *Oncomelania hupensis formosana* and *O. h. quadrasi*.

Fig. 3—Between *Oncomelania hupensis nosophora* and *O. h. formosana*.

O. h. formosana antigen; 6 bands were noticed. Fig. 4 shows 2 bands of anti-*O. h. nosophora* serum absorbed with *O. h. quadrasi* antigen. Five bands were recognized as the residual bands of anti-*O. h. quadrasi* serum absorbed with *O. h. nosophora* antigen, and same anti-*O. h. quadrasi* serum showed 8 bands after absorption with *O. h. formosana* antigen, as shown in Figs. 5 and 6.

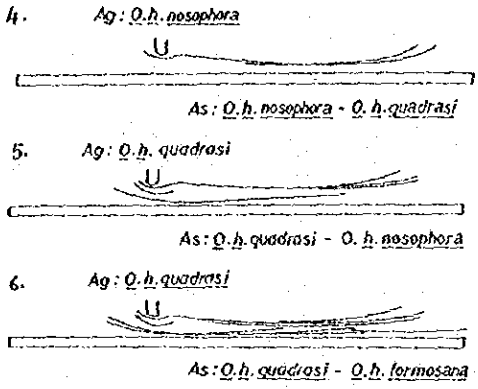

Fig. 4—Immunoelectrophoregram between *Oncomelania hupensis nosophora* and *O. h. quadrasi* using absorption technique.
Fig. 5—Between *Oncomelania hupensis quadrasi* and *O. h. nosophora.*
Fig. 6—Between *Oncomelania hupensis quadrasi* and *O. h. formosana.*

From the results of cross-reaction and absorption among *Oncomelania* species, the three subspecies of *Oncomelania* showed different patterns of immunoelectrophoretic bands.

Antigenic communities between *Oncomelania* snails and *Schistosoma japonicum*

Studies on antigenic communities between host and parasite were carried out immunoelectrophoretically with rabbit serum immunized with *S. japonicum* (Yamanashi strain) and extracts of three species of non-infected *Oncomelania.*

Seven bands were identified as common to *S. japonicum* and *O. h. nosophora* in which these larvae were grown, and five of these bands could also be identified in *O. h. quadrasi* and two bands in *O. h. formosana,* as shown in Fig. 7.

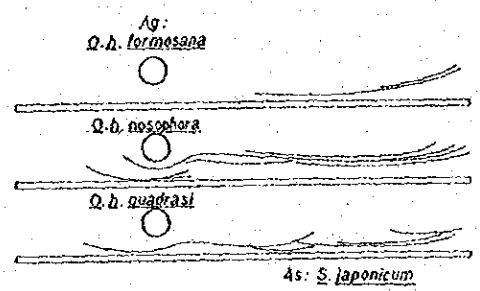

Fig. 7—Immunoelectrophoregrams between *Schistosoma japonicum* and *Oncomelania* spp.

In our laboratory, experimental infection rates of miracidia of *S. japonicum* of the Yamanashi strain to *O. h. nosophora* were 90 to 100% in the first generation of snails collected from the field, and miracidia of same strain of *S. japonicum* could infect about 30% of *O. h. quadrasi* and 0.8% of *O. h. formosana.* These infection rates are almost parallel with the number of bands representing common antigenicity between host and parasite, as shown in Table 2.

Table 2

Infection rates of *Schistosoma japonicum* miracidia (Yamanashi) to *Oncomelania* snails.

O.h. nosophora (Yamanshi)		
1st. gen.	90-100%	7 bands
2nd.- 5th. gen.	ca. 80%	
6th.- 8th. gen.	ca. 75%	
9th.- 15th. gen.	ca. 60%	
O.h. quadrasi (Leyte)		
1st. gen.	ca. 30%	5 bands
O.h. formosana (Changwa)		
1st. gen.	0.8%	2 bands

In the column chromatography of snail antigens, *O. h. nosophora* antigen was fractionated to three fractions by Sephadex G-100, as shown in Fig. 8, and it was found that the common antigenicity of *O. h. nosophora* and *S. japonicum* existed in Fractions I and II (Fig. 9). *O. h. quadrasi* antigen was fractionated to four fractions and common antigenicity of *O. h. quadrasi* and *S. japonicum* was demonstrated in Fraction II, as shown in Figs. 10 and 11.

With regard to antigenic communities between *S. japonicum* egg antigens and snails, suitable intermediate hosts (*Oncomelania* snails) were not reflected in the precipitin bands, but non-suitable hosts demonstrated precipitation, as shown in Fig. 12.

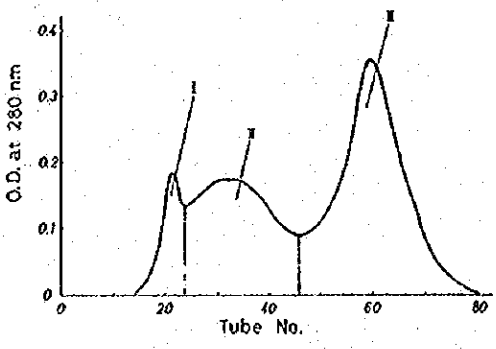

Fig. 8—Column chromatography for antigen of *Oncomelania hupensis nosophora* (Sephadex G-100).

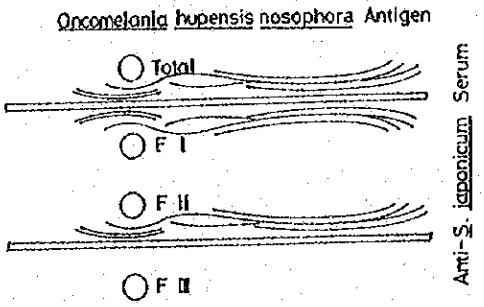

Fig. 9—Immunoelectrophoregrams between Anti-*Schistosoma japonicum* serum and fractionated antigens of *Oncomelania hupensis nosophora*.

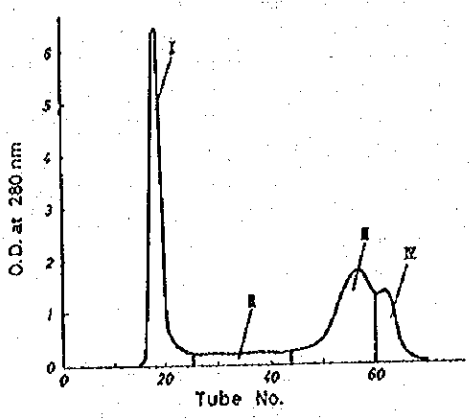

Fig. 10—Column chromatography for antigen of *Oncomelania hupensis quadrasi* (Sephadex G-100).

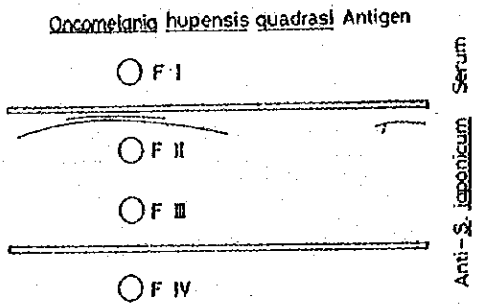

Fig. 11—Immunoelectrophoregrams between Anti-*Schistosoma japonicum* serum and fractionated antigens of *Oncomelania hupensis quadrasi*.

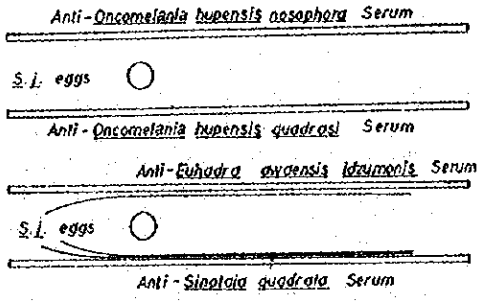

Fig. 12—Immunoelectrophoregrams between *Schistosoma japonicum* eggs and snails.

From these immunoelectrophoretical experiments it was shown that common antigenicities among three species of *Oncomelania (O. h. nosophora, O. h. quadrasi* and *O. h. formosana)* do exist, and each species also has its special antigens .

With regard to antigenic communities between intermediate host and parasite, *S. japonicum* adults shared antigens with suitable hosts, *S. japonicum* egg antigens showed precipitin bands with non-suitable hosts.

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Statistical Analysis on the Probability of Uni- and Bisexual Infections of *Schistosoma japonicum* in *Oncomelania nosophora*

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Summary: A statistical study was carried out to clarify whether occurrence of uni- and bisexual infections of *Schistosoma japonicum* in *Oncomelania nosophora* could be estimated by probability calculation. About 70 snails were exposed individually to a definite number of miracidia at each experiment. After 15 weeks, all cercariae, not exceeding 90 in number obtained from each infected snail, were subcutaneously inoculated into a mouse and the sex of adult *Schistosoma* developing in the mouse was observed 7 weeks after infection. All exposed snails were classified into 5 groups; negative infection, male, female, bisexual and undetermined sexual infections. Numbers of miracidia exposed to a snail were 1, 1, 2, 2, 3, 3, 5 and 10 respectively in 8 experiments. The theoretical probabilities of the different sexual infections except for the undetermined ones were estimated with the assumption that both sexes of *Schistosoma* develop in snails in an equal chance, and each miracidiuin has equal successful probability (p) and unsuccessful probability (q) to complete the development in the snail. The theoretical probability of snails in which r miracidia complete the development when snails were exposed to n miracidia each is calculated as $nCr\ p^r q^{n-r}$. The values of p and $q = 1-p$ are estimated by the equation $q =$ (incidence of negative infection of snails)$^{\frac{1}{n}}$ which is derived from the above formula at $r = 0$. With exposure of snails to less than 5 miracidia, the probability of snails in which 3 miracidia or more complete development was less than 5%. So the male (ML) and female (FL) infections are presumed to be single miracidial infections and the bisexual infections (B) are double miracidial infections. The male ratio (a) is estimated by the equation, $a =$ (ML + B)/(ML + FL + 2 B) and the female ratio (b) is $1-a$. The male, female, and bisexual infections are calculated by summing $a^r\ nCr\ p^r q^{n-r}$, $b^r\ nCr\ p^r q^{n-r}$ and $(1-a^r-b^r)\ nCr\ p^r q^{n-r}$ at $r = 1$ to n, respectively. The difference between the frequencies of different sexual infections from the theoretical calculation and the observed data was examined by chi square test. It was shown that the difference was not significantly large in 6 experiments in which snails were exposed to two or more miracidia each and the assumption was supported. The occurrence of bisexual infection was not less than the theoretical probability. The probability of successful development of each miracidium (p) varied from 0.1714 to 0.3066 with an average of 0.2218 in 8 experiments. Male ratio (a) was from 0.3726 to 0.6445 with an average of 0.4792. The sex of cercariae developing in a snail was independent of the sex of the host snail.

Introduction

It is generally known that cercariae of *Schistosoma* obtained from one snail quite often

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develop into adults of one sex in the inoculated animals. In routine laboratory maintenance of *Schistosoma japonicum*, cercariae collected from at least 3 snails are inoculated into mice to obtain bisexual infections.

The present study was attempted to learn whether the occurrence of the low incidence of bisexual infections of *Oncomelania nosophora* follows the rule of probability. The theoretical estimation of the occurrence of the varied sexual infections was made under the assumption that both sexes of *Schistosoma* determined at fertilization develop in an equal chance and each miracidium has an equal successful probability to complete cercarial development in the snail. Stirewalt [5] reported a different method of probability estimation on the same problem without successful result.

In connection with the sex determination of *Schistosoma*, studies on the chromosome pattern reported that both sexes have an equal number of chromosomes and the sex chromosome has not yet been demonstrated (Walton [6]). Also, in experimental infections, it is believed that a single miracidium develops into adults of one sex.

In the present study snails were individually exposed to a definite number of miracidia at one time and the incidence of male, female, bisexual and negative infections was observed. On the other hand, the theoretical probabilities of occurrence of those infections were calculated statistically following the above-mentioned assumption. From the results of 8 experimental infections of snails, the theoretically estimated frequency fitted well to the observed one and the above assumption was supported.

Materials and Methods

Schistosoma japonicum: Schistosomes used in this experiment were isolated at Kofu, Yamanashi Prefecture Japan and maintained in dogs, rabbits and mice. They have been kept mainly in mice and *Oncomelania nosophora* which were collected at Kofu and bred in this laboratory.

Infection of snails: Eggs of *Schistosoma* were collected from the mouse intestine. The intestine of the infected mice was removed, cut into pieces with scissors and emulsified in physiologic saline by metal homogenizer. The emulsion was digested with trypsin for 3 hours in a water bath at 37°C. After the emulsion was strained through two sheets of gauze, eggs were sedimented by centrifugation and washed several times with physiologic saline by mild centrifugation to remove the tissue fragments.

The sedimented eggs were then transferred with pipet into the botton of an Erlenmeyer flask which was previously filled with 0.2% NaCl solution close to the top. The flask was covered with black paper leaving the upper portion uncovered. The surface of the water in the flask was illuminated with an electric bulb. Miracidia began to float to the surface 1 hour or more after the transfer of the eggs.

In each experiment, 70 small petri dishes of 2 ml capacity half-filled with 0.2% NaCl solution were prepared. A specific number of miracidia was transferred from the Erlenmeyer flask to each of these petri dishes. The number of miracidia transferred into each petri dish was counted under a dissecting microscope.

Adult *Oncomelania* snails were placed individually into each petri dish and 0.2% NaCl was added to the top of the petri dish, covered with microscopic cover glass, and were kept overnight to expose the snails to miracidia.

Numbers of miracidia in a petri dish were 1, 1, 2, 2, 3, 3, 5 and 10 in 8 experiments with 70 snails exposed in each experiment.

The exposed snails were collected the following morning and placed on a sheet of

moistened chemical filter paper at the bottom of a large petri dish of 20 cm diameter and was covered with a metal wire mesh. Snails were fed powdered food three times a week for 15 weeks.

Infection of mice: Following infection with miracidia snails were crushed in a drop of 0.2% NaCl solution between two glass slides. All cercariae collected from an infected snail were injected subcutaneously into a mouse. When the number of emerged cercariae were too many, only 70 to 90 cercariae were used in the inoculum.

Mice were sacrificed 7 weeks after inoculation. Adult *Schistosoma* were collected from the portal and mesenteric veins under a dissecting microscope and the sex of recovered worms was determined. In instances when the inoculated number of cercariae was too small, no development of adult worms occurred and the sexual nature of the miracidium was not determined. Snails thus exposed to miracidia were classified into 5 categories; negative infection, male, female, bisexual and undetermined sexual infections.

Theoretical probability calculation: The probabilities of uni- and bisexual infections of snails were calculated using two parameters. One is the probability of successful development of each miracidium (p) in the snail host and the other is the sex ratio of miracidia (a & b). The assumption proposed for estimation is that both sexes of *Schistosoma* determined at fertilization develop in an equal chance and all miracidia have equal success (p) and unsuccess (q) of completing cercarial development in the snail.

Using these parameters the probabilities of uni- and bisexual infections were calculated. The difference between the calculated and observed frequencies of snails was examined by chi square test. If the difference is not significant, the assumption is supported. When snails are exposed individually to n miracidia each, the probability of snails in which r miracidia complete the development is calculated as $nCr\ p^r q^{n-r}$ where $r = 0$ to n and $p + q = 1$. Each probability of snails in which 0 to n miracidia complete development corresponds to each term of the expansion of the binominal equation of $(q + p)^n$ as follows:

$$(q + p)^n = q^n + npq^{n-r} + \ldots + nCr\ p^r q^{n-r} + \ldots + np^{n-1} q + p^n$$

The first term of the above expanison, q^n is the probability of no cercarial development in snails. So q and p are obtained by the following equations:

$$q = \left(\frac{\text{no. of snails uninfected}}{\text{no. of snails exposed}}\right)^{\frac{1}{n}} \qquad p = 1 - q$$

The probabilities were calculated by expanding the binominal equation using p and q obtained from observed data in 8 experiments. The number of miracidia completing development (r) and probabilities among exposed snails are shown on the first 2 columns of the upper table in TABLES 3 to 8. The probability of development of 3 or more miracidia in snails is less than 5 per cent except for experiment no. 8 in which snails were exposed to 10 miracidia each. Thus all the infected snails were regarded as having one or two miracidial infections and the unisexual infections were considered as an infection with a single miracidium and bisexual infections were two miracidial infections. The male and female ratios are calculated by the following equations:

$$\text{male ratio (a)} = \frac{ML + B}{ML + FL + 2B}$$

$$\text{female ratio (b)} = \frac{FL + B}{ML + FL + 2B} = 1 - a$$

ML: No. of snails with male infection
FL : " " " " female "
B : " " " " bisexual "

The probability of snails with r miracidial development is further divided into three classes; male, female and bisexual infections. In male infection, all invading miracidia r are male so the probability is a^r. Similarly female infection is b^r and bisexual infections are the balance remaining, $1-a^r-b^r$. The sum total of male infections at all values of r presents the probability of male infections in all snails exposed.

When each snail of one experiment is exposed to n miracidia, all probabilities of male, female and bisexual infections are given by the following formulas:

$$\text{male infection} = \sum_{r=1}^{n} a^r \, nCr \, p^r \, q^{n-r}$$

$$\text{female infection} = \sum b^r \, nCr \, p^r \, q^{n-r}$$

$$\text{bisexual infection} = \sum (1-a^r-b^r) \, nCr \, p^r \, q^{n-r}$$

Results

Snails exposed were classified into infected and uninfected groups. The infected ones were further divided into male, female, bisexual and undetermined infections. The probability of successful development of miracidium (p), unsuccessful development (q), male ratio (a) and female ratio (b) were calculated following the formulas described above.

All calculations were carried out by a mini-computer AICOM 4C programmed with AICAL language. An example of calculation of a, b, p and q values are shown in Table 1. Observed data are given after the colon as in the upper part of Table 1 and the calculated values are written after equal signs in the lower part of the Table 1. Table 2 shows all the given experimental conditions and obtained data in the 8 infection experiments including the numbers of miracidia and snails exposed, positive and negative infections, and the classification of male, female and bisexual infections. The table also gives the calculated values of p and a.

The incidence of infection in snails increased from 17.1% to 82.8% with an increase in the number of miracidia exposed to snails. The same trend is shown in the incidence of bisexual infections although they were much lower than in the former case.

The probability of successful development of each miracidium (p) varied from 0.1714 to 0.3066 with an average of 0.2218. The male ratio (a) ranged from 0.3726 to 0.6445; the average was 0.4792. The variations of these two values were small in 8 experiments and were independent of the number of miracidia exposed. The male ratio may be basically constant but the p value is considered to be much influenced by the laboratory conditions and method of infecting snails.

A single miracidium was exposed to each snail in Exp. nos. 1 and 2 (Table 2) and no bisexual infection was observed. Using the data of experimental infections with 2 or more miracidia, probabilities of uni- and bisexual infections were calculated with a computer. Table 3 shows a recording sheet of the computer in which probabilities are calculated from the data obtained in experiment no. 8. At the upper part of the table, data from Table 2 were given after colon and the computer printed out the two tables.

On the upper table of Table 3, number of miracidia which have completed development in the snail host (r) is printed out on the first column, then the value of $nCr \, p^r \, q^{n-r}$ is

TABLE 1

Recording sheet of the computer showing the given data and calculated values of a, b, p and q.

```
EXP. NO.  :6

OBSERVED DATA
NO. OF MIRACIDIA EXPOSED TO EACH SNAIL :3
NO. OF SNAILS INFECTED                 :67
  NO. OF SNAILS WITH CERCARIAL DEVELOPMENT :38
   '  '     '   WITHOUT  '          '      :29
    NO. OF SNAILS WITH MALE   INFECTION   :15
     '  '     '      '   FEMALE    '      :16
     '  '     '      '   BISEXUAL  '      :7
     '  '     '      '   UNDETERMINED '   :0


CALCULATED VALUES
    MALE RATIO (A)= 0.4889     FEMALE RATIO (B)= 0.5111
PROBABILITY OF SUCCESSFUL DEVELOPMENT OF MIRACIDIUM  (P)= 0.2436
    '        ' UNSUCCESSFUL   '       '       '      (Q)= 0.7564
```

TABLE 2

Conditions for infecting snails, observed sexual infections and calculated p and a values.

Exp. no.	No. of miracidia exposed	No. of snails exposed	No. of snails: Without cercaria	No. of snails: With cercaria	Infection: Male	Infection: Female	Infection: Bisex.	Infection: Undeterm.	p	a
				(%)			(%)			
1	1	70	50	20(28.6)	11	9	0	0	0.2857	0.5500
2	1	70	58	12(17.1)	5	6	0	1	0.1714	0.4545
3	2	70	47	23(32.9)	8	14	1(1.4)	0	0.1806	0.3750
4	2	67	38	29(43.3)	11	10	4(6.0)	4	0.2469	0.5173
5	3	69	23	46(66.7)	21	8	8(11.6)	9	0.3066	0.6445
6	3	67	29	38(56.7)	15	16	7(10.4)	0	0.2436	0.4889
7	5	64	24	40(62.5)	8	21	11(17.2)	0	0.1781	0.3726
8	10	64	11	53(82.8)	12	22	19(29.7)	0	0.1615	0.4306

Percentage is the rate to the number of snails exposed.
p: Probability of successful development of each miracidium.
a: Male ratio of miracidia.

written on the second column as total. The latter is further subdivided into male, female and bisexual infections by multiplying a^r, b^r and $(1-a^r-b^r)$, respectively. On the 1st line at $r = 0$, only total probability is presented which corresponds to the incidence of negative infection of snails. On the 2nd line at $r = 1$, the probability of bisexual infection is naturally zero because of the assumption. After the 3rd line all the 4 probabilities are written successively until the number of miracidia (r) reaches n. The sum of each column is given on the last line and these total values correspond to the theoretical probabilities of male, female and bisexual infections.

The calculation proceeds to the lower table. The calculated values on the upper table are transferred to the corresponding lines on the 1st column. These are multiplied by

TABLE 3

Results of exposure of snails to 3 miracidia (exp. no. 6); recording sheet of computer showing the given data, the calculated probabilities of different types of infection, and chi square test of the difference between the observed and theoretical frequencies.

EXP. NO. :6

N :3 SNL INF:67 + SNL:38 - SNL:29
ML:15 FL:16 B:7 U:0
(A):0.4889 (P):0.2436

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.43277	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.41812	= 0.20442	= 0.21370	= 0.00000
= 2	= 0.13466	= 0.03219	= 0.03518	= 0.06730
= 3	= 0.01446	= 0.00169	= 0.00193	= 0.01084
TOTAL	= 1.00000	= 0.23830	= 0.25081	= 0.07813

INFECTION	PROB.	FRQ. OF SNAILS CALCUL.	OBSERV.	CHI SQ
MALE	= 0.2383	= 15.966	= 15	= 0.059
FEMALE	= 0.2508	= 16.804	= 16	= 0.039
BISEXUAL	= 0.0781	= 5.2348	= 7	= 0.595
NEGATIVE	= 0.4328	= 28.996	= 29	= 0.000
UNDETERMINED	*	*	= 0	*
TOTAL	= 1.0000	= 67.000	= 67	= 0.692

$0.5 > Pr.\ (\chi^2 = 0.692) > 0.3$ DF = 1 Non-significant.

N: No. of miracidia exposed to each snail
SNL INF: No. of snails exposed
+SNL: No. of snails with cercarial development (infected)
−SNL: No. of snails without cercarial development (uninfected)
ML: No. of snails with male infection
FL: No. of snails with female infection
B: No. of snails with bisexual infection
U: No. of snails with undetermined infection
(A): Male ratio
(P): Probability of successful development of each miracidium
DF: Degree of freedom = 4−2−1 = 1

the number of snails exposed to obtain the estimated frequencies of snails of different sexual infections and are listed on the 2nd column. The observed frequencies are listed on the 3rd column and chi square is calculated on the 4th column. The observed frequency of undetermined sexual infection, if present, is written on the 5th line. Sum of each column is given on the last line and total value of chi square is obtained. The calculated frequencies are not so different from the observed data on this table and this is proven statistically by chi square test.

Data from the rest of the 5 experiments in which snails were each exposed to 2 or more miracidia were calculated in the same way (TABLES 4–8). Snails were exposed to 2 miracidia in experiment nos. 3 and 4 in TABLES 4 and 5. Calculated frequencies in varied sexual infections fitted well to the observed ones in Exp. no. 3. The fit was not

TABLE 4

Results of exposure of snails to 2 miracidia (exp. no. 3).

EXP. NO. :3

N :2 SNL INF:70 + SNL:23 - SNL:47
ML:8 FL:14 B:1 U:0
(A):0.3750 (P):0.1606

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.67142	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.29597	= 0.11099	= 0.18498	= 0.00000
= 2	= 0.03262	= 0.00459	= 0.01274	= 0.01529
TOTAL	= 1.00000	= 0.11558	= 0.19772	= 0.01529

INFECTION	PROB.	FRQ. OF SNAILS CALCUL.	OBSERV.	CHI SQ
MALE	= 0.1156	= 8.0902	= 8	= 0.001
FEMALE	= 0.1977	= 13.841	= 14	= 0.002
BISEXUAL	= 0.0153	= 1.0702	= 1	= 0.005
NEGATIVE	= 0.6714	= 46.999	= 47	= 0.000
UNDETERMINED	*	*	= 0	*
TOTAL	= 1.0000	= 70.000	= 70	= 0.008

$0,95 > Pr.\ (\chi^2 = 0.008) > 0.9$ Non-significant.

TABLE 5

Results of exposure of snails to 2 miracidia (exp. no. 4).

EXP. NO. :4

N :2 SNL INF:67 + SNL:29 - SNL:38
ML:11 FL:10 B:4 U:4
(A):0.5173 (P):0.2469

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.56716	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.37188	= 0.19237	= 0.17951	= 0.00000
= 2	= 0.06096	= 0.01631	= 0.01420	= 0.03044
TOTAL	= 1.00000	= 0.20869	= 0.19371	= 0.03044

INFECTION	PROB.	FRQ. OF SNAILS CALCUL.	OBSERV.	CHI SQ
MALE	= 0.2087	= 13.982	= 11	= 0.636
FEMALE	= 0.1937	= 12.979	= 10	= 0.684
BISEXUAL	= 0.0305	= 2.0397	= 4	= 1.884
NEGATIVE	= 0.5672	= 38.000	= 38	= 0.000
UNDETERMINED	*	*	= 4	*
TOTAL	= 1.0000	= 67.000	= 67	= 3.204

$0.1 > Pr.\ (\chi^2 = 3.204) > 0.05$ Non-significant.

TABLE 6

Results of exposure of snails to 3 miracidia (exp. no. 5).

EXP. NO. :5

N :3 SNL INF:69 + SNL:46 - SNL:23
ML:21 FL:8 B:8 U:9
(A):0.6445 (P):0.3066

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.33339	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.44224	= 0.28503	= 0.15722	= 0.00000
= 2	= 0.19555	= 0.08123	= 0.02471	= 0.08961
= 3	= 0.02882	= 0.00772	= 0.00130	= 0.01981
TOTAL	= 1.00000	= 0.37397	= 0.18323	= 0.10942

INFECTION	PROB.	FRQ. OF SNAILS CALCUL.	OBSERV.	CHI SQ
MALE	= 0.3740	= 25.804	= 21	= 0.894
FEMALE	= 0.1832	= 12.643	= 8	= 1.705
BISEXUAL	= 0.1094	= 7.5498	= 8	= 0.027
NEGATIVE	= 0.3334	= 23.004	= 23	= 0.000
UNDETERMINED	*	*	= 9	*
TOTAL	= 1.0000	= 69.000	= 69	= 2.626

$0.2 > Pr.\ (\chi^2 = 2.626) > 0.1$ Non-significant.

TABLE 7

Results of exposure of snails to 5 miracidia (exp. no. 7).

EXP. NO. :7

N :5 SNL INF:64 + SNL:40 - SNL:24
ML:8 FL:21 B:11 U:0
(A):0.3726 (P):0.1781

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.37506	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.40636	= 0.15141	= 0.25495	= 0.00000
= 2	= 0.17611	= 0.02445	= 0.06932	= 0.08234
= 3	= 0.03816	= 0.00198	= 0.00943	= 0.02676
= 4	= 0.00414	= 0.00008	= 0.00064	= 0.00342
= 5	= 0.00018	= 0.00000	= 0.00002	= 0.00016
TOTAL	= 1.00000	= 0.17791	= 0.33436	= 0.11268

INFECTION	PROB.	FRQ. OF SNAILS CALCUL.	OBSERV.	CHI SQ
MALE	= 0.1779	= 11.387	= 8	= 1.007
FEMALE	= 0.3344	= 21.399	= 21	= 0.008
BISEXUAL	= 0.1127	= 7.2113	= 11	= 1.991
NEGATIVE	= 0.3751	= 24.004	= 24	= 0.000
UNDETERMINED	*	*	= 0	*
TOTAL	= 1.0000	= 64.000	= 64	= 3.005

$0.1 > Pr.\ (\chi^2 = 3.005) > 0.05$ Non-significant.

TABLE 8

Results of exposure of snails to 10 miracidia (exp. no. 8).

EXP. NO. :8

N :10 SNL INF:64 + SNL:53 - SNL:11
ML:12 FL:22 B:19 U:0
(A):0.4306 (P):0.1615

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.17180	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.33090	= 0.14249	= 0.18842	= 0.00000
= 2	= 0.28680	= 0.05318	= 0.09299	= 0.14064
= 3	= 0.14731	= 0.01176	= 0.02719	= 0.10835
= 4	= 0.04965	= 0.00171	= 0.00522	= 0.04273
= 5	= 0.01148	= 0.00017	= 0.00069	= 0.01062
= 6	= 0.00184	= 0.00001	= 0.00006	= 0.00177
= 7	= 0.00020	= 0.00000	= 0.00000	= 0.00020
= 8	= 0.00002	= 0.00000	= 0.00000	= 0.00002
= 9	= 0.00000	= 0.00000	= 0.00000	= 0.00000
= 10	= 0.00000	= 0.00000	= 0.00000	= 0.00000
TOTAL	= 1.00000	= 0.20932	= 0.31457	= 0.30431

INFECTION	PROB.	FRQ. OF SNAILS CALCUL.	OBSERV.	CHI SQ
MALE	= 0.2093	= 13.396	= 12	= 0.146
FEMALE	= 0.3146	= 20.132	= 22	= 0.173
BISEXUAL	= 0.3043	= 19.476	= 19	= 0.012
NEGATIVE	= 0.1718	= 10.995	= 11	= 0.000
UNDETERMINED	*	*	= 0	*
TOTAL	= 1.0000	= 64.000	= 64	= 0.330

$0.7 > Pr.\ (\chi^2 = 0.33) > 0.5$ Non-significant.

TABLE 9

Comparison of infection rate of male and female snails by parasites of both sexes; (%)

Sex of snail	Sex of *Schistosoma* male	female	both	total
Male	29 (33.7)	43 (50.0)	14 (16.3)	86 (100%)
Female	40 (32.2)	56 (45.2)	28 (22.6)	124 (100%)

Sex of snail	Sex of *Schistosoma* male	female	total
Male	43 (43.0)	57 (57.0)	100 (100%)
Female	68 (44.7)	84 (55.3)	152 (100%)
Total	111 (44.0)	141 (56.0)	252 (100%)

$0.8 > Pr.\ (\chi^2 = 0.073) > 0.7$ Non-significant.

as good in Exp. no. 4 because of the larger observed frequency of the bisexual infection. The difference was, however, not statistically significant by chi square test. It is shown that the theoretical probability of bisexual infection is as small as 1.5 and 3.0% of all snails at exposure to 2 miracidia.

In Exp. no. 5 as in Exp. no. 6, snails were exposed to 3 miracidia (Tables 3 and 6). The fit of theoretical and observed frequencies is fairly good. Calculated probability of bisexual infection was also small, 7.8 and 10.9%. On Table 6, sex of miracidia was undetermined in 9 snails. In the calculation, this frequency was excluded from the observed values and totals of 69 calculated and 60 observed frequencies were compared. The difference was, however, not significant by chi square test.

In Exp. no. 7 (Table 7) the observed value was smaller in male infection and larger in bisexual infection than the calculated data. The difference, however, was not significantly large. It is shown that the probability of bisexual infection is still small, 11.3% of total snails even though they were exposed to 5 miracidia.

In Exp. no. 8 (Table 8) snails were exposed to 10 miracidia. The difference of calculated and observed frequencies was not significant statistically. On the upper Table, 9 and 10 miracidia did not complete the development, and all snails become bisexual infection when 7 and 8 miracidia complete development in a snail.

Since the calculated frequencies of uni- and bisexual infections with the assumption fit the observed frequencies in all epxeriments, the assumption that occurrence of uni- or bisexual infections is regulated by the rule of probability, is sustained.

A comparison was made whether the sex of the miracidium developing in a snail is dependent on the sex of the host snail. Different sexual infections in snails were classified by the sex of host snails (Table 9). Frequency of bisexual infections is added to those of male and female infections and the comparative table was made (Table 9). Since there was no significant difference by chi square test, the sex of *Schistosoma* developing in a snail was considered to be independent of the sex of the host snail.

Discussion

It is widely accepted that the sex of *Schistosoma* is determined at fertilization and cercariae which emerge from a snail previously exposed to a single miracidium develop into adults of one sex. However, the theoretical explanation of the proportion of uni- and bisexual infections in snails has not yet been successfully made. For this, studies have been directed to two ways: One is the observation of chromosomes; the other is the experimental infections of host snails with varying numbers of miracidia to observe the proportion of uni- and bisexual infections in these snails.

The earlier studies on the number of chromosomes in human schistosomes reported sexual differences. Numbers were 15 and 16 in male and female, respectively, or 14 and 16 in another study. More recent studies, however, shows that both sexes possessed 16 chromosomes and no recognizable sex chromosomes have yet been demonstrated (Walton [6]).

Many studies revealed that snails exposed to a single miracidium were unisexual infections (Stirewalt [5], Standen [4], El Raggal [1]). In the experimental infection of snails with varying numbers of miracidia, the proportion of uni- and bisexual infections has been disregarded except for a few papers (Schwetz *et al.* [3], Schreiber & Schubert [2], Stirewalt [5]). A theoretical explanation of the occurrence of bisexual infections was attempted by Stirewalt [5]. In that study, however, the observed incidence of bisexual in-

fections was far less than the theoretical calculation.

In the present paper, theoretical probabilities of uni- and bisexual infections were successfully calculated. In this calculation, the probability of the uninfected snails was taken into consideration, and the probabilities of negative infections, uni- and bisexual infections were simultaneously estimated using two parameters, sex ratio (a, b) and probability of successful (p) and unsuccessful (q) development of each miracidia in the snail.

In the paper reported by Stirewalt [5] negative infections were disregarded and attempts to obtain a theoretical proportion of uni- and bisexual infections among the infected snails were made. The present authors estimated the negative infections from the data of Stirewalt [5] and calculated the theoretical proportion of uni- and bisexual infections following the method discussed herein and found the observed proportion of the bisexual infections to be much higher than the theoretically calculated probability.

As was described earlier, the incidence of bisexual infections in the total number of exposed snails is usually low and increases with the increase in the incidence of infected snails. In most laboratories, the incidence of positive infections is about 30% when snails are exposed to 5 miracidia each. The theoretical probability in such a case is calculated in TABLE 10.

The value of p is as small as 0.0688 calculated from $1-\sqrt{1-0.3}$. The probabilities of uni- and bisexual infections are 13.9% and 2.1% in all exposed snails. The result indicates that the incidence of bisexual infections is very low in routine laboratory infections

TABLE 10

Theoretical probabilities of uni- and bisexual infections of snails when exposed to 5 miracidia each and 30% are successfully infected.

EXP. NO. :THEOR.

N :5
(A):0.5 (P):0.0688

NO. OF MIRACID.	PROBABILITY OF INFECTION TOTAL	MALE	FEMALE	BISEXUAL
= 0	= 0.70019	= 0.00000	= 0.00000	= 0.00000
= 1	= 0.25866	= 0.12933	= 0.12933	= 0.00000
= 2	= 0.03822	= 0.00956	= 0.00956	= 0.01911
= 3	= 0.00282	= 0.00035	= 0.00035	= 0.00212
= 4	= 0.00011	= 0.00001	= 0.00001	= 0.00009
= 5	= 0.00000	= 0.00000	= 0.00000	= 0.00000
TOTAL	= 1.00000	= 0.13925	= 0.13925	= 0.02132

The calculated value of p is 0.0688 and male ratio (a) is regarded as 0.5.

of snails especially when the incidence of infection is less than 30%.

In the present study, it is shown that the sex of *Schistosoma* developing in the snail is independent of the sex of host snails, similar to the report by El Raggal [1].

From the results of the present study, it is concluded that each miracidium develops in the snail without influence from other developing miracidia or from the sex of the host snails, and uni- and bisexual infections which occur follow the rule of probability.

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CULTURE OF *SCHISTOSOMA JAPONICUM* (PHILIPPINE STRAIN) FROM THE CERCARIAL STAGE AND THE EFFECTS OF IMMUNE RABBIT AND HUMAN SERA *IN VITRO*: A PRELIMINARY REPORT

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Schistosomula were cultured after allowing cercariae of *Schistosoma japonicum* (Philippine strain) to penetrate through pieces of prepared rat skin. The schistosomula were cultured in tubes in a medium containing equal parts of human serum and NCTC 109, penicillin (100 units/ml), streptomycin (100 gm/ml), at 37°C and pH 7.2 in a flowing atmosphere of 8% carbon dioxide in air. On day 4, 1% washed human blood cells were added to the tubes and from day 7 onwards most worms began to feed. The schistosomula in culture reached the "gut-closed" stage by day 12, equivalent to that in the mouse, and attained a relatively late stage of development at which males were sexually mature and mated with females but the female worms did not produce eggs.

Three normal rabbits were given 300 cercariae each and samples of serum taken before infection and thereafter at monthly intervals. Every month the sera taken from the three rabbits were compared for lethal effects on schistosomula with a control containing normal serum. In the sera of the rabbits at the end of the 3rd month after infection, a high level lethal effect as well as a membraneous sheath around schistosomula were observed although there was considerable variation between individual sera. Some of the schistosomula which broke free of their sheaths remained viable. The heating of the sera at 56°C for 30 minutes did not abolish the lethal effect or the perioschistosomular envelope formation. Sera from patients infected with *S. japonicum* in Leyte, Philippines, killed 25.8 ± 4.7% of schistosomula during a 4-day culture period compared with 13.2 ± 4.8% in control cultures with normal human sera.

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LABORATORY TRIALS OF TWO NEW MOLLUSCICIDES, THE BARK OF "GOGO" *(ENTADA PHASHOLOIDES)* AND POLYNACTIN, AGAINST *ONCOMELANIA* SNAILS : A PRELIMINARY REPORT

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The present paper briefly describes our studies on two new molluscicides which have been done recently in Japan and the Philippines.

The bark of "gogo", *Entada phasholoides*

Many plants have been reported from different parts of the world to possess varying degrees of molluscicidal potency and mammalian and fish toxicity. Although plant molluscicides may not complete with other existing and expensive synthetic molluscicides, they may be useful in the control of schistosomiasis in rural areas on a "self-help" basis.

Entada phasholoides, "gogo" in Tagalog, is widely distributed and abundant in the Philippines. The bark of the vine has largely been used by the natives for hair shampoo. The fish toxicity of "gogo" bark was first reported in 1906 by Bacon and the molluscicidal properties of the bark have recently been announced by Garcia (1973).

The bark was repeatedly extracted with hot methanol, from which several fractions, (1) ether soluble, (2) benzene soluble, (3) ethylacetate soluble, (4) acetone soluble, (5) water soluble, and (6) n-butanol soluble, were prepared. Of the fractions tested, the n-butanol soluble extract was the most toxic with an LC_{50} of 5.8 (4.7-7.5) and 3.6 (2.8-4.6) ppm. Analytical work on the n-butanol fraction by thin layer chromatography (Liebermann-Burchard reaction) indicated that the molluscicidal agents are at least two kinds of saponins. No remarkable loss in the toxicity was found in hard water, a 24-hour exposure to ultraviolet rays, or the presence of yeast cells. Alkaline waters reduced the toxicity.

Because of the promising results obtained from the above laboratory tests, it would be of value to know the fate of this toxicant under field conditions. Field trials are thus being carried out at a small pond in Leyte, Philippines.

Polynactin

Polynactin, an antibiotic extracted from *Streptomyces aureus*, has recently been developed as a miticide by Chugai Pharmaceutical Co. Ltd., Tokyo. Since this antibiotic has been shown to exert a promising lethal effect on mites, one might anticipate that it would also be useful against schistosome-bearing snails. Preliminary laboratory tests have been started very recently to evaluate the molluscicidal activities of the antibiotic against *Oncomelania quadrasi*.

From the results of duplicate immersion tests with Polynactin, using a 20% w/v emulsifiable concentrate, the LC_{50} and respective 95% confidence limits were computed as 0.99 (0.75-1.30) ppm and 0.99 (0.82-1.20) ppm in juvenile snails and 1.12 (0.82-1.20) ppm and 1.60 (1.23-2.08) ppm in adults. Evidence from

studies on the biochemical mechanism of the insecticidal action of this antibiotic indicates that NADH oxidative enzymes are inhibited in the presence of the chemicial at a higher concentration. It is also demonstrated that Polynactin has properties as an uncoupler of oxidative phosphorylation in the muscle of a cockroach, *Blattella germanica*.

Further studies are now underway to compare molluscicidal values for Polynactin with four other molluscicides now in use.

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Laboratory and Field Assessment of the Molluscicidal Activity of Gogo *(Entada phaseoloides)* Against the Amphibious Snail Intermediate Host of *Schistosoma japonicum*[1]

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Summary: A molluscicidal fraction occurs naturally in the bark of a vine (gogo in Tagalog), *Entada phaseoloides*, which grows indigeneously and abundantly in the Philippines. Butanol fraction of the methanol extracts of the bark was most toxic against *Oncomelania quadrasi* with the LC_{50} of 3.6–5.8 ppm. Analytical work on the butanol fraction by thin layer chromatography indicated that the active molluscicidal agents contained at least two kinds of saponins. The potency of *E. phaseoloides* remained rather stable over a wide range of pH values, in the presence of minerals and yeast cells and after ultraviolet irradiation of solutions. Our preliminary field trials, however, showed that doses as higher than 40 g per square meter would be needed to produce a satisfactory molluscicidal effect under field conditions.

Introduction

At present, control of schistosomiasis depends to a large extent on chemical molluscicides. Although effective molluscicidal compounds are commercially available, almost all of them are factory products in developed countries where no schistosomiasis is endemic. The high cost of these existing molluscicides, and the large quantities that must be continually applied in order to have adequate snail control, are some of the major factors limiting thier use in developing countries. An effective and safe molluscicide that can be produced locally, rather than imported, would be an important contribution to the struggle against the highly endemic and rapidly spreading schistosomiasis in these countries [5].

Entada phaseoloides, gogo in Tagalog, is widely distributed and abundant in the Philippines. The wood of the vine is cut into thin strips, which are then beaten between stones in order to separate the fiber. In this condition gogo is brought into the markets and has largely been used by the natives for hair shampoo. The fish toxicity of gogo bark was first reported in 1906 by Bacon[1] and the molluscicidal properties of the bark have recently been announced by Garcia[2]. The present investigation was undertaken to evaluate the molluscicidal activity of the bark against *Oncomelania* snails.

Materials and Methods

The bark of gogo purchased on the market in Manila was torn and ground to a powder. Nine hundred g of the ground bark was extracted three times with 4 liters of hot methanol and brought to dryness in vacuo, yielding about 142 g of a brown powder upon evaporation. The methanol extract was successively extracted

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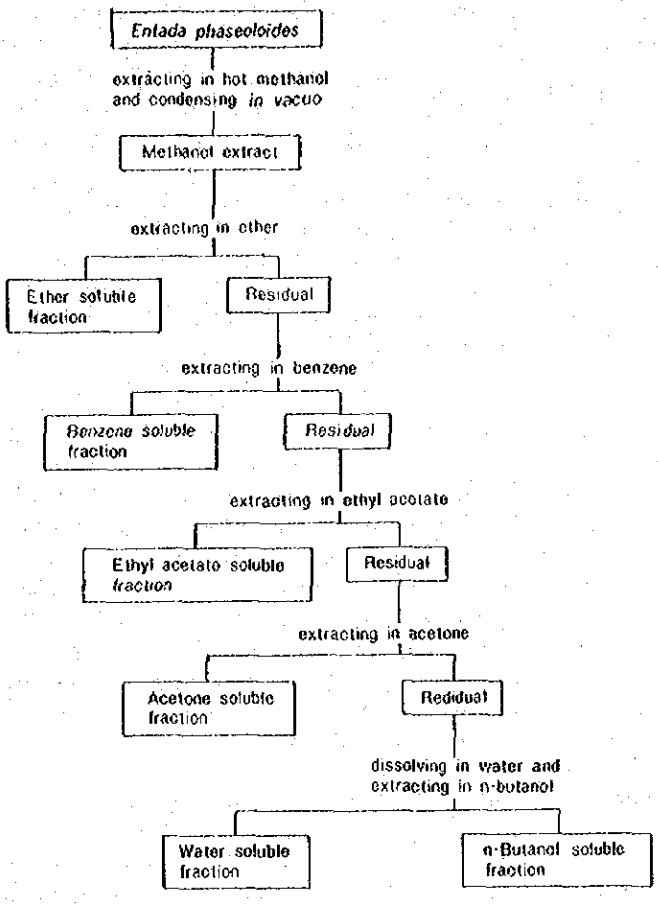
Fig. 1. *Procedures for stepwise extraction from the bark of E. phasholoides.*



with 0.5 liters each of hot ether, benzene, ethyl acetate and acetone as shown in Fig. 1. After final extraction with hot acetone, the residue was dissolved in 0.5 liters of water and again extracted seven times with 0.5 liters of n-butanol saturated with water. The n-butanol phase was withdrawn and concentrated to dryness in vacuo, about 85 g of a powder was obtained. Evaporation in vacuo of the aqueous solution gave about 25 g of a brown powder.

As the ether, benzene and ethyl-acetate fractions were hardly soluble in water, a 500 ppm dilution was prepared by emulsifying 50 mg of each fraction in 2 ml of 10% gum arabic solution in a mortar and then by bringing it up to 1,000 ml by the addition of water, from which suitable two fold dilution series were made with stirring. The acetone, n-butanol and water fractions were each dissolved in water and a series of appropriate twofold dilutions was prepared.

O. quadrasi used in this experiment were obtained from field collections in Leyte, Philippines. Their viability was assured by selecting only those snails which crawled out of water-filled Petri dishes.

For the test ten mature snails (3.5 to 5 mm in length) were exposed to 100 ml of solution of the desired concentration in aged tap water at a temperature of 25°C. The testing procedure was essentially same as described by Komiya *et al.* [3], using 30 snails for each concentration, with 10 snails per dish. Snails were exposed to test solutions for 48 hr. Following the exposure period the snails were rinsed and placed in aged tap water for a 48-hr recovery period, after which the reading of dead and living snails was made. The data were analyzed by probit analysis method [6]. All experiments involved appropriate controls.

RESULTS

1. A comparison of the molluscicidal activity of various extracts from the bark.

Tests were made in duplicate to assess the relative molluscicidal activity of various fractions of the bark and the LC_{50} values and its 95% confidence limits are given in TABLE 1. Of the fractions tested, n-butanol extract was most toxic with the LC_{50} of 5.8 and 3.6 ppm. The acetone fraction was slightly toxic showing the LC_{50} of 120 and 124 ppm. The rest of the four fractions, ether, benzene, ethyl-acetate and water extracts, were ineffective as molluscicides at the maximum concentration tested (500 ppm). Based on these results, tests with frac-

tions other than n-butanol extract were discontinued.

2. *Degradation of gogo n-butanol extract by physicochemical factors.*

Influence of ultraviolet rays: Petri dishes (250 mm in diameter) each containing 600 ml of a 100 ppm of n-butanol extract solution, in a layer 11 mm thick, were exposed to a germicidal lamp (Toshiba GL-15) with a peak at 254 nm for 24 hr. The dishes were covered with a thin layer of vinyl sheet and placed about 70 cm apart from the germicidal lamp. The irradiated sample and corresponding unirradiated controls were tested for their molluscicidal activity. Parallel tests with niclosamide and sodium pentachlorophenate (NaPCP) were run.

Gogo and niclosamide showed no appreciable loss, while NaPCP suffered 64% loss (TABLE 2).

TABLE 1 Comparison of the LC50 values of different fractions from the bark for *Oncomelania quadrasi*.

Fraction	1st test	2nd test
Ether fraction	more than 500 ppm	more than 500 ppm
Benzene fraction	more than 500 ppm	more than 500 ppm
Ethyl-acetate fraction	more than 500 ppm	more than 500 ppm
Acetone fraction	124 (104–147) ppm	120 (92–156) ppm
Water fraction	more than 500 ppm	more than 500 ppm
n-Butanol fraction	5.8 (4.7–7.5) ppm	3.6 (2.8–4.6) ppm

TABLE 2 Comparison of molluscicidal activities of irradiated and untreated control dilutions of gogo n-butanol extract, niclosamide and NaPCP.

Molluscicide	LC50 (95% confidence limits) in ppm	
	Irradiated*	Untreated
Gogo	6.9 (4.1–11.7)	5.5 (4.1–7.4)
Niclosamide	0.10(0.08–0.12)	0.09(0.08–0.10)
NaPCP	0.66(0.55–0.79)	0.24(0.17–0.32)

* Exposed to a germicidal lamp (Toshiba GL-15) with a peak at 254 mμ for 24 hrs.

Influence of hydrogen ion concentration of water: The pH levels were adjusted using sodium hydroxide or hydrochloric reagents. The water was first adjusted to the desired pH, then gogo extract was added. Snails were introduced and the pH was again recorded; after 24-hr exposure, the pH was again noted. The LC_{50}s were 9.6 (7.7–11.0) ppm at pH 7.6–8.5 and 4.2 (3.3–5.1) ppm at pH 4.2–5.5. The results indicate that alkaline waters decrease the toxicity.

Influence of minerals of water: To determine the degree of degradation by minerals of water hardness, the appropriate twofold dilution series of n-butanol extract were prepared in a hard water (600 ppm calcium chloride and 139 ppm magnesium chloride). Parallel tests were run with deionized water. The LC_{50}s were 3.6 (2.8–4.6) ppm with the hard water and 3.7 (2.9–4.7) ppm with deionized water. High concentrations of minerals had no effect on the activity of the gogo extract.

Influence of yeast cells: Commercial yeast has been selected as a test organism for investigating the absorption of molluscicides by living cells in water and the results have indicated that the toxicity of niclosamide and organotin compounds decreased in the presence of the yeast. [7, 8]. Experiments were performed to determine whether the efficacy of gogo extract is reduced by the presence of yeast cells. The test solutions were made as follows: 100 mg of the extract was dissolved in 1 liter of water, to which 1 g of dried commercial yeast was added. The yeast was maintained in suspension at 25°C by means of a magnetic stirrer. After 24 hr of agitation, the yeast cells were removed and the toxicity of the supernatant was tested. Parallel runs were made using both the extract in water without yeast and yeast alone in water. The LC_{50}s in the presence and absence of yeast cells were 7.5 (6.4–8.8) ppm and 8.6 (6.9–10.8) ppm, respectively. There was no apparent enhanced reduction in toxicity due to the presence of yeast cells.

TABLE 3 Toxicity of gogo n-butanol extract compared with that of niclosamide against Japanese killifish, *Oryzias latipes*.

	LC_{50} (with 95% confidence limits)	Fish toxicity index
Gogo n-butanol extract	1.3(1.2–1.5)	0.44
Niclosamide	0.3(0.2–0.4)	2.9

3. Toxicity to fish.

Japanese killifish, *Oryzias latipes*, in group of 20 were exposed for 48 hr to appropriate twofold dilution series of n-butanol extract. Parallel runs were made with niclosamide. The fish index was calculated according to the following formula.

$$\text{Fish toxicity index} = \frac{LC_{50} \text{ to fish}}{LC_{50} \text{ to snail}}$$

The LC_{50}s and the fish toxicity index are give in TABLE 3. It can be seen that the fish was remarkably more susceptible to gogo n-butanol than to niclosamide.

4. Analysis of the n-butanol fraction.

To isolate and characterize the active molluscicidal component in the bark; the n-butanol fraction was analyzed by thin layer chromatography as follows: An aliquot of the fraction was applied to Silica Gel HR (E. Merck AG) plate, developed with a solvent mixture of chloroform : methanol : water (65 : 35 : 10, by vol.) at 26°C. Spots were detected by Kieberman-Burchard reagent. Two light-red spots (Rf 0.15 and 0.18) developed immediately after the reaction, and it was found that the n-butanol fraction contained at least two kinds of saponins.

5. Preliminary field trials

Preliminary field trials with the n-butanol fraction were carried out at Caibaan, Tacloban City, Leyte. Twelve quadrats (1 square meter each) were measured, delineated by wooden boards to prevent snails from crawling out. Three hundred snails were released into each quadrat and made to acclimatize for 2 days prior

TABLE 4 Field evaluation of molluscicidal effect of gogo on *Oncomelania quadrasi* at Caibaan, Tacloban City, Leyte.

Chemical	Trial No.	Dose (g/m²)	Pre-treatment			Post-treatment								
						1 week			2 week			3 week		
			No. of snails collected	No. of dead snails	Per cent mort.	No. of snails collected	No. of dead snails	Per cent mort.	No. of snails collected	No. of dead snails	Per cent mort.	No. of snails collected	No. of dead snails	Per cent mort.
Gogo n-butanol extract	1st	10	34	2	5.9	28	15	53.4	21	17	81.0	17	10	58.8
		20	20	1	5.0	51	11	21.6	21	8	38.1	16	2	12.5
	2nd	40	20	1	5.0	23	10	43.5	14	7	56.0	26	8	30.8
Control	1st		34	2	5.9	45	3	6.7	36	1	2.8	23	1	4.4
	2nd		25	1	4.0	18	1	5.6	31	2	6.5	32	1	3.1

Chemical	Trial No.	Dose (g/m²)	Post-treatment 4 week		
			No. of snails collected	No. of dead snails	Per cent mort.
Gogo n-butanol extract	1st	10	32	9	28.1
		20	33	5	15.2
	2nd	40	12	4	33.3
Control	1st		33	1	3.0
	2nd		20	1	5.0

to application of the chemical. Measured doses of the chemical were dissolved in 1 liter of water and sprayed in each quadrat. Samplings were made by scraping the top soil surface of 9 rings (13.5 cm in diameter) after a period of 1, 2, 3, and 4 weeks from chemical application. Samples were washed in the laboratory to recover the snails. The first trial was done during months, August to September, and the second trial September to October.

Results of the two trials are summarized in TABLE 4 where the number of snails found alive or dead is given for each observation made before and after the application of the chemical. The n-butanol fraction at a dose as high as 40 g per square meter was not significantly effctive against *O. quadrasi* under field conditions.

DISCUSSION

Many plants from different parts of the world are known to be lethal to snails. Among these, the Ethiopian endod *(Phytolacca dodencandra)* has been the most extensively investigated. Lemma (1970) evaluated an aqueous extract of the berries in Ethiopia under laboratory and field conditions and has reported good results against several species of snails including *Biomphalaria glabrata* [4]. Butanol extraction of the aqueous suspension of the berries increased its potency by 7- to 10- fold [5]. The LC_{50} of the extraction against *O. nosophora* was 1.85 (1.5–2.4) ppm [9].

In the present study, butanol extraction of the bark of *E. phaseoloides* was found to possess molluscicidal properties against *Oncomelania* snails although its potency seemed a little lower than that of endod. The working stability in the presence of various physicochemical factors appeared to be satisfactory. Our preliminary field trials, however, showed that doses as higher than 40 g per square meter would be needed to produce a satisfactory molluscicidal effect under field conditions. Additional disadvantage was that this was toxic to fish below its molluscicidal level. The question then arises as to the usefulness of the bark as a potential molluscicide.

Many attempts have been made in the past to use plant molluscicides for snail control but the majority have met with little success. Although plant molluscicides may not compete with synthetic molluscicides presently available, they may be useful for snail control in the rural areas of some developing countries on a self-help basis. Experiments are now under way so as to evaluate the molluscicidal activity of *Croton tiglium* and *Jatropha curcas*, which grow in the Philippines and will be reported in a subsequent communication.

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A Quantitative Sampling Method for *Oncomelania quadrasi* by Filter Paper[1]

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Summary: Filter paper was found to attract *Oncomelania quadrasi* in waters the same way as fallen dried banana leaves, although less number of other species of snails was collected on the former than on the latter. Snails were collected in limited areas using a tube (85 cm² area at cross-section) and a filter paper (20×20 cm) samplers. The sheet of filter paper was placed close to the spot where a tube sample was taken, and recovered after 24 hours. At each sampling, 30 samples were taken by each method in an area and sampling was made four times. The correlation of the number of snails collected by the tube and that by filter paper was studied. The ratio of the snail counts by the tube sampler to those by the filter paper was 1.18. A loose correlation was observed between snail counts of both methods as shown by the correlation coefficient $r = 0.6502$. The formulas for the regression line were $Y = 0.77X + 1.6$ and $X = 0.55Y + 1.35$ for 3 experiments where Y is the number of snails collected by tube sampling and X is the number of snails collected in the sheet of filter paper.

The type of snail distribution was studied in the 30 samples taken by each method and this was observed to be nearly the same in both sampling methods. All sampling data were found to fit the negative binomial distribution with the values of the constant k varying very much from 0.5775 to 5.9186 in $(q - p)^{-k}$. In each experiment, the constant k was always larger in tube sampling than in filter paper sampling. This indicates that the uneven distribution of snails on the soil surface becomes more conspicuous by the filter paper sampling.

Introduction

For the purpose of determing the population density of *Oncomelania quadrasi*, methods using ring and tube samplers with a 13.5 cm diameter for both, were studied and established by Pesigan *et al.* [6] and have been widely used in connection with the evaluation of snail control in the Philippines. However, difficulties in observing the snail density on the submerged soil surfaces and in the submerged vegetation have remained. To collect snails in water, fallen dried banana leaves are being utilized as attractant and this method gave a hint to solve the above-mentioned problem.

In the present study, filter paper was used as an attractant for *O. quadrasi*. The number of snails collected by the filter paper method was compared with that collected by the tube method to determine whether the filter paper sampling method is useful for the determination of the population density of snails on the submerged areas.

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MATERIALS AND METHODS

Filter paper sampling: The filter paper measuring 60×60 cm (Toyo Roshi No. 131) was cut into appropriate sizes at each experiment. For comparison with tube sampling, 20×20 cm filter paper was used and this was placed close to the spot where a soil sample was taken by a tube sampler. Each piece of filter paper was collected on the following day, placed in a separate polyethylene bag and brought to the laboratory where the snails were picked out by forceps.

Tube sampling: The metal tube, 60 cm long and 10.42 cm in diameter, was pushed into the soil for a distance of 15 to 20 cm and with the bottom of the tube supported by a hand or a hoe, the tube was raised and the soil plug was removed and placed in a polyethylene bag. The area of the cross-section of the tube was 85 cm² or 1/117 m². The soil samples were washed with tap water through a seive with 20 meshes per inch. The washed samples were transferred into a white enameled tray and the debris were poured out a few times. The snails were then collected by forceps from the sediment.

Measurement of the shell length of O. quadrasi: The shell length of the snails were measured by an ocular micrometer under a dissecting microscope up to 0.1 mm. The sex of the snails were observed under a dissecting microscope after crushing.

RESULTS

1. Comparison of filter paper and banana leaf as attractants for O. quadrasi.

Filter paper which was cut into different sizes and fallen dried banana leaves were set in the water along the banks of the South Main Irrigation canal at Bo. Gacao, Palo, Leyte and were taken out after 2 days. As shown in TABLE 1, the number of *O. quadrasi* attracted per 100 cm² in filter paper appears to be more than on the banana leaf, but other species of snails were fewer in number on the filter paper. The 30×15 cm size of filter paper seems to be the best for collecting the snails.

A similar comparative study was made at Batang stream in Tanauan, Leyte using filter paper and dried banana leaves of about the same size each as the filter paper (TABLE 2). The filter paper was taken out on the following day in this experiment. The number of snails obtained per 100 cm² of both attractants were compared. The average number on filter paper and that on banana leaves were 3.06 and 2.40/100 cm², respectively and this difference was not statistically significant by the *t* test. Less number of *Gyraulus* sp. was found on filter paper.

Both attractants of the same size were submerged in the South Main Canal and the attractivity was compared (TABLE 3). The average number of *O. quadrasi* on filter paper paper and on banana leaf were 65.5 and 58.5,

TABLE 1 Comparison of banana leaf and filter paper as attractants of *O. quadrasi*. Attractants were submerged for 2 days.

Attractant	Size (cm)	No. of sample	Total no. of *O. quadrasi* obtained	No. of *O. quad.* per 100 cm²	Other Snails obtained
Filter paper	60×30	1	47 (16,20,10,1)*	2.6	*Brotia* sp. 1
	30×15	3	224 (57,100,46,21)	16.6	—
	30×10	2	17 (6,9,2,0)	2.8	—
Banana leaf	96×65	1	156 (112,18,20,6)	2.5	*Gyraulus* sp. 5 *Viviparus leytensis* 9 *Thiara scabra* 2 *Brotia* sp. 1

April 1974 at South Main Canal, Gacao.
* Classification of snails by size: large, moderate, small, tiny.

TABLE 2 Comparison of banana leaf and filter paper as attractants of *O. quadrasi*. Attractants were submerged for 1 day at Batang stream, Kiling, Tanauan in April 1974.

Attractant	Size (cm)	No. of *O. quadrasi* obtained per sample	No. of *O. quad.* per 100 cm²**	Other snails obtained
Filter paper (x)	30×15	7 (2,3,2,0)	1.6	*Gyraulus* sp. 1
		15 (5,6,3,1)	3.3	1
		22 (10,9,1,2)	4.9	0
		20 (8,12,0,0)	4.4	0
		7 (6,1,0,0)	1.6	0
		11 (4,6,1,0)	2.4	1
		12 (6,4,2,0)	2.7	0
		16 (9,7,0,0)	3.6	1
Banana leaf (y)	34×16	5 (2,2,1,0)	1.0	*Gyraulus* sp. 5
	34×12	5 (4,1,0,0)	1.2	1
	30×20	17 (7,8,1,1)	2.8	1
	30×20	16 (10,4,2,0)	2.7	4
	35×14	28 (17,6,3,2)	5.7	1
	35×15	5 (3,2,0,0)	1.0	3

** $\bar{x}=3.06$, $\bar{y}=2.40$, $t=0.8186$, $0.5>Pr(t)>0.4$, d.f.$=12$
Difference is not significant.

TABLE 3 Comparison of banana leaf and filter paper as attractants of *O. quadrasi*. Attractants were submerged for 1 day in South Main Canal at Cogon, Palo in April 1974.

Attractant size in cm.	No. of *O. quadrasi*** obtained per sample	No. of *O. quadrasi* per 100 cm²	Other snails obtained
Filter paper 30×15 (x)	67 (3,25,24,15)	14.9	—
	64 (7,14,23,20)	14.2	—
	51 (10,20,10,11)	11.3	—
	56 (8,23,22,3)	12.4	—
	65 (7,16,24,18)	14.4	*Gyraulus* sp. 1
	90 (8,19,34,29)	20.0	—
Banana leaf 30×15 (y)	46 (7,12,14,13)	10.2	—
	47 (4,10,11,22)	10.4	*Vivipara* sp. 1
	108 (14,41,27,26)	24.0	*Gyraulus* sp. 1
	30 (3,8,12,7)	6.7	*Vivipara* sp. 1
	75 (5,6,16,34)	16.7	*Gyraulus* sp. 1
	45 (7,20,15,3)	10.0	—

** $\bar{x}=65.5$, $\bar{y}=58.5$, $t=0.5471$, $0.6>Pr(t)>0.5$, $d.f.=10$
Difference is not significant.

respectively, but the difference was not significant. Less snails of other species were also found on the filter paper.

The collected *O. quadrasi* were classified into 4 groups according to the shell length; large, moderate, small and tiny (TABLE 4). The proportions of the different sizes to the total number of snails were not so different on both the filter paper and on the banana leaf.

TABLE 4 Comparison of distributions of shell length of *O. quadrasi* collected by banana leaf and filter paper

Attractant	Total no. of snails	Distribution of size (%) large 4.0– above	moderate 3.0– 3.9mm	small 2.0– 2.9mm	tiny 1.0– 1.9 mm
Filter paper	791	21.7	37.2	25.8	15.3
Banana leaf	569	34.3	24.3	21.4	20.0

2. Correlation of snail counts by the filter paper and the tube samplers.

Since the tube sampling method is known to be very reliable in estimating the snail population density, the number of snails collected by filter paper was compared to that collected by tube sampling. For this com-

parative studies, 30 samples were taken by both methods at about 1 m distance between sampling sites.

The correlation of snail counts by both methods is shown in Fig. 1. A loose correlation was observed between snail counts of both methods as the coefficient was very small, *i.e.*, $r = 0.3830$.

Comparative studies were made 3 times at Tibak depression taking 30 samples by both methods each time. No correlation was found in one of the 3 experiments as shown in Fig. 2 where the variation of snail count per tube was limited while that by filter paper was large. The correlation existed in the other 2 experiments and the coefficients were 0.5976 and 0.4842, respectively (Figs. 3 and 4).

The ratio of the number of snails by tube

Fig. 1. Correlation of the number of snails collected by tube and filter paper methods in Cogon-Anahaway rice field beside the irrigation lateral canal A-O in May 1974.

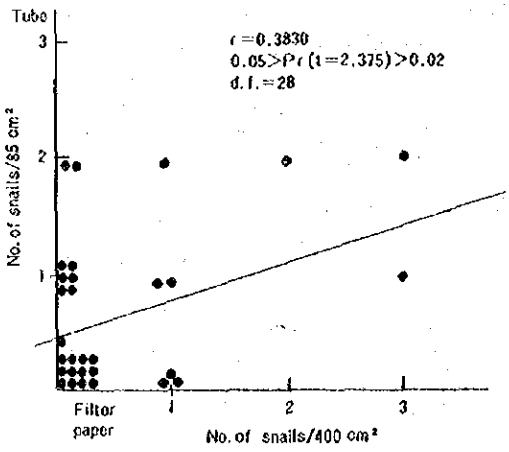


Fig. 2. Correlation of the number of snails collected by tube and filter paper methods at Tibak depression (no. 1) in June 1974.

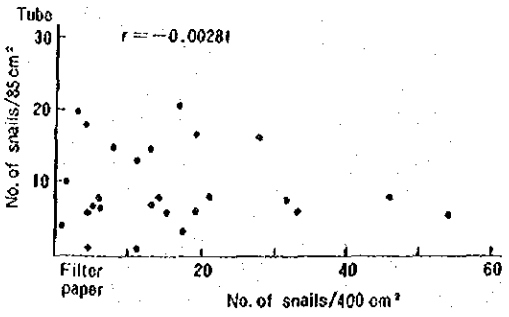


Fig. 3. Correlation of the number of snails collected by tube and filter paper methods at Tibak depression (no. 2) in June 1974.

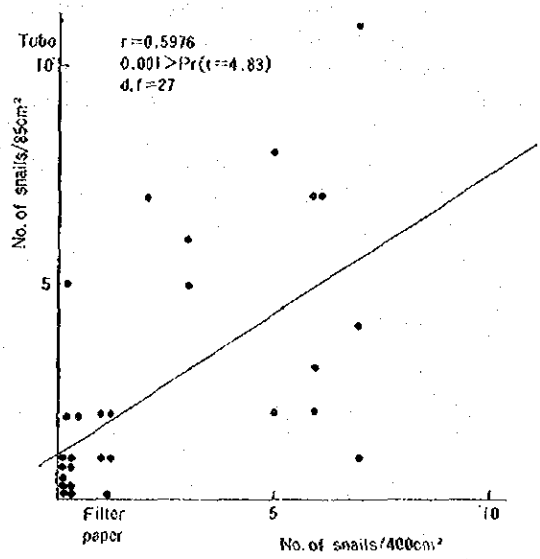
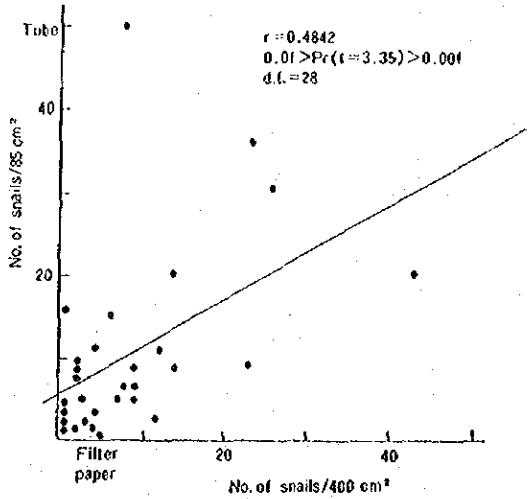


Fig. 4. Correlation of the number of snails collected by tube and filter paper methods at Tibak depression (no. 3) in June 1974.



method to that by filter paper; the correlation coefficient and the formula for the regression line were calculated and are shown in TABLE 5. Because these values differed widely in the three experiments, these were consolidated as a total of 3 experiments which showed the presence of correlation between the tube and the filter paper methods.

Using the ratio of snail counts between the two methods, the number of snails in soil at an area of 85 cm² collected by the tube method can be approximately estimated by multiplying 1.18 with the number of snails collected on a sheet of filter paper (20×20 cm). A more precise relation between the number of snails at 85 cm² area (Y) and that on a sheet of filter paper (X) can be obtained by the following two formulas:

$$Y = 0.77\,X + 1.57$$
$$X = 0.55\,Y + 1.35$$

3. Distribution of snail sizes by both sampling methods.

It was shown that more young snails could be collected by the tube method than by the ring method [6]. The size of all snails collected by both methods in the present study was measured in both sexes and the frequency distribution by snail size was made (TABLE 6). The size distribution thus observed did not differ much between the two sampling methods. The results showed that the snails attracted to the filter paper have nearly the same age composition as in the natural environment which was well observed by the tube sampling method [6].

4. Type of snail distribution on the soil.

From the snail sampling data, the type of distribution of snails on the soil was examined. The distribution at each collection by both

TABLE 5 Correlation of the number of snails collected by "tube method" and "filter paper" method.

Exp. No.	Place	Tube (Y)* / Filter paper (x)	Correlation coefficient (r)	t value	d.f.	Probability	Formula of regression line
1	Ricefield along canal	1.3571	0.3830	2.375	28	0.05>	Y=0.31 x+0.49
2	Tibak depression 1	0.6064	−0.0028	0.014	25	>0.9	—
3	〃 2	1.0548	0.5976	4.830	27	0.001>	Y=0.65 x+1.03
4	〃 3	1.2047	0.4842	3.347	28	0.01>	Y=0.57 x+5.36
Total	1)+3)+4)	1.1789	0.6502	10.506	87	0.001>	Y=0.77 x+1.57

* Ratio of the number of snails by tube method (Y) to that by filter paper method (x).
** Area of cross-section of a tube sampler 85 cm². Area of filter paper 20 cm×20 cm=400 cm².

TABLE 6 Distribution of shell length of *O. quadrasi* in both sexes obtained by filter paper and tube method.

Shell length (mm)	No. of snails				% total			
	Male		Female		Male		Female	
	FP	T	FP	T	FP	T	FP	T
1.0—	0	1	11	16	0.0	0.8	5.2	6.0
1.5—	5	5	31	55	4.4	3.9	14.6	20.5
2.0—	21	29	18	36	18.6	22.8	8.5	13.4
2.5—	7	5	6	8	6.2	3.9	2.8	3.0
3.0—	13	15	5	9	11.5	11.8	2.4	3.4
3.5—	45	50	13	16	39.8	39.4	6.1	6.0
4.0—	22	22	37	49	19.5	17.3	17.5	18.3
4.5—			63	53			29.7	19.8
5.0—			27	26			12.7	9.7
5.5—			1	0			0.5	
Total	113	127	212	268	100	100	100	100

* FP: Filter paper method. ** T: Tube method.

sampling methods was analysed separately. At first, the frequency distribution on the snail count per sample (x) was made and the cumulative percentage of frequency (y) was calculated. The results were plotted on the statistical paper to know whether the cumulative percentages on x line up on a straight line. Since they did not make a straight line at any collection data, the type of distribution was found to be other than the normal distribution. The cumulative percentages were then plotted on a log statistical paper on log (1 + x). In all cases, the plotted percentages lined up approximately straight so that the distribution was presumed to be either Poisson's type or the negative binominal type [1, 2].

Following the results in the report by Pesigan *et al.* [6], the snail sampling data in the present study were tried to fit the negative binomial distribution, *i.e.*, expansion of $(q - p)^{-k}$. In the present study the k value was obtained by the equation $k = \bar{x}^2/(s^2 - \bar{x})$ at first and when k value thus obtained was smaller than 1.0 or if the obtained datum did not fit the negative binomial, the k value was corrected to satisfy the following equation by trial and error method:

$f_0/\Sigma f = (1 + \bar{x}/k)^{-k}$ where $f_0/\Sigma f$ is the proportion of 0 frequency to the total number of samples and $\bar{x}$ is an arithmetic mean of the snail count per sample [2–4].

The calculation was carried out without difficulty by the use of a table calculator (Hewlett-Packard Model 35).

The result of the snail collection in a ricefield and goodness of fit to the negative binomial are shown in TABLE 7 as well as mean

TABLE 7 Distribution of snails in a ricefield by the lateral A-O canal at Anahaway observed by filter paper and tube sampling methods.

No. of snails per sample	Method of sampling			
	Filter paper		Tube	
	Fo*	Fnb**	Fo	Fnb
0	21	21.00	16	16.44
1	6	5.85	9	9.40
2	1	1.99	5	3.14
3	2	1.16	0	1.02
Total	30	30.00	30	30.00
$\bar{x}$	0.4667		0.6333	
s^2	0.7156		0.5656	
k	0.8750		5.9186	
corrected k	0.6923		/	
p	0.6741		0.1070	
χ^2	1.105		2.151	
Pr.	0.3–0.2		0.2–0.1	

* Fo; Frequency observed.
** Fnb; Frequency calculated by negative binomial distribution.

TABLE 8 Distribution of snails at Tibak depression (no. 1) observed by filter paper and tube sampling methods.

No. of snails per sample	Method of sampling					
	Filter paper			Tube		
	Fo		Fnb	Fo		Fnb
0	1			1		
1	1			2		
2	0			0		
3	1			1		
4	3			1	5	6.50
5	1	7	6.14	0		
6	2			5		
7	0			6	11	6.80
8	1			4		
9	0			0		
10	0			1		
11	2	5	6.34	0	5	7.58
12	0			0		
13	2			1		
14	1			0		
15	1			2		
16	0			0		
17	2			2		
18	2			1		
19	1	9	5.94	0		
20	0			1		
21	0			1		
22	0			0		
23	0			1	9	9.12
28	1					
32	1					
33	1					
46	1					
54	1	5	7.58			
Total	26		26.00	30		30.00
$\bar{x}$	15.5385			9.4000		
s^2	177.4024			36.5733		
k	1.4917			3.2517		
p	10.4166			3.8908		
χ^2	2.86			3.82		
Pr.	0.1–0.05			0.1–0.05		

TABLE 9 Distribution of snails at Tibak depression (no. 2) observed by filter paper and tube sampling methods.

No. of snails per sample	Method of sampling: Filter paper Fo	Filter paper Fnb	Tube Fo	Tube Fnb
0	11	11.00	7	7.64
1	5	5.17	7	5.82
2	1		6	
3	2		1	7.42
4	0		1	
5	3	8.57	1	
6	4		1	
7	3	4.26	3	
8			1	
9			0	
10			0	
11			1	8.12
Total	29	29.00	29	29.00
$\bar{x}$	2.5172		2.6552	
s^2	7.2152		9.2604	
k	1.3487		1.0673	
corrected k	0.5775		/	
p	4.3588		2.4878	
χ^2	2.54		0.32	
Pr.	0.2–0.1		0.7–0.5	

TABLE 10 Distribution of snails at Tibak depression (no. 3) observed by filter paper and tube sampling method.

No. of snails per sample	Method of sampling: Filter paper Fo	Filter paper Fnb	Tube Fo	Tube Fnb
0	5		1	
1	0		3	
2	4	10.72	2	7.87
3	2		3	
4	3		1	
5	1		3	
6	1	6.77	2	
7	1		1	8.25
8	2		2	
9	3		3	
11	0		2	
12	2	4.83	0	
14	2		0	
15	0		1	5.82
16	0		1	
20	0		2	
23	2		0	
26	1		0	
30	0		1	
36	0		1	
43	1		0	
50	0	7.68	1	8.06
Total	30	30.00	30	30.00
$\bar{x}$	8.4667		10.2000	
s^2	89.1822		123.9600	
k	0.8881		0.9146	
corrected k	0.6946		/	
p	12.1893		11.1524	
χ^2	2.73		2.16	
Pr.	0.1–0.05		0.2–0.1	

($\bar{x}$), variance (s^2), constant k, value of χ^2 test and its probability of the reliability (Pr.). Data from both filter paper and tube samplings fitted well the negative binomial distribution. The big difference between both methods was that k value was much larger by the tube than by the filter paper. This means that the uneven distribution of snails in the natural environment became more exaggerated by the filter paper sampling.

Other data of snail distribution are the observation at the bank of a narrow stream at Tibak depression as shown in TABLES 8, 9 and 10. The distribution of snails in these experiments fitted well to the negative binomial distribution by both sampling methods. The constant k values varied largely among the different snail collections. This finding disagrees with the result reported by Pesigan *et al.* [6] who regarded it to be always about 1. The constant k was always smaller by filter paper than by the tube method and such large values as 5.9 and 3.3 were observed by tube sampling (TABLES 7 and 8). It can be said that snails sometimes distribute closely following the Poisson's type which is one of the uniform distribution in the natural environment since the negative binominal distribution approaches the Poisson's when the k value becomes larger than 4 and can be regarded as Poisson's itself when k is over 8.

The type of snail distribution thus obtained can be regarded as uneven and fitted the negative binomial distribution, although the degree of clustering varies much.

DISCUSSION

For collecting a large number of *O. quadrasi* snails from wet soil surface or narrow ditches,

fallen dried banana leaves have been utilized as a routine method in the Schistosomiasis Control Pilot Project in the Philippines. In the present study, filter paper was examined whether it can be used as a tool of quantitative collection of snails. By the comparative study, it was shown that the filter paper attracted *O. quadrasi* as well as the fallen banana leaf and collected less number of other species of snails than the banana leaf. In the population study of *Oncomelania* snails, tube sampling method is considered to be best. Especially on the submerged soil surface, this must be the only method. However, only 30 samples can be collected at most in a day by the difficulty of isolating snails from collected soil samples. Since the procedure of the filter paper sampling is simple, more than 100 samples could be readily taken in a day.

As it was found that snail counts in a piece of filter paper loosely correlated to those by a tube sampling method. The age distribution of collected snails by filter paper method was nearly the same as that by tube sampling. By the above advantages, the filter paper sampling is considered to be useful for the quantitative ecological study of *O. quadrasi* on the submerged soil surface, for instance, on the wet bank and in the rice field and possibly in waters. Mori *et al.* [5] observed the population density of *O. nosophora* by filter paper in rice fields and ditches at Kofu district Japan, found that snails were more populated around the entrance of irrigation water in the rice field and considered that snails might be carried by irrigation water.

It can be said generally that *O. quadrasi* distributes unevenly in the field following negative binomial distribution with varied degree of clustering. Snail distribution, however, was sometimes found to be close to a uniform distribution in the field. This type of distribution was observed by the tube sampling but not by the filter paper method. The filter paper sampling method must be used for a field survey in consideration of that uneven distribution of snails are always exaggerated by this method.

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Migration of *Oncomelania quadrasi* Observed by the Mark and Release Method in a Wet Bank of a Stream in Leyte, Philippines[1)]

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Summary: The migration and population size of *Oncomelania quadrasi* were studied at two sites in a submerged bank of a stream in Leyte Philippines. The snails collected by filter paper in an experimental area were marked with manicure and released in the same area. Collection was repeated every 4 days in the same area for 5 more times and snails without mark were marked with manicure of different colors at each time. The immigration and emigration rates and population were calculated from the collection data. One experimental site with an area of 5.86 m^2 was established at the edge of the water pathway, 3 sides of which were surrounded by wooden planks except that side facing the water pathway to determine the migration between the bank and water pathway. The other experimental site was 7.54 m^2 in area, located in a submerged place close to high land and was left open in all sides. The ranges of the population, immigration and emigration rates during a 4 day period were 3845 to 7638, 0 to 0.4448 and 0.2263 to 0.8911, respectively in the former site. The results in the latter area were 1336 to 2468, 0.1261 to 0.6725 and 0.1349 to 1.2340, respectively. It was observed that the replacement of individual snails in a habitat was largely due to migration which in turn was influenced much by the change of water level in the bank. The emigration increased with the elevation of water level. On the other hand, the immigration increased with the lowering of water.

Introduction

For the purpose of controlling *Schistosoma japonicum* in the Philippines, the ecology of its intermediate host, *Oncomelania quadrasi*, was extensively studied by Pesigan *et al.* [5]. By their study, the population of *Oncomelania quadrasi* was found to be comparatively stable in each habitat. The change of population was interpreted by the change of reproduction and mortality rates and of life span in their work. In the present study, migration of snails was studied using the mark and release method to determine whether it plays a certain role in the change of population on a wet soil surface along a water pathway.

Methods

The migration of snails was observed in a wet bank along a narrow stream at Tibak depression in Sta Fe, Leyte Philippines. The stream is about 1 meter wide with low banks spreading at both sides. The bank was wet in most portions and the level of water on the soil surface fluctuated much daily according to the amount of rain-fall. The population of snails was observed at two sites in the bank as shown in Fig. 1.

In the present study, both immigration and emigration rates, and the population size were

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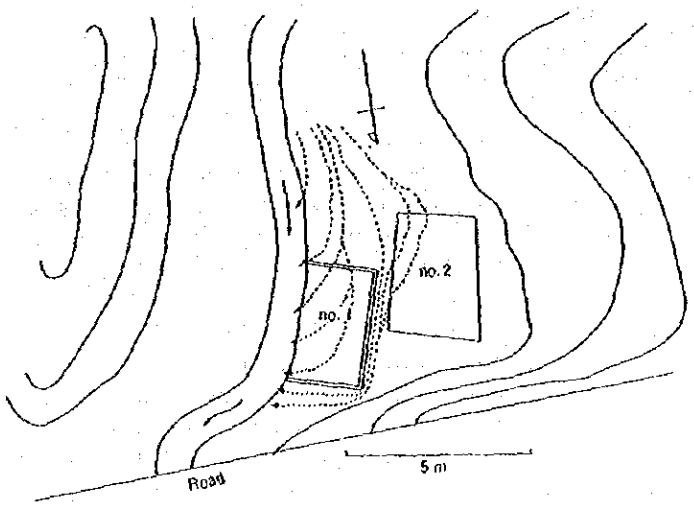

Fig. 1. Topography of the experimental area at Tibak depression in Sta Fe, Leyte Philippines.

observed by the method of mark, release and recapture. Snails were collected by the filter paper sampling method [6] and collection was repeated every 4 days for 6 times in a period of 22 days. At each collection 30 sheets of filter paper 20×20 cm in size were placed in an experimental area and were recovered after 24 hours. Snails found on the filter paper were isolated by running tap water and collected in a vessel. After the snail count was recorded, snails smaller than 1.9 mm in shell length were excluded. The rest of the snails were marked with red manicure at the initial collection (collection 0) and were released into the same experimental area. At collection 1, 4 days after collection 0, unmarked snails larger than 1.9 mm were marked with white manicure and released. The collection was repeated 4 more times changing the color marking each time. The colors of manicure used for marking were red, white, pink, orange and violet successively. The violet manicure was replaced by green at the 2nd experiment due to the difficulty of noticing the violet mark.

The immigration and emigration rates and the population were calculated by the method of Leslie [2]. The calculation for estimating the above values are as follows and the coding used in these calculations are shown in TABLE 1.

TABLE 1 Coding of collection of snails by mark and release method and estimation of population, immigration and emigration rates.

Time of collection		t	0	1	2
Mark	red	0		k_{01}	k_{02}
	white	1			k_{12}
Number of snails with mark		S_t	S_0	S_1	S_2
Number of snails without mark		B_t	B_0	B_1	B_2
Number of snails collected (excluding tiny snails)		C_t	C_0	C_1	C_2
Number of tiny snails		T_t	T_0	T_1	T_2
Remaining rate		P_t	P_0		
Increasing rate		Z_t		Z_1	
Immigration rate		Q_t	Q_0		
Emigration rate		B_t		B_1	
Population (less tiny snails)		N_t		N_1	

For obtaining one set of the population, immigration and emigration rates, 3 consecutive collections are necessary. As shown in TABLE 1, using the data of collections 0, 1 and 2, the immigration rate at the time of collection 0 (Q_0), the emigration rate (B_1) and the population (N_1) at the time of collection 1 can be calculated.

The population at the next collection (N_1) is obtained by the product of the present population (N_0) multiplied by the remaining rate (P_0) and the increasing rate (Z_0), *i.e.*, $N_1=N_0P_0Z_0$. The emigration rate B_t is defined as the increasing rate Z_t minus 1 and the

immigration rate Q_t is 1 minus the remaining rate P_t. The codings of k_{ij} in TABLE 1 are the number of snails captured and marked at collection i and recaptured at collection j. Calculations of the population and emigration rate at collection 1 and the immigration rate at collection 0 are available from the 3 collections in TABLE 1 by the following formulas:

$$N_1=\frac{k_{02}C_1R_1}{k_{01}k_{12}} \quad P_0=\frac{k_{02}R_2}{k_{12}R_0} \quad Z_1=\frac{k_{01}C_2}{k_{02}C_1}$$

$$Q_0=1-P_0 \qquad B_1=Z_1-1$$

The daily rates of immigration and emigration are as follows:

$$DQ_t=1-(P_t)^{\frac{1}{d}} \qquad DB_t=(Z_t)^{\frac{1}{d}}-1$$

where d is the number of days between two collections. In this calculation the reproduction rate is included in the emigration rate and the mortality rate is in the immigration rate. Q_t and B_t are the rates during a period from the collection t to the next collection. An example of the calculation is shown in TABLE 1 using collections 0, 1 and 2. The same calculations can be performed using any consecutive 3 collections successively.

RESULTS

The first experiment was conducted along the bank close to the edge of a stream in a site with an area of 5.86 m² as shown in Fig. 1. The whole area was submerged during the observation period. Movement of water was always slow in this area and the water level fluctuated much day by day. Three sides of this area were surrounded by narrow wooden planks and one side facing the stream was left open for the purpose of determining the migration of snails between the bank and water pathway. The observed immigration rates in a 4 day period were 0 to 0.448 while the emigration rates were from 0.2263 to 0.8911. The population was observed to change between 3845 and 7638. It was shown that changes of both immigration and emigration rates were large as well as the fluctuation of the population (TABLE 2).

In the second experiment, a site with an area of 7.54 m² was prepared in the same bank, but close to the high land (Fig. 1). The area was not surrounded by wooden

TABLE 2 Results of snail collections by the mark and release method and calculation of population, immigration and emigration rates in experimental area no. 1 at Tibak depression from Aug. 26 to Sep. 16, 1974.

Time of collection t		0	1	2	3	4	5
Mark red released at	0	/	13	8	9	7	3
white " "	1	/	/	8	7	14	0
pink " "	2	/	/	/	11	11	20
orange " "	3	/	/	/	/	15	12
violet " "	4	/	/	/	/	/	6
Number of snails with mark		0	13	16	27	47	41
Number of snails without mark		295	243	212	233	217	218
Number of snails collected (excluding tiny snails)		295	256	228	260	264	259
Number of tiny snails		58	32	92	64	50	41
Remaining rate		0.8237	0.5552	0.8060	1.8626	/	/
Increasing rate		/	1.4473	1.3033	1.0153 (1.8911)	1.2263	/
Immigration rate		0.1763	0.4448	0.1940	−0.8626 (0)	/	/
Emigration rate		/	0.4473	0.3033	0.0153 (0.8911)	0.2263	/
Daily immigration rate		0.0473	0.1368	0.0525	(0)	/	/
Daily emigration rate		/	0.0968	0.0685	(0.1727)	0.0523	/
Population (less tiny snails)		/	4785	3845	4039	7638	/
Corrected population (including tiny snails)		/	5383	5396	5033	9085	/

TABLE 3 Results of snail collections by the mark and release method and calculation of population, immigration and emigration rates in experimental area no. 2 at Tibak depression from Sep. 19 to Oct. 10, 1974.

Time of collection t		0	1	2	3	4	5
Mark red released at	0	/	5	4	1	0	2
white " "	1	/	/	11	5	1	1
pink " "	2	/	/	/	11	6	4
orange " "	3	/	/	/	/	6	5
green " "	4	/	/	/	/	/	4
Number of snails with mark		0	5	15	17	13	16
Number of snails without mark		151	136	237	113	79	146
Number of snails collected (excluding tiny snails)		151	141	252	130	92	162
Number of tiny snails		99	95	101	51	41	31
Remaining rate		0.3275	0.7921	0.4768	0.8739	/	/
Increasing rate		/	2.2340	1.1349	1.2974	2.1130	/
Immigration rate		0.6725	0.2079	0.5232	0.1261	/	/
Emigration rate		/	1.2340	0.1349	0.2974	1.1130	/
Daily immigration rate		0.2435	0.0566	0.1690	0.0331	/	/
Daily emigration rate		/	0.2226	0.0321	0.0673	0.2057	/
Population (less tiny snails)		/	1395	2468	1336	1514	/
Corrected population (including tiny snails)		/	2137	3457	1860	2189	/

Interval of collections in 4 days.
Experimental area; 7.54 m².

planks this time to allow the snails to migrate in any direction. The population fluctuated between 1336 and 2468, the immigration rates in a 4 day period changed from 0.1261 to 0.6725 and the emigration rate varied from 0.1349 to 1.2340. The change of immigration and emigration rates were large compared with that of the population (TABLE 3).

In the first experiment, the population increased at the time of collection 4. The water level was observed to rise after collection 3 and a sudden increase of the emigration rate (0.8911) was also observed. The same change was found at collection 1 in the second experiment. After collection 1, when the water level rose, the emigration rate increased enormously to 1.2340 and the population increased at collection 2.

In these experiments, it was found that population change is influenced much by the fluctuation of water level in the wet bank. A more remarkable fact observed was that snails in an area were always being replaced rapidly by other population from adjacent areas. The emigration of snails increased much by the elevation of water level and the immigration increased by the lowering of the water.

DISCUSSION

In the present study, the mortality rate of the snails is included in the immigration rate. To know the actual immigration rate, the mortality should be subtracted from the calculated immigration rate. By a different method, the mortality rate of adult *Oncomelania quadrasi* observed by Pesigan *et al.* [5] ranged from 0 to 0.00193 daily with an average of 0.0090. Since the mortality rate is small compared with the daily immigration rates, the obtained immigration rates could be regarded nearly as the actual immigration.

In the emigration rate, the reproduction rate is involved. The reproduction rate studied by Pesigan *et al.* [5] ranged from 0.0001 to 0.0095 per female with an average of 0.002361. The number of reproduced youngs in the present study may be at most 10 per 1000 female per day. This rate of reproduction is considered to be negligible in the

present study compared with large values of the emigration rate. The emigration rates observed could be regarded as the actual proportion of the emigrating snails.

Observing the successive change of the rates of migration in connection with the fluctuation of water level in the bank, it can be said that snails living in the wet bank migrate to the high land with the rise of water level and the snails in the submerged bank migrate towards the water pathway with the lowering of the flood water. The migration between the bank and water pathway may cause the dispersal of snail colony through the water pathway. The shift of population of *O. nosophora* by the change of water level was also observed by Nakao [4] in the bank of water logged area in Japan.

The dispersal of *O. nosophora* was also examined by Mori *et al.* [3] by observing the distribution of *Oncomelania* in the rice field and in water in the irrigation canal. They considered that snails might be carried by the running water through an irrigation canal and snail population might be accumulated at the inlet of irrigation water in a rice field. Dense population of *O. nosophora* was also observed by Ito [1] at the same portion in the rice field.

The stability of a snail colony may be affected much by the varied environments of the soil surface. The snail colony was reported to be comparatively stable by Pesigan *et al.* [5] and less possibility of migration or dispersal was observed by Ito [1] in the snail colony in the rice field in Japan.

The migration and the resultant dispersal of *Oncomelania* should not be ignored in the population study when the snail habitat is connected with the water pathway. Migration may play an important role in the repopulation of snails after controling snails by either molluscide or engineering methods.

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POPULATION RESEARCH ON *ONCOMELANIA QUADRASI* IN LEYTE, PHILIPPINES

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For the evaluation of snail control measures, determination of the population size of snails before and after control is important. In this connection, Pesigan *et al.*, (1958) studied the ecology of *Oncomelania quadrasi* in the field and under experimental conditions. However, difficulty still exists in estimating a population size properly by a simple, practical and satisfactory method. As an approach to solve this difficulty, the ecology of *O. quadrasi* was studied as a part of the Philippine-Japan cooperative project. The present paper deals with a few aspects of population research on *O. quadrasi*, such as the determination of type of snail distribution, establishment of a snail sampling method by filter paper, observation of snail distribution in and around waters and migration of snails on the wet bank.

Type of snail distribution in the field

For the determination of the population density of snails in the field, sampling has been carried out using either a metal ring or a metal tube 13.5 cm in diameter in the Philippines. The type of snail distribution was analysed from the collection data by the ring method.

At Cada-atan, Matagob in Leyte, snails were collected by using the ring at 5 m intervals along the edge of a water-logged area in a rice field. The area of the depression was about 3 ha, total length of the edge was about 750 m and the number of ring samples was 150. The frequency distribution of samples in relation to the number of snails per ring did not fit a uniform type of distribution; normal or Poisson's distribution. The distribution showed a good fit to the negative binomial distribution, a type of uneven distribution, of which estimated frequencies can be obtained by the expansion of $(q-p)^{-k}$. The negative binomial distribution can be represented by two parameters; one is the mean number of snails per ring sample ($\bar{x}$) and the other is a constant k in the above formula. The value of k becomes smaller when snails cluster more. At Cada-atan, $\bar{x}$ and k were 2.18 and 0.5142, respectively.

A sampling datum along one side of the bank along 2.7 km of Caibaan stream showed 3 habitats of snails, i.e., at a distance of 400 m upstream, 330 m in the middle portion and 400 m downstream. The snail distribution fitted the negative binomial up- and downstream. The values of x and k were 1.37 and 0.5867 upstream and 0.56 and 0.2857 downstream, respectively. A snail colony in the middle portion did not fit any type of distribution and was considered to be a mixture of colonies.

In 4 sampling data at Tibak depression using the tube method, the distribution also fitted well to the negative binomial. The values of $\bar{x}$ and k were 0.63/tube and 5.9186, 9.4 and 3.2517, 2.66 and 1.0673, and 10.2 and 0.9146 in 4 collections, respectively. The degree of clustering of snails varied greatly as was shown by the wide range of k values, from 0.29 to 5.92 at different habitats.

The efficiency of the ring sampling method was examined under experimental conditions. A known number of snails was released in an area of 4 m^2 surrounded by wooden planks.

Snails were collected by 36 samples 3 days after release. The ratios of the number of snails released to the number estimated by ring sampling were 1.31, 1.29, 1.90, 2.30 and 2.42 in 5 experiments. In field collections, this ratio or the correction factor can be regarded as about 2.

Sampling method by filter paper

Filter paper was tried as a simple sampling method for *O. quadrasi* on wet soil and in water. Filter paper was found to attract *O. quadrasi* in water the same as fallen banana leaves, although fewer other species of snails were collected on filter paper than on dried banana leaves. Snails were collected in limited areas using a tube (85 cm^2 area at cross-section) and filter paper (20 × 20 cm) samples. The sheet of filter paper was placed close to the spot where a tube sample was taken, and recovered after 24 hours. At each location, 30 samples were taken by each method in an area and sampling was done four times. The correction of the number of snails collected by the tube and that by the filter paper was studied. The ratio of the snail counts by the tube sampler to those by the filter paper sampler was 1.18. A loose correlation was observed between snail counts of both method as shown by the correlation coefficient, $r = 0.6502$. The formulas for the regression line were $Y = 0.77 X + 1.6$ and $X = 0.55 Y + 1.35$ for the 3 experiments where Y is the number of snails collected by tube sampling and X is the number of snails collected on the sheet of filter paper.

The type of snail distribution was studied in the 30 samples taken by each method. All sampling data were found to fit the negative binomial distribution. In each experiment, the constant k was always larger in the tube sample than in the filter paper sample. This indicated that the uneven distribution of snails on the soil surface was emphasized by the filter paper sampling.

Distribution of snails in water

Snail distribution in water was observed by filter paper sampling. For snail collections, 20 cm × 20 cm filter paper was used at the edge of the water and the same size of filter paper, folded between two sheets of wire mesh, was placed at the bottom of the water or on the thick, floating vegetation in the water. Bamboo poles, 7 cm in diameter, wrapped with filter paper were stuck into the bottom to determine the vertical distribution of snails. The submerged filter paper was recovered after 24 hours. In observations at 3 water pathways, snails were abundant at the edge of water in the bank, small in number at the upper portion in vegetation near the bank and rarely found in the moving water.

In a shallow artificial pond, 8 m by 28 m and 20 cm deep where aquatic vegetation was sparse, snails were collected only at the edge of water on the gently sloping bank and none was in water or at the bottom of the water.

In a part of a huge water-logged area close to the bank, 10 m by 28 m and no deeper than 70 cm, where no vegetation existed, the snail distribution was the same as in the above artificial pond. In the remainder of this water-logged area, no deeper than 50 cm, where thick floating vegetation occupied the water from the surface to the bottom, snails were detected visually, by bamboo poles and filter paper in wire mesh, at a distance from the bank of 50 m. Snails were found only on the surface of vegetation at all observing spots. The snail density, however, was lower on the vegetation than in the sloping bank.

Migration of *O. quadrasi* on the wet bank

The migration and population size of *O. quadrasi* were studied at two sites on a submerged bank of a stream at Tibak depression in Leyte. Snails collected by filter paper in an experimental area were marked with fingernail lacquer and released in the same area.

Collection was repeated every 4 days in the same area 5 more times and each time snails without marks were marked with fingernail lacquer of different colors. The immigration and emigration rates and population were calculated from the collection data. One experimental site with an area of 5.86 m^2 was established at the edge of the water pathway; 3 sides were surrounded by wooden planks but the side facing the water pathway was left open to determine the migration between the bank and water pathway. The other experimental site was 7.54 m^2 in area, located in a submerged place close to a highland and was left open in all sides. The population and the immigration and emigration rates during a 4-day period ranged from 3,845 to 7,638; 0 to 0.4448 and 0.2263 to 0.8911, respectively, in the first site. The results in the second area were 1,336 to 2,468, 0.1261 to 0.6725 and 0.1349 to 1.2340, respectively. It was observed that the replacement of individual snails in a habitat was largely due to migration which in turn was influenced much by the change of water level in the bank. Emigration increased with the elevation of the water level and immigration increased with the lowering of the water.

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Distribution of Oncomelania quadrasi in waters in the Philippines

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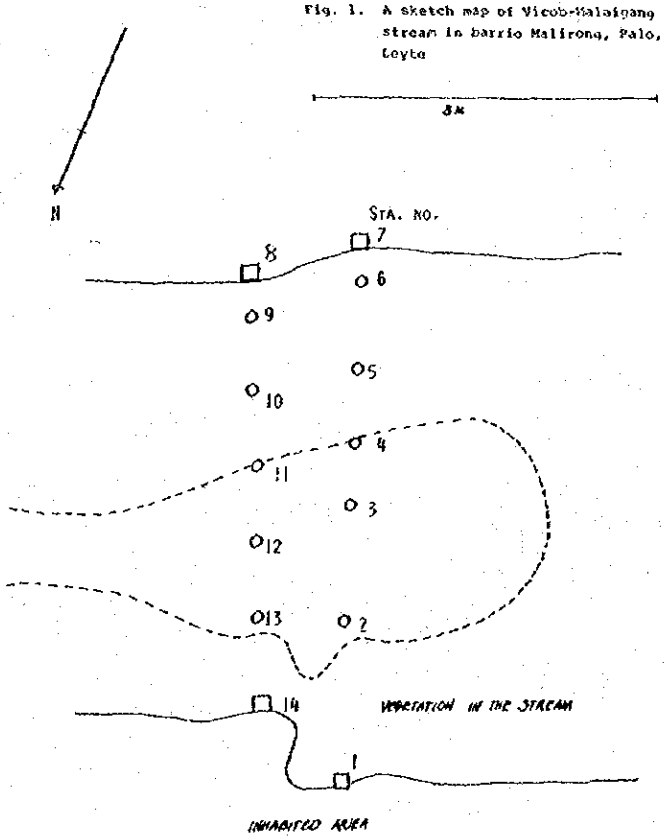
The horizontal and vertical distribution of Oncomelania quadrasi, the intermediate snail host of Schistosoma japonicum, was studied in both standing and running water habitats using filter paper sampling method. Filter paper with a size of 20 x 20 cm was used in the most collection sites. The filter paper inserted in wire mesh folder was placed at the bottom of the water and the bamboo poles of 7 cm diameter covered with filter paper were stood at the bottom to examine the horizontal and vertical distribution of snails in water, respectively. In the studies made in the three streams surveyed, that is, in Vicob-Malaigang Stream, Juber Creek and Gacao South Main Canal, snail density was found to be highest at the margin of the water in the sloping bank and in the near-by wet bank. The density of snails became lesser at the higher portion of the moist bank. Snails were also found in the water plants growing from the bank and shallow places close to the bank. In the running water habitat only a few snails were noted on the surface or at the bottom of water. In a shallow Gacao Pond with few standing vegetation and where water is 20 cm at its deepest, no snails were noted although abundant in the sloping bank and a few in the steep bank. In a portion of the huge water-logged area in Sab-a Basin, it was noted that snails were many in a near-by stream, some in boggy area and only a few at a deep watery portion where only standing vegetation was dense. In a huge water-logger area in Tabontabon, snails were densely accumulated along the moist banks, less only on the surface of accumulated floating vegetation and none below the surface of the floating vegetation and decreased by the distance from the bank

following a regression line between log density and the distance. The density in the moist bank reduced to 1/10 at a distance of 50 m from the bank. It can be generally concluded that the snail density in waters cannot be regarded as the same as in the bank or that the population is rather negligible in streams, in ponds and in the open watery portion with standing vegetation only in the water-logged area except on the surface of the accumulated floating vegetation where the snail density decreased enormously by the distance from the bank. From this result, O. quadrasi is determined to be not so aquatic in habitual character than was presumed before, although it must be more aquatic than O. nosophora in Japan.

This is only the summary of a paper to be published in Japanese Journal of Experimental Medicine 48 (1978). Figures and tables in this paper are reproduced here to inform more details.

Fig. 1. A sketch map of Vicob-Malaigang stream in barrio Malirong, Palo, Leyte



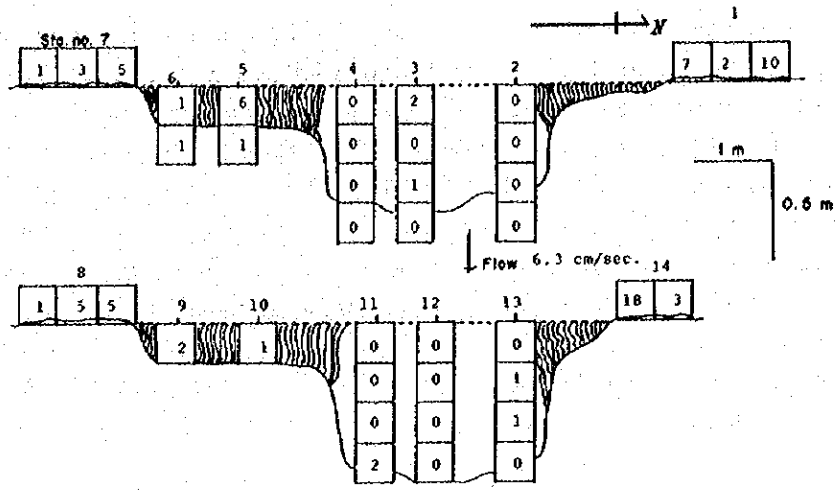
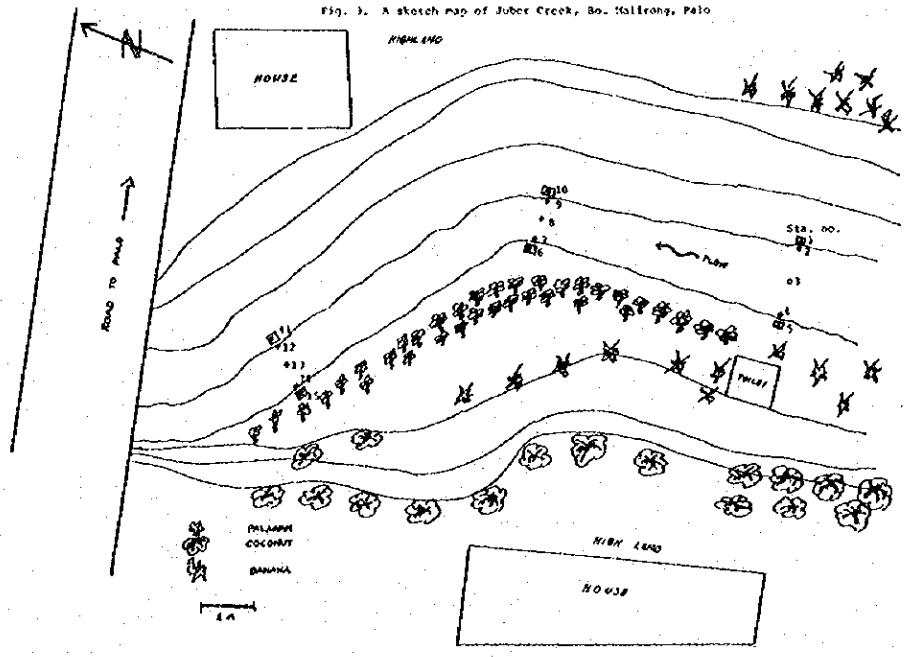


Fig. 2. Distribution of Q. quadrasi observed by filter paper collection at cross-section of Vicob-Malaigang Stream, barrio Malirong, Palo, Leyte in July 1974



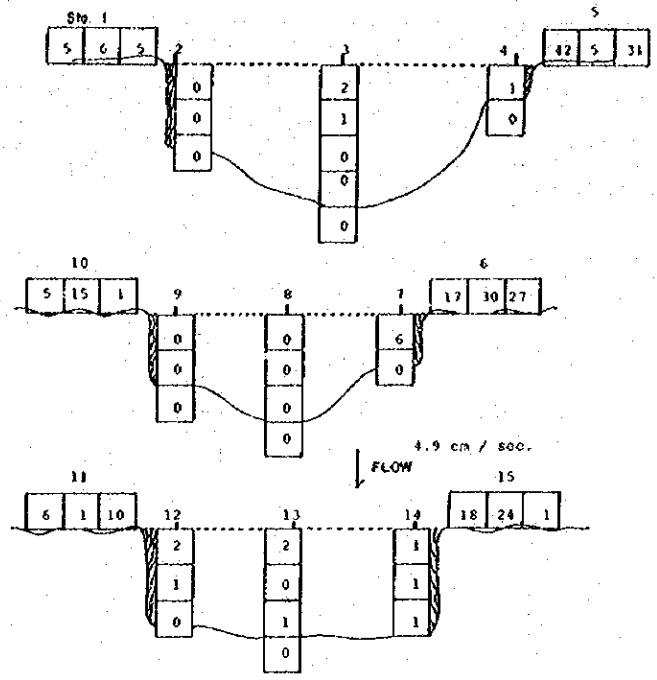


Fig. 4. Cross-section of Juber Creek and the number of snails collected by filter paper. July 1974

Fig. 5. A sketch map of South Main Canal in Gacao, Palo, Leyte

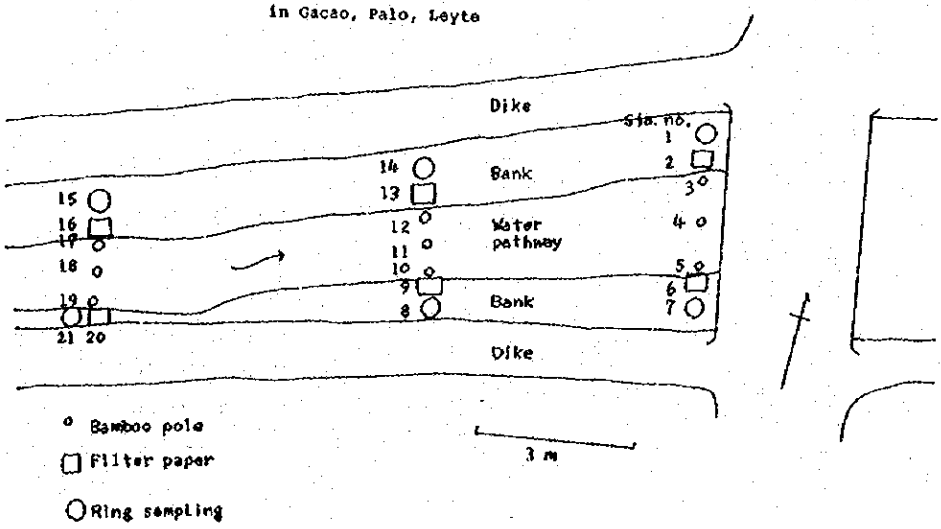


Fig. 6. Distribution of O. quadrasi observed by filter paper sampling at cross-sections in the South Main Canal in Gacao Leyte. July 1974.

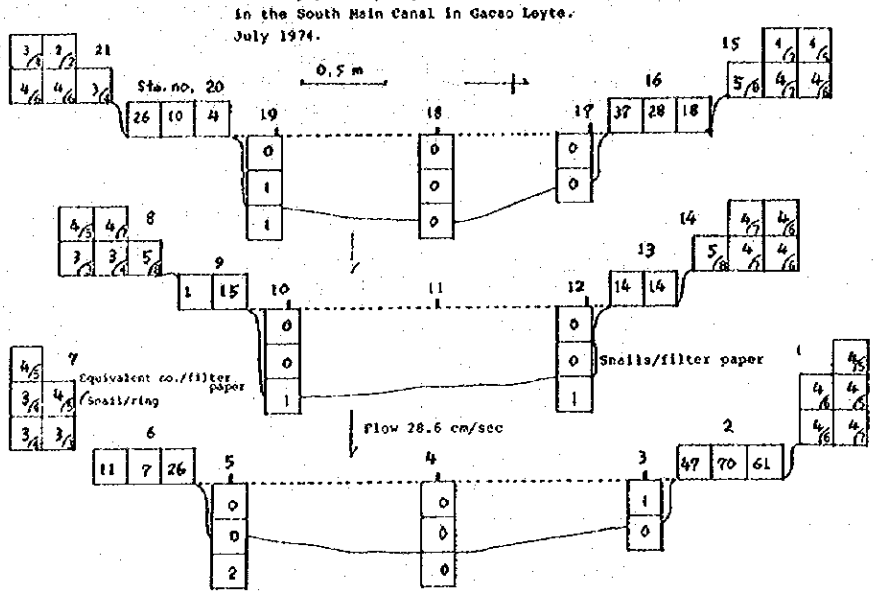


Fig.8. Snail distribution observed by filter paper sampling at Sab-a Basin, Purac, Alangalang. Feb. 1976

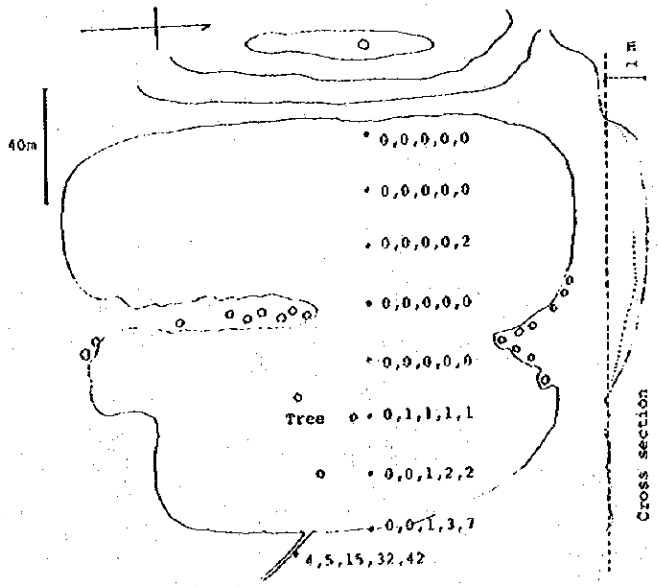
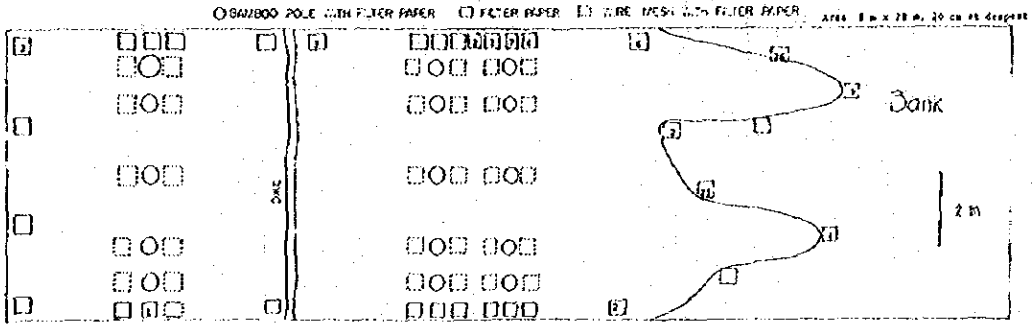


Fig. 1. Distribution of *G. Gundersi* collected by filter paper method in Cacao Pond Leyte in Oct. 1974. The number of snails/sample.



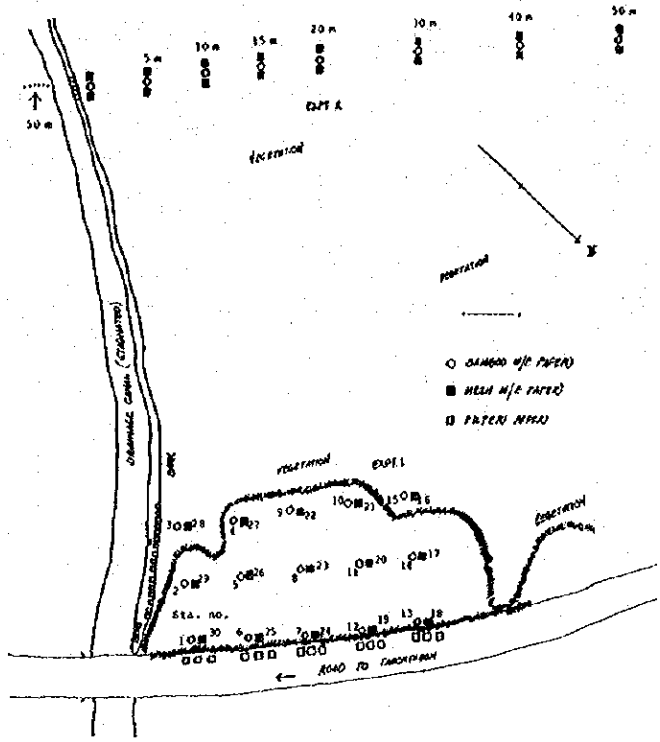


Fig. 9. Sketch map of the surveyed site in a water logged area at Capaho-an, Tabontabon, Leyte. Feb. 1975

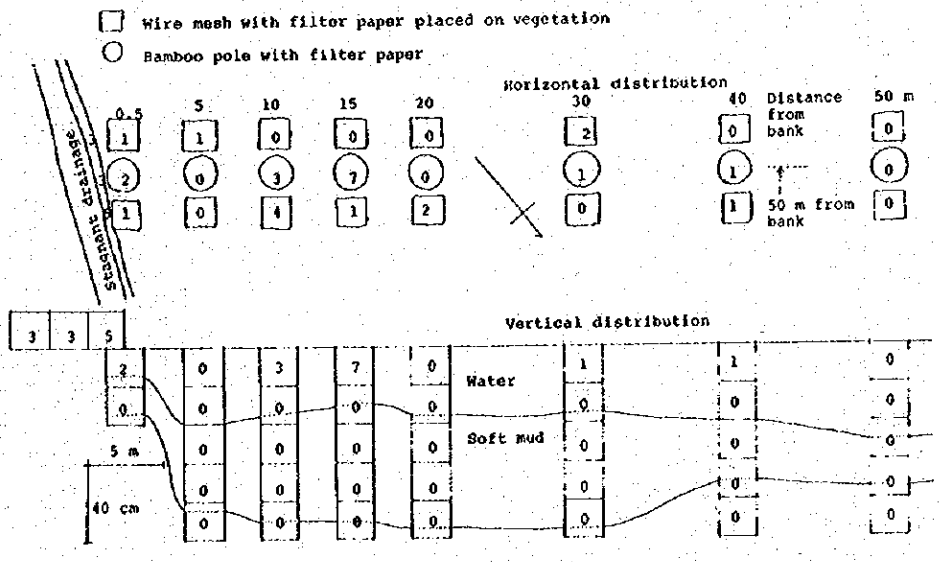


Fig. 10. Distribution of Q. quadrasi in a water logged area in Capaho-an, Tabontabon. Water is full of standing and floating vegetation. (Feb. 1975)

Fig. 11. Distribution of O. quadrasi at a part of a huge water logged area where no vegetation exists in Tabontabon (Feb.1975)

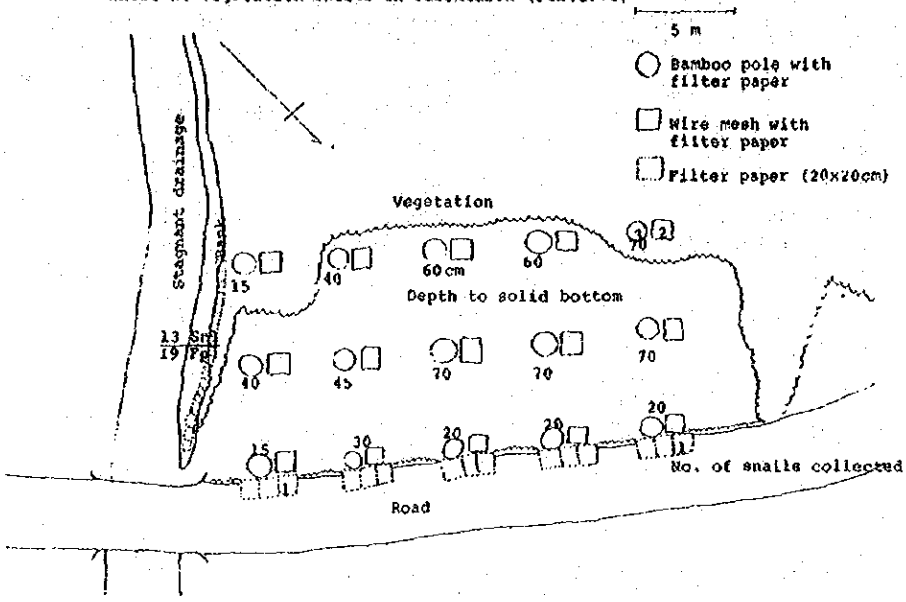


Fig. 12. Sampling of O. quadrasi on the surface of heavy vegetation by grid using filter paper method at water logged area in Tabontabon, Leyte. Aug. 1975. No. of snails / filter paper

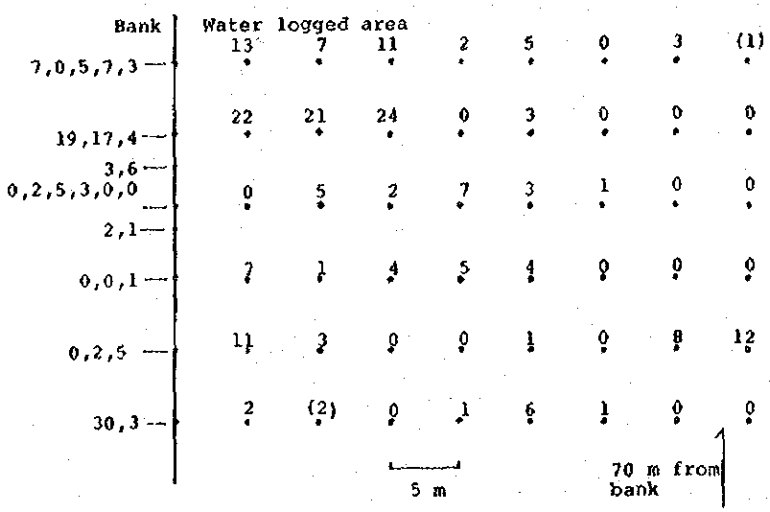


Table 1

Difference of densities of O. quadrasi on the bank and vegetation by analysis of variance of collection data transformed by log(x + 1)

	Bank (J_1)	Vegetation (J_2)
No. of sample	38	46
Total no. of snails collect	149	195
Mean density	5.32	4.24
Mean of transformed data	0.5857	0.4806

Factor	SS	DF	MS
J	0.1924	1	0.1924
R(J)	14.6522	72	0.2035
	14.8446	73	

$F_s = 0.9455 < 3.98 = F^1_{72}(0.05)$ not significant

Table 2

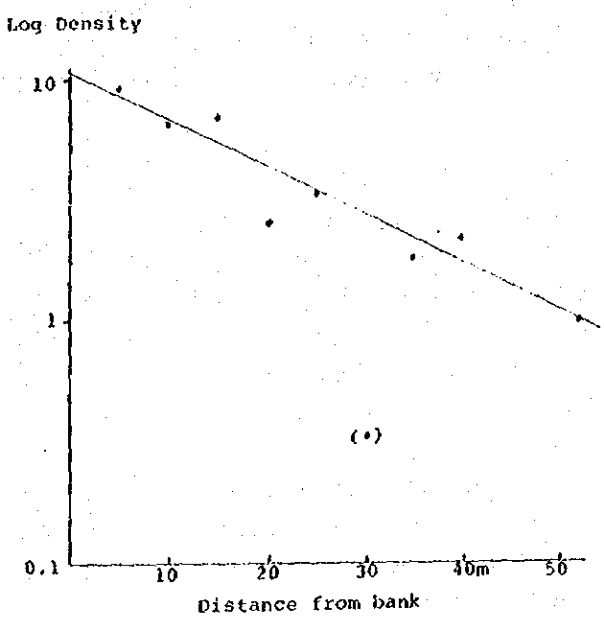
Difference of densities of O. quadrasi at different distance from the bank tested by analysis of variance of collection data transformed by log(x + 1)

Distance (A)	No. snl collected	No. of samples	Mean density
1	55	6	9.17
2	39	6	6.50
3	41	6	6.83
4	15	6	2.50
5	22	6	3.67
6	2	6	0.33
7	11	6	1.83
8	13	6	2.17

Factor	SS	DF	MS
A	2.9058	7	0.4151*
R(A)	6.4475	40	0.1612
AR	9.3533	47	

$Fs = 2.5751 > F^7_{40}(0.05) = 2.25$ Significant

Fig. 13. Relationship between the snail density and the distance from the bank on the floating vegetation in a water logged area



(•) The density is omitted for the regression line

Type of Distribution, Transformation of Sampling Data and Population Estimation of *Oncomelania quadrasi* in the Philippines

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SUMMARY

The type of distribution of *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* in the Philippines was studied in 18 sampling data; 3 samplings along a bank of a river, 1 at the margin of a depression, 5 in experimental plots by ring sampling method, 5 by tube method and 4 by filter paper method in the field. All the data but 1 colony by a river were found to fit with the negative binomial distribution. Values of exponent constant k of the negative binomial varied from 0.63 to 5.92 by the tube method, from 0.29 to 1.88 by ring and from 0.58 to 1.49 by filter paper methods. Considering the k values, snail distributions observed by the tube sampler were comparatively even than by the other methods.

To determine significance of difference in the comparative studies of densities in different snail colonies, the transformation of the sampled data is necessary. For this, 4 formulas of transformation were compared to select an efficient and simple method by which standard deviations are stable by the big different mean values of snail density in 17 sampling data. It was found that 2 formulas, $\log(x+1)$ and $\sqrt{k}\ \sinh^{-1}\sqrt{kx}$, gave a comparatively satisfactory result.

A known number of snails were released into a 4 m^2 quadrat which was surrounded by plywooden frame and 36 ring samples were collected after 3 days. The populations estimated by the ring were always far below the released number in 5 experiments. The correction factor calculated averaged to 1.79. The error of population estimation by using this correction factor showed a range from 43.1 % over- to 30.6 % underestimation at a 95 % reliable

level. In the field survey, however, the correction factor must be regarded to be larger than this as about 2 and the range of errors must be much greater.

This is only the summary of a paper to be published in a monograph titled "Research in Filariasis and Schistosomiasis Vol. 3". The most important tables in this paper are reproduced here to inform more details.

Table 11.
Distribution of snails in a rice field collected by 270 tube samples (Pesigan et al., 1958)

No. of snl. per tube	Fo	Fnb		No. of snl. per tube	Fo	Fnb	
0	60	59.99		26	1	1.03	
1	39	34.32		27	1	0.92	
2	23	25.38		28	2	0.84	
3	25	20.22		29	0	0.73	
4	18	16.66		30	0	0.68	
5	9	14.01		31	1		
6	12	11.93		36	2		
7	9	10.26		46	1	10	6.01 10.21
8	8	8.88			270		270.00
9	5	13	7.75 16.63				
10	3		6.78			$\bar{x}_2$	6.233
11	7	10	5.94 12.72			s	67.3419
12	5		5.24			k	0.6358
13	4		4.62				
14	4	13	4.08 13.94		Corrected	χ^2	0.6297
15	5		3.62			$\Sigma\chi^2$	6.845
16	3		3.21			df	11
17	2		2.86			Pr.	0.9 - 0.8
18	4	16	2.54 12.23				
19	1		2.27				
20	2		2.03				
21	2		1.78				
22	4		1.59				
23	2		1.43				
24	1		1.27				
25	1	13	1.13 11.50				

Table 12.
Goodness of fit of snail distribution observed by filter paper samplings with negative binomial distribution (Tanaka et al., 1975)

Sampling locality	Rice field Anahaway	Tibak depression No.1	No.2	No.3
No. of samples	30	26	29	30
$\bar{x}$	0.4667	15.5385	2.5172	8.4667
s^2	0.7156	177.4024	7.2152	89.1822
k	0.6923	1.4917	0.5775	0.6946
χ^2	1.105	2.86	2.54	2.73
Degree of freedom	1	1	1	1
Pr.	0.3 - 0.2	0.1 - 0.05	0.2 - 0.1	0.1 - 0.05

Table 13.
Error of estimation of snail population using arithmetic mean and the correction factor of 1.79 in experimental condition.

Exp. no.	No. snl. released	No. of snl. estimated	% error
1	980	1337	+ 36.4
2	500	696	+ 39.2
3	800	752	- 6.0
4	300	265	- 11.7
5	677	501	- 26.0

Table 14.
Evaluation of ring sampling method for estimation of snail population by experimental release and sampling.

Exp. no.	No. of snl. released	Arithmetic mean/ring	Population estimated	No. released / No. estimated	Williams mean/ring	Population estimated	No. released / No. estimated
1	980	2.6667	746.7	1.3125	1.6031	448.9	2.1833
2	500	1.3889	388.9	1.2857	0.6565	183.8	2.7201
3	800	1.5000	420.0	1.9048	1.0175	284.9	2.8080
4	300	0.5278	147.8	2.0300	0.3475	97.3	3.0832
5	677	1.0000	280.0	2.4179	0.5397	151.1	4.4799
Mean (Correction factor)				1.7902			3.0549
Standard deviation				0.4353			0.7700
Coefficient of variance (%)				24.3			25.2

Table 13.

Relationship between mean and standard deviation in different transformation methods of density of *O. quadrasi* in 17 samplings

Sampling no.	Method of sampling	No. of samples	$k^{1)}$	Arithmetic x̄	Arithmetic SD	log (x + 1) x̄	log (x + 1) SD	log (x + k/2) x̄	log (x + k/2) SD	$\sqrt{k}\sinh^{-1}\sqrt{kx}$ x̄	$\sqrt{k}\sinh^{-1}\sqrt{kx}$ SD	$\sinh^{-1}\sqrt{x/k}$ x̄	$\sinh^{-1}\sqrt{x/k}$ SD	Remarks
1	Ring	79	0.5867	1.3671	1.8200	0.2673	0.2963	-0.0540	0.5041	0.3866	0.4044	0.7252	0.7463	Upstream of Caibaan stream
2	"	80	0.2857	0.6500	1.2437	0.1378	0.2371	-0.5117	0.5442	0.1075	0.1780	0.4833	0.7803	Downstream "
3	"	150	0.5142	2.1800	3.0567	0.3469	0.3549	0.0330	0.5875	0.4275	0.4044	0.9148	0.8366	Cada-atan depression
4	"	36	0.6990	2.6667	3.2514	0.4155	0.3639	0.2034	0.5356	0.6584	0.5217	0.9889	0.7666	Experimental condition
5	"	36	0.5503	1.3889	3.6667	0.2192	0.2983	-0.1412	0.4883	0.3174	0.3683	0.6537	0.7189	"
6	"	36	1.9837	1.5000	1.6648	0.3048	0.2897	0.2892	0.2987	1.1781	1.0426	0.5566	0.5015	"
7	"	36	0.8012	0.5278	0.9407	0.1295	0.2019	-0.1786	0.3294	0.2766	0.4085	0.3647	0.5362	"
8	"	36	0.4767	1.0000	2.3905	0.1875	0.2662	-0.2407	0.4912	0.2377	0.3081	0.5831	0.7289	"
9	Tube	270	0.6297	6.2333	8.2215	0.6171	0.4646	0.4472	0.6318	0.8298	0.5449	1.3608	0.8481	Rice field, Pesigan et al.,1958
10	"	30	5.9186	0.6333	0.7649	0.1698	0.1937	0.5465	0.0881	1.9742	2.1629	0.2122	0.2363	Tanaka et al., 1975
11	"	30	3.2517	9.4000	6.1510	0.9265	0.3178	0.9670	0.2808	4.0466	1.0042	1.1980	0.3672	"
12	"	29	1.0673	2.6552	2.9553	0.4358	0.3373	0.3173	0.4256	1.0100	0.6609	0.9369	0.6167	"
13	"	30	0.9146	10.2000	11.3241	0.8734	0.4021	0.8192	0.4569	1.5198	0.5236	1.6673	0.5612	"
14	Filter	30	0.6923	0.4667	0.8604	0.1163	0.1940	-0.2494	0.3414	0.2183	0.3482	0.3454	0.5477	Tanaka et al., 1975
15	paper	26	1.4917	15.3846	13.5383	1.0595	0.4097	1.0426	0.4307	2.4916	0.7370	1.6783	0.5388	"
16	"	29	0.5775	2.5172	2.7337	0.4004	0.3721	0.1366	0.5897	0.5341	0.4622	0.9922	0.8377	"
17	"	30	0.6946	8.4667	9.6051	0.7687	0.4602	0.6531	0.6026	1.0753	0.5737	1.5689	0.8074	"
Slope function, A[2)]					0.864		0.215		-0.066*		0.263		0.093*	
Correlation coefficient[3)]					0.957		0.758		0.209		0.589		0.246	

1) Exponent constant k of negative binomial distribution
2) A in the equation of the regression line, SD = A x̄ + B
3) The correlation between x̄ and SD
* Correlation is absent

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Studies on the Fresh Water Cercariae in Leyte Island, Philippines[1)]

I. Collection of Snails

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Summary: During the past two years of 1975 and 1976, approximately sixteen thousand specimens of fresh water snails were examined for the presence of cercariae in Leyte Island, Philippines. These snails comprised about 14 species belonging to 7 families, and ultimately 43 species of cercariae were found, namely, 6 species from *Oncomelania quadrasi*, 13 species from Thiaridae, 9 species from Viviparidae and Pilidae, 8 species from Planorbidae, and 7 species from Lymnaeidae and Bulinidae. As a total, out of 15,925 sanils examined, 761 or 4.78% were found infected with cercariae.

Up to the present, 22 species of cercariae had been reported from the Philippines, and almost all of them are from Luzon Island only. Therefor, except for the cercaria of *Schistosoma japonicum*, all of the cercariae in the present study are new discoveries, and most of them are considered as new species.

In this paper, the historical review on the cercarial study in the Philippines was made first, then dates and localities of snail collection in the present study were shown in details with some map and table, and general method of cercarial observation was described for the benefit of succeeding reports of this series.

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Introduction

During the past two years from 1975 to 1976, we had an opportunity to examine many fresh water snails for the presence of cercariae in Leyte, especially in the northeastern part of the island. Altogether 15,925 specimens of snails covering 7 families and about 14 species were collected from every possible snail habitat, and examined for the cercariae. Ultimately 43 species of cercariae were found, including 7 species of furcocercariae, 2 species of amphistome cercariae, 2 species of monostome cercariae, 9 species of echinostome cercariae, 3 species of gymnocephalous cercariae, 3 species of heterophyid cercariae, 1 species of *Paragonimus* sp., 1 species of cercariaea, and 15 species of xiphidiocercariae.

Except for one species of cercaria of *Schistosoma japonicum*, all of them are new discoveries from Leyte Island, and most of them are considered as new species, though experimental studies on their life histories are wanting at present. And it seems very necessary to describe here all of these cercariae in order to be of benefit to further investigations in the future.

At first it was planned to give them a proper scientific name, but later on decided to number them instead, such as "*Cercaria leyteensis no. 1*" to "*Cercaria leyteensis no. 43*", to avoid some confusions in the future. The proper name of cercariae is more useful in reports dealing with a few species, so that, in this study identifying them by numbers was considered better, as like as *Cercaria Indicae I–LXII* by Sewell (1922), or *Cercaria helvetica I–XXIV* by Dubois (1929, 1934), or *Cercaria caribbea I–LI* by Cable (1956), in as much as this study concerns as many as 43 species.

This report is divided into six parts. Part 1 is about the collection of snails. Part 2 deals with 6 species of *Cercaria leyteensis no. 1–6* from *Oncomelania quadrasi*. Part 3 includes 13 species of *Cercaria leyteensis no. 7–19* from Thiaridae. Part 4 concerns 9 species of *Cercaria leyteensis no. 20–28* from Viviparidae and Pilidae. Part 5 is about 8 species of *Cercaria leyteensis no. 29–36* from Planorbidae. and the last part 6 is on 7 species of *Cercaria leyteensis no. 37–43* from Lymnaeidae and Bulinidae.

Historical Review

Studies on cercariae in the Philippines were initiated by Tubangui (1928), who reported nine species of cercariae each with a proper scientific name. It was composed of four new species from *Melania* spp., and five new species from *Pila luzonica* (=*Ampullaria lagunaensis*). The former group was *Cercaria parvomelania*, *C. philippindica*, *C. melaniasperata* and *C. maquilingui*. The latter group was *Cercaria redicystica*, *C. lagunaensis*, *C. rarissima*, *C. maitimensis* and *C. dorsocauda*. Four years later, Tubangui (1932) reported also the cercaria of *Euparyphium ilocanum* with a detailed experiment on its life cycle, indicating the first intermediate snail host, *Planorbis compressus* and *P. umbilicalis*. This was again reported in details by Tubangui & Pasco (1933). Tubangui (1932) made again a report on the life histories of *Euparyphium murinum* and *Echinostoma revolutum*. In this report it was clarified that both cercariae were found from the snail, *Lymnaea peregra* in Luzon, the former, possessing 45 collar spines, can develop in white rat, while the latter, 37 collar spines, can develop in pigeon experimentally. Thus all reports mentioned above were carried on in Luzon Island only.

The first study in Leyte Island was also made by Tubangui (1932) who reported the cercaria of *Schistosoma japonicum* from *Oncomelania quadrasi* in Palo with about 2.0% of infection rate.

After the World War II, Tubangui, Cabrera & Yogore (1950) made a report on the human lung fluke in the Philippines. In this study it was found out that 2 out of 1,986 snails, *Brotia asperata*, from Sorsogon were harbouring the lung fluke cercaria. Unfortunately no morphological description was made about the cercaria by them.

Recently eight papers have been made on the life histories of trematodes by Velasquez continuously. The first report (Velasquez, 1961) was on the life cycle of *Transversotrema laruei*, making the description of its peculiar furcocercaria from *Thiara riquetti* in Rizal. The second report (Velasquez, 1964) was the description of cercaria of *Euparyphium paramurinum* from *Vivipara angularis*, with a feeding experiment to guinea pig. The third one (Velasquez, 1964) was the cercaria of *Acanthoparyphium paracharadrii* from the brackish water snail, *Cerithium ornata*. The fourth one (Velasquez, 1964) was the report of cercaria of *Plagiorchis dilimanensis* from *Lymnaea philippinensis* in Rizal. The fifth one (Velasquez, 1969) was about the cercaria of *Paramonostomum philippinensis* from *Thiara riquetti* in Rizal too. The sixth one (Velasquez, 1969) was the description of cercaria of *Cloacitrema philippinum* from *Cerithium ornata*, with a detailed report about its life cycle. The seventh one (Velasquez, 1973) was about the cercaria of *Haplorchis taichui* from *Melania juncea*, and the last eighth one (Velasquez, 1973) was the cercaria of *Procerovum calderoni* from *Thiara riquetti* in Rizal too.

Altogether only 22 species of cercariae have

been reported from the Philippines up to this date, according to the references mentioned above. And all of them were from Luzon Island only, except one species, namely, the cercaria of *Schistosoma japonicum* in Leyte Island.

Identification of Snails Collected

Though it was not our main purpose, the identification of fresh water snails was one of the most difficult problem. Snails collected from the field and brought into the laboratory of the Project in Palo, were classified tentatively before crushing for the cercarial ex-examination. Later at about the end of this study, a specialist on the molluscan taxonomy was asked for the identification. Some snail specimens were sent to Dr. Tadashige Habe, a specialist of conchology in the National Science Museum in Tokyo, who responded kindly to our request as follows.

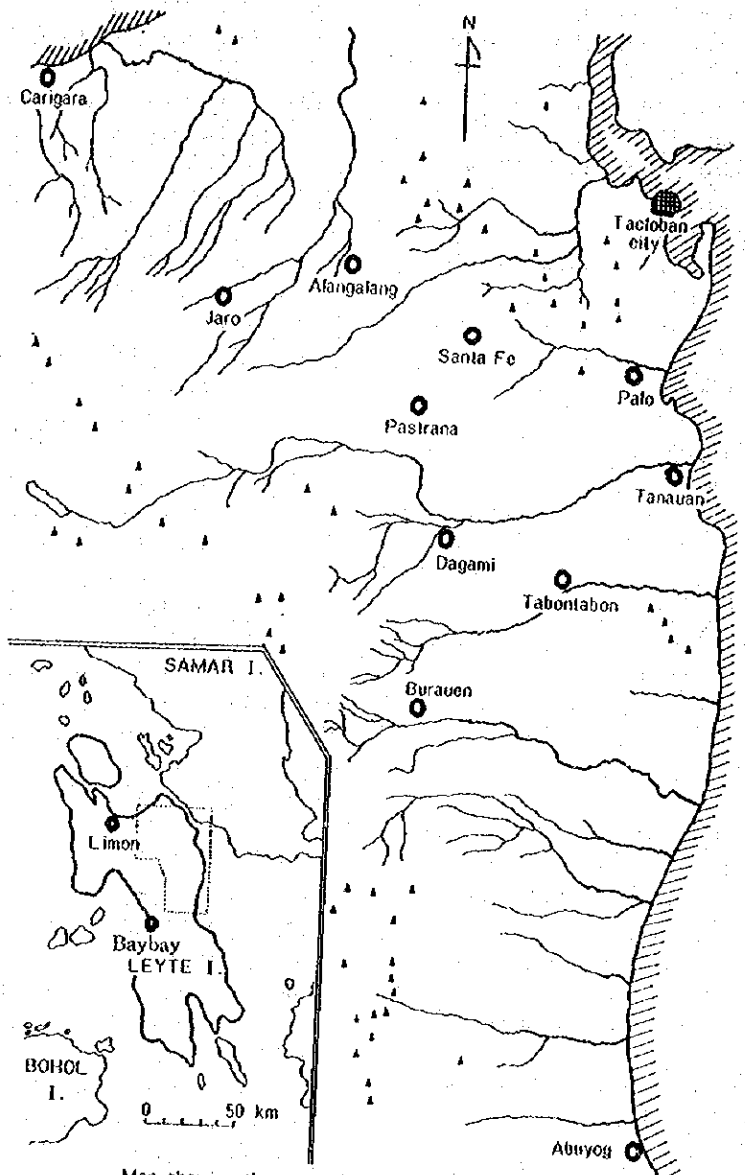
The most accurate scientific name for the snail vector of *S. japonicum* in the Philippines seems to be *Oncomelania nosophora quadrasi* (Möllendorff, 1895), but the name *O. quadrasi* is still adequate customarily. As to the snails belonging to Thiaridae, at least six species are distinguished. The commonest one is *Melanoides tuberculatus* (Müller, 1774) and *Antemelania dactylus* (Lea). Next two species, *Antemelania asperata* (Lamarck) and *Thiara* (*Plotiopsis*) *scabra* (Müller, 1774) are also common. Among them the following two species, *Stenomelania costellaris* (Lea, 1850) and *S. biflammatus* (Reeve) are rarely found. About the snails of Viviparidae, it will be most reasonable to identify as *Bellamya philippinensis* (Nevill), though here remains some problems because of much confusion among Viviparidae. As for the Pilidae, there are two species. One is *Pila ampullacea* (Linnaeus, 1858) which is bigger and brownish in the inside, and the another one is *Pila luzonica* (Reeve) which is smaller and whitish in the inside. The majority of Lymnaeidae is identifiable as *Austropeplea philippinensis* (Clessin, 1886) and *Mixas cumingianus* (Pfeiffer, 1845). The former resembles closely *Fossaria ollula* (Gould, 1859), but it is difficult to distinguish them by the shell alone. The latter, a proper species in the Philippines, is usually called *Lymnaea auricularia* (Linnaeus, 1758), but the name *Myxas cumingianus* seems to be more suitable. Only a few specimens of *Radix auricularia rubiginosa* Michelin, 1831 are found among the Lymnaeidae. One species of Bulinidae, *Physastra hungerfordiana* (Nevill, 1891) is found commonly. This is easily distinguishable because of the counterclockwise coil of the shell. Lastly, as to the snails of Planorbidae, three species are found which are rather easy to distinguish each other. These are, *Indoplanorbis exustus* Deshayes, 1832, *Segmentina hemisphaerula* (Benson, 1842), and *Gyraulus convexiusculus* Hutton, 1849. The name *G. convexiusculus* is adopted here because it is rather popular, though the name *G. chinensis* Dunker, 1848 is more reasonable from the viewpoint of priority.

Thus according to Dr. Habe, 17 species covering 7 families were identified from our specimens, but unfortunately only 14 species could be distinguished by us. The photographs of the species are illustrated on the plates (cf. plate 1 & 2).

Date and Locality of Snail Collection

Leyte Island is one of the Visayan group of islands, and is nearly 8,000 km². The area from Palo south to Abuyog (52 km) and from Palo north to Carigara (40 km) is a long stretch of plain which is nearly 1,200 km², constituting approximately one-sixth of the total area of the Island of Leyte. This is the main surveyed area of our present study, it being the endemic area for schistosomiasis in the province. Across this area run numerous perenneal rivers, deep enough for light boats to navigate as far as 10 km or more inland. They often swell and cause floods during the period of heavy rains (November—January). In many places wide depressions exist into which the water drains, causing swamps to develop (cf. map).

Dates and localities of our snail collection



Map showing the surveyed area in Leyte Island

were listed in the following. The locality was noted as town or poblacion, with parenthesized barrio name.

October, 1975

8....Palo (Gacao)
10....Dagami (Hinulogan)
14....Palo (Vicob), Pastrana (Socsocon)
15....Pastrana (Socsocon)
27....Palo (Cogon), Pastrana (Socsocon)
28....Palo (Gacao), Pastrana (Socsocon)
31....Santa Fe (Maslog)

November, 1975

4....Pastrana (Socsocon)
10....Pastrana (Socsocon)

13....Pastrana (Socsocon)
14....Palo (Naliwatan), Javier (Javier)
17....Santa Fe (Maslog)
18....Palo (Vicob), Santa Fe (Maslog), Dagami (Hinulogan), Pastrana (Socsocon), Tanauan (Kiling), Burauen (Buri)
24....Santa Fe (Maslog)
25....Pastrana (Socsocon), Santa Fe (Maslog)
27....Palo (South Main Canal), Santa Fe (Tibak)
28....Palo (South Main Canal)

December, 1975
2....Palo (Naliwatan)
3....Palo (Cogon)
5....Pastrana (Socsocon), Tanauan (Batom)
9....Limon (Limon)
10....Baybay (Baybay)
12....Palo (Vicob), Pastrana (Socsocon), Santa Fe (Maslog)
17....Pastrana (Socsocon)
22....Alangalang (Alangalang)

January, 1976
6....Pastrana (Socsocon)
8....Palo (Upper Hubang), Pastrana (Socsocon), Santa Fe (Tibak)
12....Palo (Upper Hubang), Santa Fe (Tibak)
13....Palo (Upper Hubang), Santa Fe (Cogon na South)
14....Palo (South Main Canal)
15....Palo (Nariwatan)
19....Pastrana (Socsocon), Santa Fe (Maslog, Tibak)
20....Palo (Hubang)
23....Palo (Upper Hubang)
26....Dagami (Dagami)
28....Santa Fe (Maslog), Dagami (Dagami, Maliwaliw, Cabuluran)
29....Dagami (Maliwaliw, Cabuluran)

February, 1976
2....Dagami (Maliwaliw, Central II)
3....Palo (Vicob), Pastrana (Socsocon), Santa Fe (Maslog)
6....Santa Fe (San Isidro)
9....Palo (Hubang)
11....Pastrana (Socsocon), Santa Fe (Tibak)
13....Palo (South Main Canal, Naliwatan)
18....Pastrana (Socsocon), Santa Fe (Tibak, Maslog, San Juan)
23....Palo (Upper Hubang), Santa Fe (San Juan)
25....Palo (Vicob), Pastrana (Socsocon), Santa Fe (Maslog)

March, 1976
1....Jaro (Malobago)
2....Tabontabon (Capohu-aw)
3....Pastrana (Socsocon), Santa Fe (San Juan)
8....Tabontabon (Capohu-aw)
10....Palo (Upper Hubang)
12....Tabontabon (Capohu-aw)
15....Jaro (Malobago)
16....Palo (Upper Hubang)

On the table, total number of examined and infected snail was shown according to locality and snail species. Totally 761 out of 15,925, or 4.78% of snails were infected with cercariae, with some disparity from locality to locality on each species of snail.

Method of Cercarial Observation

Cercariae and parthenitae were obtained by crushing the snails. These were immersed in 0.4% NaCl-solution for preserving several hours. In order to make the ventral view visible, a drop of the solution containing the cercariae was put on a cover slip which is as thin and of good quality as possible. A slide-glass was placed very slowly over the loaded cover slip until it comes in contact with it. The surface tension exerted by the mounting fluid naturally lifted the cover slip to come into contact with the surface of the glass slide. The glass slide then was turned upside down and the excess mounting fluid between the cover slip and the glass slide was blotted by a strip of filter paper at the end of cover slip. This was done until the cercariae inside became very thin, flat, and transparent, thereby making it suitable for morphological observa-

A summary of snail examination for the cercariae in Leyte Island.

(Results of 15,925 snail examination during 1975–1976)

Species of snail	Palo	Pastrana	Santa Fe	Dagami	Tabon-tabon	Jaro	Alang-alang	Limon, Baybay	Tana-uan	Bura-uen	Total	Infection rate
Pomatiopsidae												
Oncomelania quadrasi	1719(87)	1769(50)	2517(49)	304(20)	70(2)	0	0	0	31(5)	13(0)	6423(213)	3.32%
Thiaridae												
Melanoides tuberculatus & *Antemelania dactylus*	434(56)	772(25)	527(39)	360(21)	9(1)	205(6)	40(0)	59(8)	0	5(0)	2411(156)	6.47%
Thiara (*Plotiopsis*) *scabra*	73(0)	35(1)	0	9(0)	0	0	33(0)	0	0	0	150(1)	0.67%
Antemelania asperata & *Antemelania dactylus*	0	0	0	0	0	143(11)	0	0	0	0	143(11)	7.69%
Viviparidae												
Bellamya philippinensis	755(49)	479(7)	606(33)	59(2)	155(18)	0	3(0)	0	4(1)	14(1)	2075(111)	5.35%
Pilidae												
Pila ampullacea & *Pila luzonica*	9(1)	27(0)	18(0)	136(9)	22(2)	0	0	0	5(0)	2(0)	219(12)	5.48%
Planorbidae												
Segmentina hemisphaerula	1137(51)	737(76)	280(10)	47(1)	13(0)	0	0	0	0	0	2214(138)	6.23%
Gyraulus convexiusculus	512(14)	271(35)	45(2)	9(0)	0	0	0	0	0	0	837(51)	6.09%
Indoplanorbis exustus	0	0	0	177(0)	102(0)	0	0	0	0	0	279(0)	
Lymnaeidae												
Austropeplea philippinensis & *Myxas cumingianus*	230(20)	220(3)	270(28)	226(2)	59(0)	0	0	0	1(0)	0	1006(53)	5.27%
Bulinidae												
Physastra lungerfordiana	36(1)	81(4)	51(10)	0	0	0	0	0	0	0	168(15)	8.93%
Total	4905(279)	4391(201)	4314(171)	1327(55)	430(23)	348(17)	76(0)	59(8)	41(6)	34(1)	15925(761)	4.78%
Infection rate	5.69%	4.58%	3.96%	4.14%	5.35%	4.89%		13.56%	14.63%	2.94%		

Figure indicates the number of snail examined, and parenthesized one is the number of snail infected.

tion under the microscope. The cercariae were restricted in their movements by flattening them to such an extent as not to injure their body. This preparation was sealed with vaseline around the cover slip to prevent the evaporation of the mounting fluid for the duration of a few hours' observation.

Precise measurements of cercariae, which should be performed on the fixed specimens, may frequently serve as an essential classification key for the identification of closely related cercariae. Description of measurements is, therefore, indispensable for the specific diagnosis. In the present paper, all measurements were taken on specimens fixed in 10% hot formalin, because the results obtained by this way were relatively constant. The number of individuals used for such measurents were 10 to 20 in each species.

All drawings were scaled to such measurements, and were illustrated semidiagrammatically, not by camera lucida. In some drawings, the gland cells were omitted on the right side, and the flame cells on the left side, to make the figure clear.

Acknowledgements

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Explanation of Plates

Plate I. Fresh water snails in Leyte Island.

Fig. 1.	*Oncomelania quadrasi* (Möllendorff)	Pomatiopsidae
Fig. 2.	*Melanoides tuberculatus* (Müller)	Thiaridae
Fig. 3.	*Thiara* (*Ploiopsis*) *scabra* (Müller)	Thiaridae
Fig. 4.	*Antemelania dactylus* (Lea)	Thiaridae
Fig. 5.	*Antemelania asperata* (Lamarck)	Thiaridae
Fig. 6.	*Indoplanorbis exustus* Deshayes	Planorbidae

Plate II. Fresh water snails in Leyte Island.

Fig. 7.	*Bellamya philippinensis* (Nevill)	Viviparidae
Fig. 8.	*Pila ampullacea* (Linnaeus)	Pilidae
Fig. 9.	*Austropeplea philippinensis* (Clessin)	Lymnaeidae
Fig. 10.	*Myxas cumingianus* (Pfeiffer)	Lymnaeidae
Fig. 11.	*Physastra hungerfordiana* (Nevill)	Bulinidae
Fig. 12.	*Segmentina hemisphaerula* (Benson) and *Gyraulus convexiusculus* Hutton	Planorbidae


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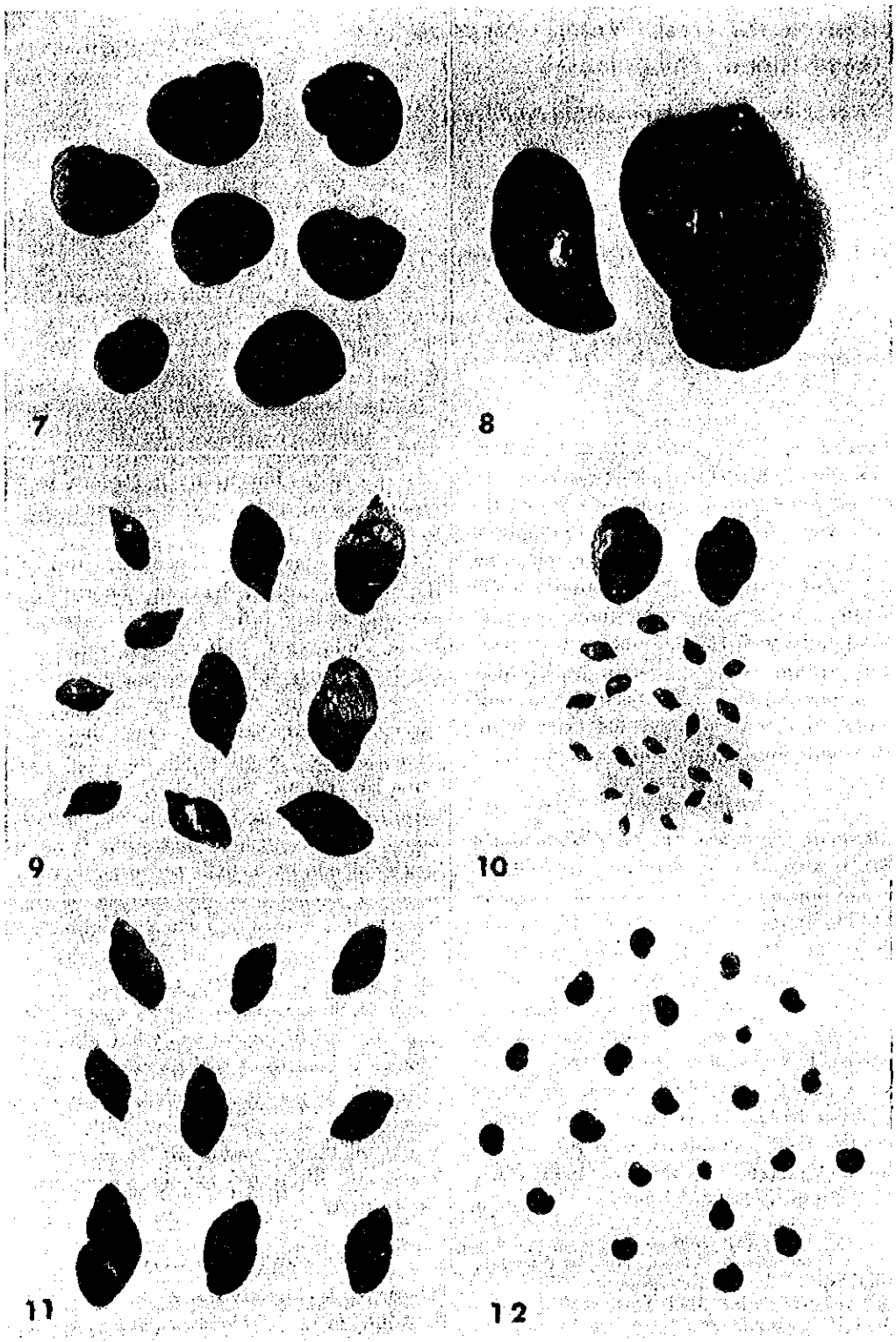
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[Japan. J. Exp. Med.
Vol. 47, 3, p. 151-162, 1977]

Studies on the Fresh Water Cercariae in Leyte Island, Philippines[1)]

2. Cercariae from *Oncomelania quadrasi*

(Received for Publication, February 15, 1977)

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Summary: From 1975 to 1976, more than six thousand snails of *Oncomelania quadrasi* were observed for cercarial fauna in the north-eastern part of Leyte Island, Philippines. Altogether, six species of cercariae including that of *Schistosoma japonicum* were found, namely two furcocercariae, one monostome cercaria, two xiphidiocercariae and one tail-less cercaria. In this paper a description of their morphology, infection rate, locality, and some remarks on the presumptive life cycle is included with illustrations and photographs.

Introduction

The snail, *Oncomelania quadrasi* (Möllendorff, 1895), is a well-known snail because it is the only intermediate host of *Schistosoma japonicum* in the Philippines. In the course of epidemiologic studies of this fluke, many senior investigators have encountered some other cercariae aside from *S. japonicum*. Thus from *Oncomelania nosophora* in Japan, besides the cercaria of *S. japonicum*, three other species were reported; namely, *Cercaria longissima* (Suzuki et Nishio, 1914) Faust, 1924, *Cercaria okabei* Ito, 1949, and cercaria of *Maritreminoides caridinae* (Yamaguti et Nishimura, 1944) Chen, 1957. From *Oncomelania hupensis* in China, only one species, *Cercaria sensa* Komiya, 1952 was reported. But from *O. quadrasi* in the Philippines no such report has been made up to the present time.

During the past two years of 1975 and 1976, the present authors had an opportunity to examine a lot of the snails, *O. quadrasi* in Leyte, Philippines. These snails were collected from nearly all of the endemic areas in Leyte, increasing the number to 6,423. Six species of cercariae including that of *S. japonicum* were found to be infected. These cercariae except that of *S. japonicum* are surmised to be non-pathogenic for the human body. By artificially increasing these five species of non-pathogenic cercariae there may result a decrease in the number of snails, because it is known that when a snail harbours some cercariae, this snail will be destroyed as a result of parasitic castration. So the study of these cercariae is very necessary, not only for the biological study but also for the biological control of schistosomiasis.

In this paper the detailed morphological descriptions of each cercariae, with some remarks on their presumptive life history, were made and illustrated for the convenience of future study.

Description of Cercariae

Cercaria leyteensis no. 1

Adult form: *Schistosoma japonicum* Katsu-

[1)] This work was performed as a part of the RP-Japan Cooperative Studies supported by the Japan International Cooperative Agency.

伊藤二郎（静岡大学教育学部衛生学）
安羅岡一男（筑波大学基礎医学系医生物学）

rada, 1904

Snail host: *Oncomelania quadrasi*

Infection rate: On the whole, 193 out of 6,423, or 3.00%

Measurements:

body	160 (138–180) × 64 (60–75)μm
anterior organ	50 (40– 60) × 36 (32–45)μm
acetabulum	19 (18– 20) × 20 (18–22)μm
tail stem	113 (100–140) × 23 (20–28)μm
tail furca	67 (65– 75) × 10 (8–13)μm

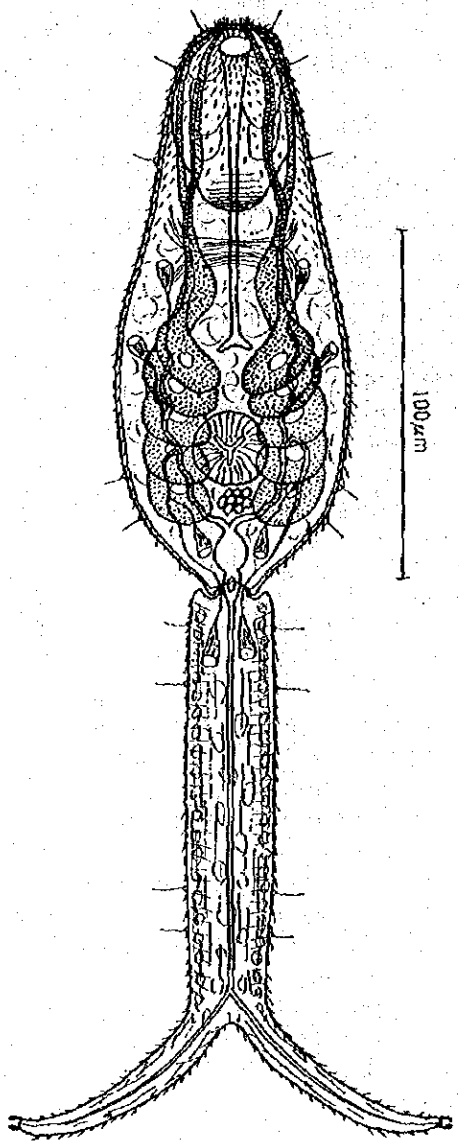
Specific description: (Fig. 1)

Nonoculate, apharyngeal brevifurcate distome furcocercaria. Body is elongated oval in shape, with many backward directed minute spines on the surface of body and tail. In addition, nearly 5 pairs of sensory hairs are on the body surface. Anterior organ is delimited posteriorly by a cup shaped posterior portion which is provided with a thick layer of circular muscles and a thinner layer of longitudinal muscles. Within the anterior organ are several pairs of cephalic glands. Acetabulum is situated at the middle of posterior half of body. There is no pharynx. A short rhabdocoel esophagus ends in front of acetabulum with a short bifurcation. Five pairs of penetrating glands occupy the posterior half of body, the anterior two of which contain coarse granules and the posterior three contain fine granules. Five openings of the ducts were observed in circular arrangement on each side of the oral cavity, each opening in close connection with a sharp-pointed penetrating spine. A mass of genital primordia is on the ventral side behind the acetabulum. Excretory bladder is small. Island of Cort is present. Flame cell formula is 2[(1+1)+(1+[1])]=8. Several pairs of cilia are in the main collecting tubes.

Tail stem is shorter than the body, and is provided with many minute spines and about 4 pairs of sensory hairs. Tail furca is nearly half length of the tail stem. It is beset with many minute spines but no sensory hair. Tail excretory tubule is bifurcating near the posterior end of the tail stem, with each branch opening at the tip of furca with an ampulla.

Remarks: The first discovery of this cercaria in the Philippines was made by Tubangui (1932), who undertook a survey of schistosomiasis in Palo, Leyte, and determined the snail vector, *Oncomelania quadrasi*, as its intermediate snail host. He reported the infection rate as 2.0%, made illustrations of the cercaria, but no description of this cercaria was

Fig. 1. *Cercaria leyteensis no. 1* (Cercaria of *Schistosoma japonicum*).



furnished.

In other endemic countries, the cercaria of *S. japonicum* was first described by Miyairi and Suzuki (1914) from *Oncomelania nosophora* in Japan. After that the description of this cercaria had been made from time to time by several investigators, such as Suzuki (1919), Cort (1919), Faust and Meleney (1924), Takahashi (1928), Tang (1938), etc. Comparing the present cercaria with those of the references mentioned above, no fundamental difference could be found.

Cercaria leyteensis no. 2

Presumptive adult form: Diplostomatidae (*Diplostomum* or *Alaria*)

Snail host: *Oncomelania quadrasi*

Date, locality and infection rate:

On the whole, 5 out of 6,423, or 0.08%

Oct. 8, 1975, Palo (Gacao), 3 out of 79, or 3.8%
Nov. 18, 1975, Tanauan (Kiling), 2 out of 31, or 6.5%

Measurements:

body .....114 (106–128)×62 (52–70)μm
oral sucker .... 26 (20– 30)×24 (19–28)μm
acetabulum .... 20 (17– 22)×24 (20–30)μm
tail stem .....148 (130–160)×36 (30–40)μm
tail furca .....165 (140–190)×18 (15–22)μm

Specific description: (Fig. 2)

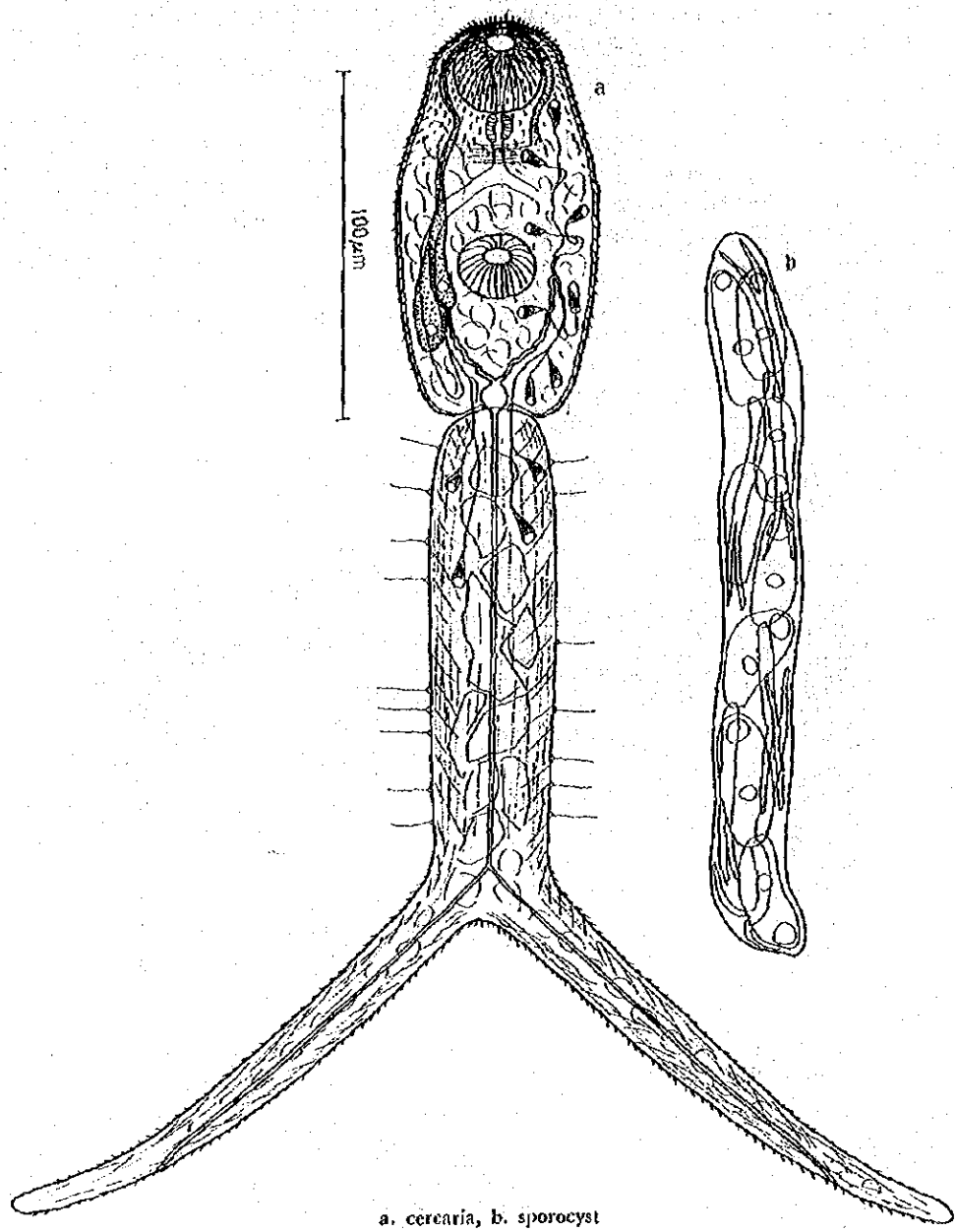
Nonoculate, pharyngeal longifurcate distome furcocercaria. Body is ellipsoidal in shape, tapering slightly in the anterior part of body. The surface is covered with many backward directed minute spines, more densely at the anterior part. Oral sucker is spherical, situated at the anterior portion of body. Acetabulum is slightly smaller than the oral sucker, and is located slightly posterior to the middle of body, the ratio of anterior and posterior being 7:4. A distinct pharynx follows the oral sucker directly, without any prepharynx. It is followed by a short esophagus dividing into two caeca reaching to the posterior end of body. Two pairs of penetrating glands are at the posterior half of body, and their ducts extend to the tip of body terminating in each opening. Nervous system is observed as a transverse commissure at the level of esophagus.

Excretory bladder is small. Main collecting tube divides anteriorly and posteriorly, the anterior tube receives four flame cells grouping in two, and the posterior tube does so too. Moreover two flame cells at the tail stem send off a tubule to join to the posterior tube. Thus the flame cell formula is constructed as $2[(2+2)+(2+2+[2])]=20$. Island of Cort is absent. Tail stem is longer than the body, and is provided with about 8 pairs of sensory hairs, but without spine. Tail furca is much longer than the body, and is provided with many minute spines, but no sensory hair.

Sporocyst is whitish filamentous, with fairly mobile, and about the same diameter throughout. It contains many cercariae of nearly the same developmental stage.

Remarks: The general feature of this cercaria indicates that the presumptive adult form is a member of Diplostomatidae. The life cycles of so many genera and species of this family have already been clarified throughout the world, and the present cercaria seems to be mostly related to the genus *Diplostomum* or *Alaria*. Up to now the life cycles of nearly ten species of *Diplostomum* have been reported around the world. Their first intermediate hosts are all fresh water snails aside from *O. quadrasi*. According to the references, most of these flukes have a fresh water fish as their second intermediate host, and some of them have a tadpole of *Rana* as their intermediate host. Cysts were reported to be found not from the muscle but from the eye lens or brain of those vertebrate hosts. Adults were obtainable from birds such as pigeon, duck, chick, etc., after feeding them with the metacercariae or by natural infection cases. In the case of genus *Alaria*, six or more species were reported from *Planorbis* or *Helisoma* in other countries, but not from *Oncomelania*. These cercariae penetrate into the tadpole of *Rana* spp., in which they become mesocercariae. These mesocercariae develop into metacercariae and adults after infecting dog, cat, fox, mink, and so on according to the species.

Fig. 2. *Cercaria leyteensis, no. 2.*



a. cercaria, b. sporocyst

Komiya and Ito (1967) made a redescription of *Cercaria longissima* Faust, 1924, which was found from *O. nosophora* in Japan. It is very similar to the present one but differs in some part. Unfortunately they did not mention about its life cycle.

In view of the reports mentioned above, the present cercaria is presumed to be a new species because it is the only species from *Oncomelania quadrasi*. The experiment on the

life cycle study should be started, therefore, by letting the cercaria come in contact with the fresh water fish or the tadpole of *Rana*.

Cerearia leyteensis no. 3

Presumptive adult form: Notocotylidae
Snail host: *Oncomelania quadrasi*
Date, locality and infection rate:
On the whole, 8 out of 6,423, or 0.12%

Oct. 8, 1975, Palo (Gacao),
3 out of 79, or 3.8%
Oct. 28, 1975, Palo (Gacao),
3 out of 162, or 1.9%
Jan. 28, 1976, Santa Fe (Maslog),
1 out of 185, or 0.5%
Feb. 18, 1976 Pastrana (Socsocon),
1 out of 200, or 0.5%

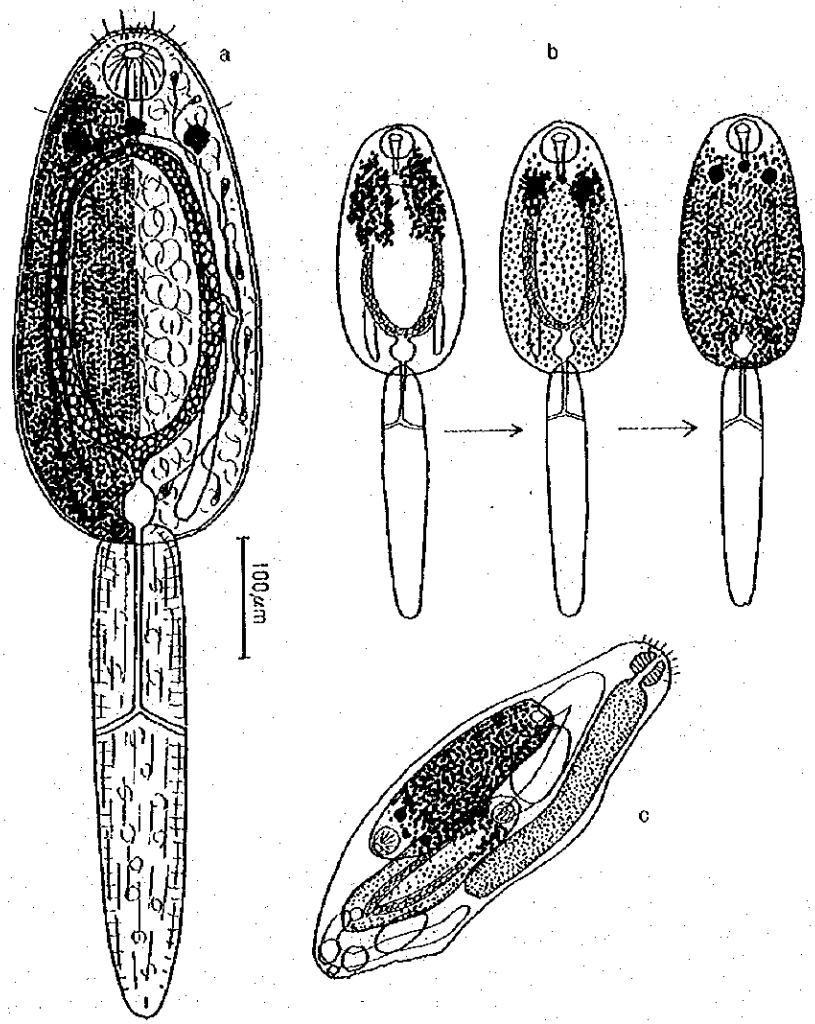
Measurements:

body .....433 (400–500) × 214 (190–230)μm
oral sucker..... 53 (48– 60) × 48 (45– 50)μm
tail .....424 (360–450) × 70 (60– 80)μm

Specific description: (Fig. 3)

Large, trioculate monostome cercaria belonging to Notocotylidae. Body is oval in shape, without any adhesive pocket at the

Fig. 3. *Cercaria leyteensis no. 3.*



a. cercaria, b. developmental stage of cercaria, c. redia

postero-lateral portion of body. Cuticle is aspinose, but with several pairs of sensory hairs at the anterior part of body. A subglobular oral sucker is followed by a short esophagus which is divided into two ceca terminating in blind at the posterior end of body. Well developed cystogenous materials and heavily pigmented granules make the body very opaque, though in the young cercaria these pigmented granules are present only in the anterior part of body. Paired eye spots are prominent, with a median one being smaller, but these are not clear in immature cercaria.

Non-epithelial excretory vesicle is small, sending off a short median stem anteriorly, which is divided into two long collecting tubes, connected with each other dorsally near the intestinal bifurcation, and send a short median vessel anteriorly. These tubes are densely provided with many refractile excretory concretions. From the middle of each branch, a secondary tubule arises to receive 8 pairs of flame cells. The flame cell formula is 2[(2+2)+(2+2)]=16. Tail is about the same length as the body, with neither spine nor hair. Caudal excretory tube divides into two short branches which open at about the middle of tail.

Redia is plump shape, with many sensory hairs around the mouth opening. Pharynx is at the anterior end of body, and is followed by a sausage shaped gut compacting the digested snail tissue. Locomotor appendages are rudimentary in the old redia whicn is less motile than the young one. A few cercariae of various developmental stages are contained in the redia.

Remarks: This cercaria apparently belongs to the family Notocotylidae. The life cycles of about twenty species of this family have already been reported throughout the world. The intermediate snail host belongs to many species of snails, but never to *Oncomelania quadrasi*. In the Philippines, Velasquez (1969) made a report of the life history of *Paramonostomum philippinensis* belonging to this family. According to her, a trioculate monostome cercaria was obtained from a brackish water snail, *Thiara riquetti*, in Luzon Island. She obtained the adult worm from the ceca of chicks and ducklings 12 to 15 days after experimental feeding with the metacercariae which was scratched up from the glass container. Thus it has been already known that the cercaria of this group can encyst easily in the outside without any second intermediate host, and that the duck and chicken serve as the final host for most of the species of this family, and mouse, muskrat, or guinea pig does as that for some few species of the family.

In view of the above, the present cercaria is suspected to be a new one belonging to the family Notocotylidae. For the benefit of future study of life cycle, it is acqaintable that the first attempt to feed the duckling with this metacercaria should be carried on.

Cercaria leyteensis no. 4

Presumptive adult form: Lecithodendriidae

Snail host: *Oncomelania quadrasi*

Date, locality and infection rate:

On the whole, 1 out of 6,423, or 0.02%

Feb. 25, 1976, Santa Fe (Maslog), 1 out of 200, or 0.5%

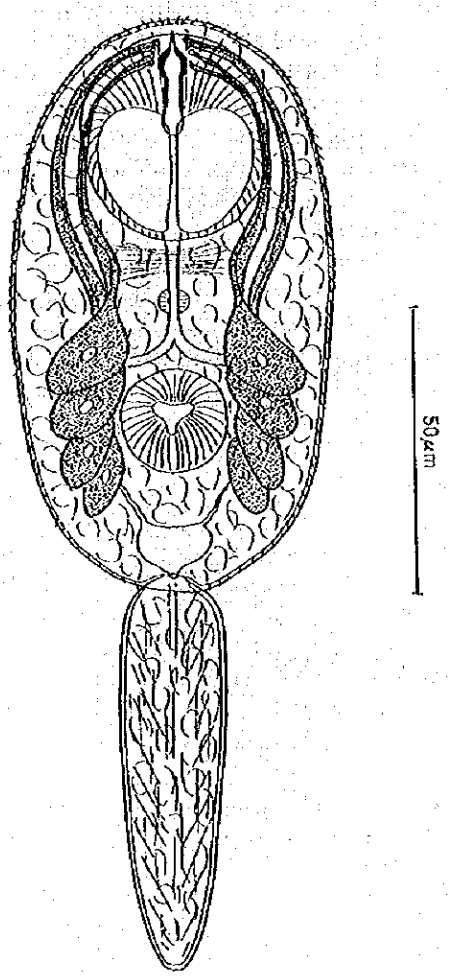
Measurements:

body .....97 (94–101) × 55 (49–62)μm
oral sucker .....36 (33–39) × 33 (29–36)μm
stylet .....13–16 μm in length
acetabulum .....18 (16–20) × 19 (16–23)μm
tail .....69 (59–81) × 18 (16–23)μm

Specific description: (Fig. 4)

Small sized virgulate xiphidiocercaria belonging to Paravirgula subgroup of Sewell (1922). Body is ellipsoid in shape, covered with many minute spines. Relatively large oral sucker is at the subterminal anterior end of body, and contains a large flat-shaped virgula organ in it. A strong shouldering stylet is at the dorso-median portion of the oral sucker. Acetabulum is smaller than the oral sucker, situated at the posterior third. Prepharynx is long, connected to a small pharynx and a short esophagus which is divided into

Fig. 4. *Cercaria leyteensis no. 4.*



two faint ceca. Four pairs of penetrating glands, two pairs in the ventral side, and two other in the dorsal side, are situated around the acetabulum. Their ducts, in two bundles, open beside the stylet. Nervous commissure is observed across the prepharynx dorsally.

Excretory vesicle is cup-shaped, opening dorsoterminally. The flame cell formula is not determined yet. Tail is tapering posteriorly, without spine or hair, and is inserted slightly into the postero-dorsal end of body. No excretory tube is observed in the tail.

Sporocyst is in the shape of a simple sac, with thin smooth wall, and contains a few cercariae of approximately equal developmental stage.

Remarks: Up to the present, no report of virgulate xiphidiocercaria from the snail, *Oncomelania* spp. has been made in the Orient. So, this is the first report and is considered as a new species. Because of its general appearance, this cercaria presumably belongs to the family Lecithodendriidae. Life histories of so many genera and species of this family have already been clarified throughout the world. Aqueous insect larvae or shrimps were reported to serve as the second intermediate host for all members of Lecithodendriidae. And experimental mammals such as rat and mouse serve as the final host for majority of them, but frogs do for *Loxogenes*, *Pleurogenes*, and night-hawk or canary do for *Mosesia*.

In view of the above, the first step in clarifying the life cycle of this cercaria should be carried on by a contact experiment on some aqueous insect larvae, such as stone fly, dragonfly, firefly, alderfly, *Chironomus* larvae, and or shrimps.

Cercaria leyteensis no. 5

Presumptive adult form: Microphallidae (*Maritreminoides*)

Snail host: *Oncomelania quadrasi*

Date, locality and infection rate:

On the whole, 4 out of 6,423, or 0.06%

Dec. 12, 1975, Santa Fe (Maslog), 1 out of 100, or 1.0%
Jan. 12, 1976, Palo (Upper Hubang), 2 out of 63, or 3.2%
Jan. 19, 1976, Santa Fe (Maslog), 1 out of 240, or 0.4%

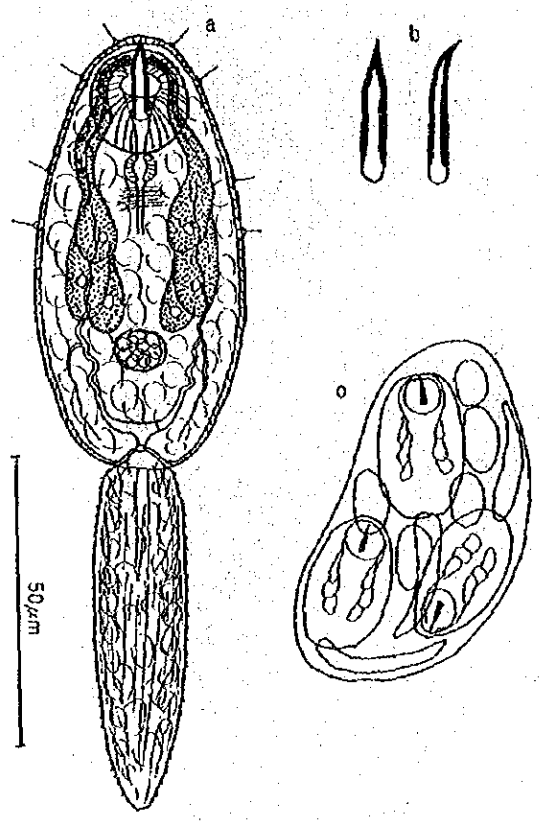
Measurements:

body	75 (70–83) × 34 (32–35)μm
oral sucker	18 (17–20) × 17 (16–19)μm
stylet	13 (12–14) × 3 (3– 4)μm
acetabulum	8 (6–10) × 8 (7– 8)μm
tail	63 (50–80) × 17 (15–20)μm

Specific description: (Fig. 5)

Small sized xiphidiocercaria belonging to Microcotyle group of Lühe (1909). Body is elongated oval in shape, its posterior end being slightly blunt than the anterior end.

Fig. 5. *Cercaria leytensis no. 5.*



a. cercaria, b. stylet, dorsal and lateral view, c. sporocyst

Body surface is covered with minute spines all over the surface, and is provided with four pairs of sensory hairs on the anterior half of body. A well developed oral sucker is at the anterior end of body. A solid, sharply pointed stylet is embedded in the median dorsal side of oral sucker. It is indistinctly shouldered and bent slightly ventralward when observed laterally. No prepharynx but a small pharynx follows directly the oral sucker. A faintly developed short esophagus is recognized but the remainder of digestive system is not differentiated. An acetabulum is observed as a cell mass at the posterior two thirds of the body. Three pairs of penetrating glands occupy the middle part of body. Their ducts run forward across the dorsal side of oral sucker and open near both sides of the stylet shouldering. Nervous commissure is faintly recognized across the esophagus transversely. Excretory vesicle is U-shape, and opens at the junction of body and tail by a short duct. A pair of main collecting tubes runs forward dividing into anterior and posterior collecting tubes at both sides of acetabulum. The flame cell formula is not yet determined. Tail is shorter than the body, with fine cuticular annulations, without any excretory tubule, and is connected ventro-posteriorly with the body. Sporocyst is an irregular sack shaped body, containing a few cercariae and germ balls. It measures 150–300 μm in length.

Remarks: The cercaria of *Maritreminoides caridinae* (Yamaguti et Nishimura, 1944) Chen, 1957 was reported from *Oncomelania nosophora* in Japan by Ito (1952). The cercaria in the present study resembles greatly this cercaria in detailed structure, such as the measurements, shape of the stylet which is bent ventrally, the rudimentary acetabulum, and so on. Moreover both of them develop in the same genus of snail, *Oncomelania*. Because of the close resemblance of these two cercariae, the second intermediate host of the present one is presumed to be some kind of fresh water shrimp, such as *Neocaridina*. Mice served as an experimental final host, though the natural final host of *Maritreminoides caridinae* was already reported to be a snipe, *Rostratula benghalensis* in Japan.

According to Ito (1952), it was reported that among 5,500 snails collected from the endemic area of schistosomiasis in Kyushu, Japan, the incidence ratio of xiphidiocercariae was 1.8%, while that of schistosome cercaria 11.1%, with no case of double infection. He also noted that the dominant area of xiphidiocercariae was the more or less recessive area of schistosome cercariae. If this condition is the same in endemic areas of schistosomiasis in the Philippines, it will offer some useful suggestions for the control of schistosomiasis in future.

Cercaria leyteensis no. 6

Presumptive adult form: Monorchidae?
Snail host: *Oncomelania quadrasi*
Date, locality and infection rate:
On the whole, 2 out of 6,423, or 0.03%
Oct. 28, 1975, Pastrana (Socsocon),
1 out of 168, or 0.6%
Jan. 8, 1976, Pastrana (Socsocon),
1 out of 114, or 0.9%

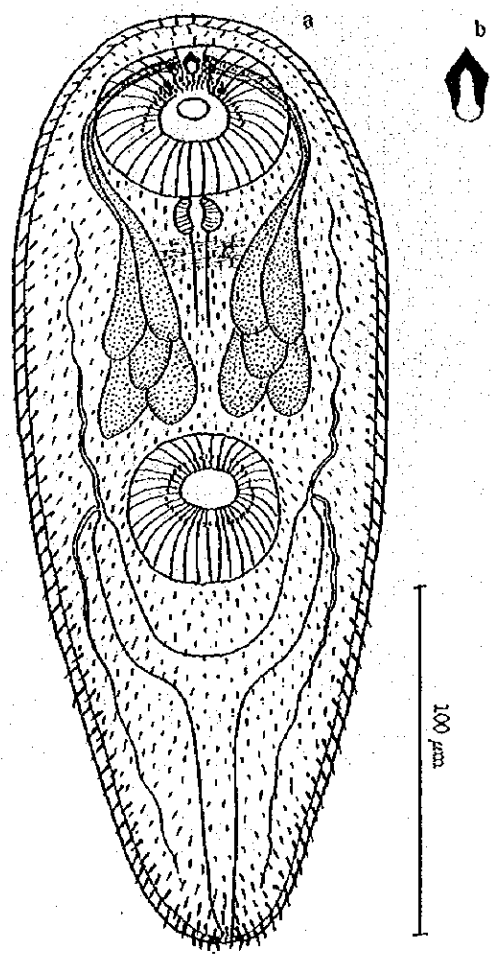
Measurements:

body .....267 (220–320)×103 (85–130)μm
oral sucker..... 43 (37– 50)× 54 (45– 60)μm
acetabulum .... 44 (40– 48)× 44 (40– 48)μm

Specific description: (Fig. 6)

Nonoculate, tailless cercaria with a very short stylet. Body is elongated ellipsoidal, being more blunt in the anterior part. The surface is covered with many backwardly directed spines of 2–3 μm long, which is increasing in number and size posteriorly. No sensory hair is observed. A well developed oral sucker is located subterminally, on the antero-medina of which is a very short rhomboidal, single pointed stylet, measuring about 7×4 μm in size. The anterior half margin of mouth opening is beset with approximately 40 minute spines. A globular acetabulum, smaller than the oral sucker, is situated slightly posterior at the middle of body. Its opening is also provided with about 50 minute spines arranged circularly. The mouth is followed by a faintly developed pharynx and a short esophagus. No cecum is observed.

Fig. 6. *Cercaria leyteensis no. 6.*



a. cercaria, b. stylet

A list of cercariae from *Oncomelania quadrasi* in Leyte Island, Philippines.
(Results of 6,423 snail examination during 1975–1976)

Species	Presumptive adult form	Number of snails infected	Body size	Locality
Cercaria leyteensis no. 1	Schistosomatidae (*S. japonicum*)	193 (3.00%)	160× 64 μm	Everywhere of surveyed area
Cercaria leyteensis no. 2	Diplostomatidae (*Diplostomum* or *Alaria*)	5 (0.08%)	114× 62 μm	Palo, Tanauan
Cercaria leyteensis no. 3	Notocotylidae	8 (0.12%)	433×214 μm	Palo, Santa Fe, Pastrana
Cercaria leyteensis no. 4	Lecithodendriidae	1 (0.02%)	97× 55 μm	Santa Fe
Cercaria leyteensis no. 5	Microphallidae (*Maritreminoides*)	4 (0.06%)	75× 34 μm	Palo, Santa Fe
Cercaria leyteensis no. 6	Monorchidae?	2 (0.03%)	267×103 μm	Pastrana

Five pairs of penetrating glands are observed between two suckers. The bundles of their ducts run forward and open at the side of stylet. A nervous commissure is recognized transversely across the esophagus. A large Y-shaped excretory vesicle is at the posterior half of body. Its anterior extremity ends at the side of acetabulum, from where an anterior and a posterior collecting tube arises. The flame cell formula is not determined yet.

Remarks: It has already been known that the group of non-tailed cercaria does not show any classificational group, but shows only divergent adaptational characters. According to the references, non-tailed cercariae have been found sporadically in a wide range of trematodes, such as Gorgoderidae, Zoogonidae, Brachylaimidae, Monorchidae, etc. Some cercariae of Gorgoderidae and Zoogonidae develop usually in sea clams such as *Musculium*, and some of Brachylaimidae in land snails such as *Helix*. Some members of Monorchidae develop in fresh water snails like *Lymnaea*, *Amnicola*, etc., in which the cercariae made their cysts to develop to metacercaria, and adult worms were reported from fresh water fishes.

The present cercaria is the first recorded discovery from *Oncomelania quadrasi*, and seems to be related to the family Monorchidae, especially to the genus *Asymphylodora*, though there is some differences between them.

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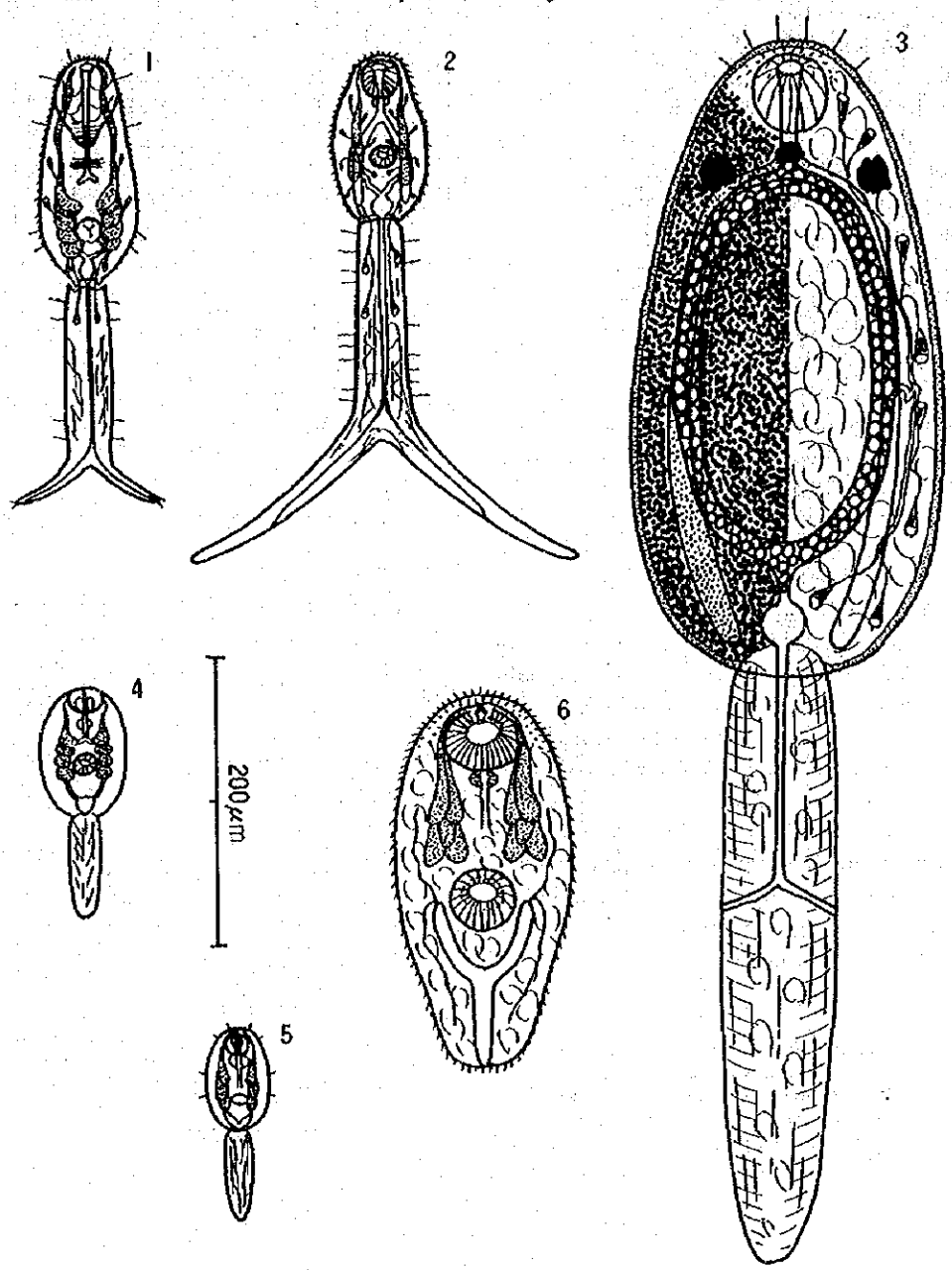
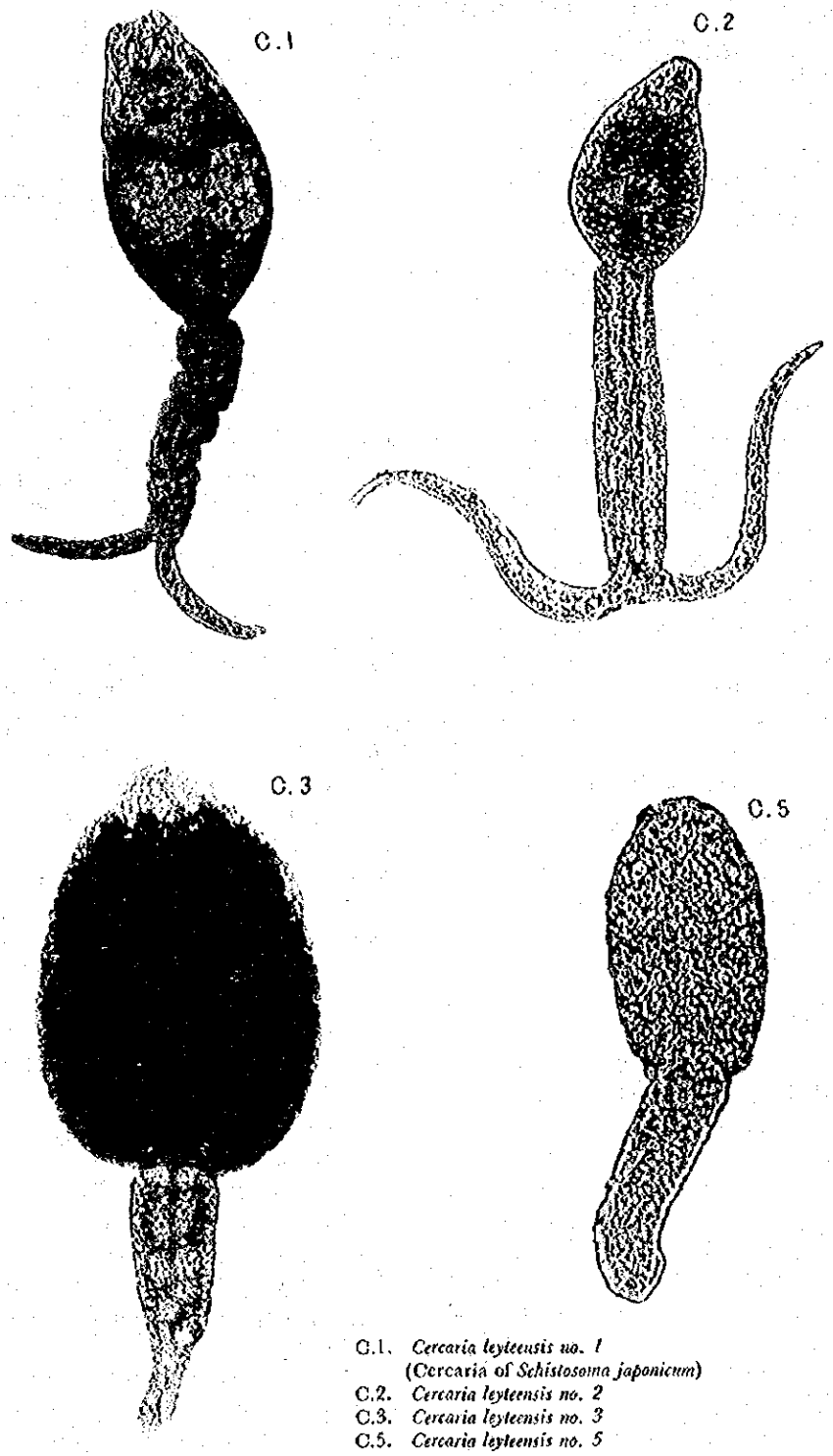
Plate 1. Cercariae from *Oncomelania quadrasi*, semidiagrammatic drawings by the same scale.



1. *Cercaria leyteensis no. 1* (Schistosomatidae) (Cercaria of *Schistosoma japonicum*)
2. *Cercaria leyteensis no. 2* (Diplostomatidae)
3. *Cercaria leyteensis no. 3* (Notocotylidae)
4. *Cercaria leyteensis no. 4* (Lecithodendriidae)
5. *Cercaria leyteensis no. 5* (Microphallidae)
6. *Cercaria leyteensis no. 6* (Monorchidae ?)

Plate 2. Cercariae from *Oncomelania quadrasi*, photographs by various scales.



C.1. *Cercaria leyteensis no. 1*
(Cercaria of *Schistosoma japonicum*)
C.2. *Cercaria leyteensis no. 2*
C.3. *Cercaria leyteensis no. 3*
C.5. *Cercaria leyteensis no. 5*

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Vol. 47, 4, p. 223-248, 1977]

Studies on the Fresh Water Cercariae in Leyte Island, Philippines[1)]

3. Cercariae from Thiaridae

(Received for Publication, February 15, 1977)

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Summary: From 1975 to 1976, about three thousand snails of Thiaridae were examined for cercarial fauna in the northeastern part of Leyte Island, Philippines. The thiarid snails examined were *Melanoides tuberculatus*, *Thiara* (*Plotiopsis*) *scabra*, *Antemelania dactylus* and *A. asperata*, and a total of 13 species of cercariae including some important human parasites were found. These cercariae are consisted of one philophthalmid cercaria, two echinostome cercariae, three heterophyid cercariae, five xiphidiocercariae, one paragonimid cercaria and one unknown cercaria. In this paper a description of their morphology, infection rate, locality, and some remarks on the presumptive life cycle are included with illustrations and photographs.

Introduction

To date in the Philippines, eight species of cercariae have already been reported from both fresh and brackish water snails of Thiaridae. Among them three species, namely, the cercaria of *Transversotrema laruei*, that of *Paramonostomum philippinensis* and that of *Procerovum calderoni* were reported from the brackish water snail, *Thiara riquetti* by Velasquez in 1961, 1969 and 1973 respectively. The other four species were reported from the fresh water snails of *Melania* spp. by Tubangui in 1928, who gave a proper name for each species as *Cercaria philippindica*, *C. parvomelania*, *C. melaniasperata*, and *C. maquilingui*. The remaining one species is cercaria of *Paragonimus* sp. which was reported by Tubangui, Cabrera and Yogore in 1950, from *Antemelania asperata* (=*Brotia asperata*). But all of these above mentioned cercariae were reported only from Luzon Island but not from Leyte Island yet.

During the past two years of 1975 and 1976, a total of 2,704 fresh water snails of Thiaridae were examined and 13 species of cercariae were detected on Leyte Island. Some cercariae of the important human parasites such as *Haplorchis*, *Centrocestus*, *Paragonimus* were found to exist commonly among these 13 species of cercariae on Leyte Island too. As already pointed out by Africa and others (1938), the heterophyid flukes are the important pathogens for inhabitants who eat raw fish on Luzon Island. It is revealed by the present study that this may be also the same condition on Leyte Island too. With regard to human paragonimiasis on Leyte Island, even though the prevalence of this disease was reported by Yogore (1958), or by Cabrera and Fevidal (1974), the detailed epidemiologic study seems to be still insufficient. Moreover the specific name of *Paragonimus* on Leyte, as well as on Luzon, is not determined yet at present.

These are some of the problems in connection with studies on cercariae obtained from Thiaridae. For the benefit of further investigation,

1) This work was performed as a part of the RP-Japan Cooperative Studies supported by the Japan International Cooperation Agency.

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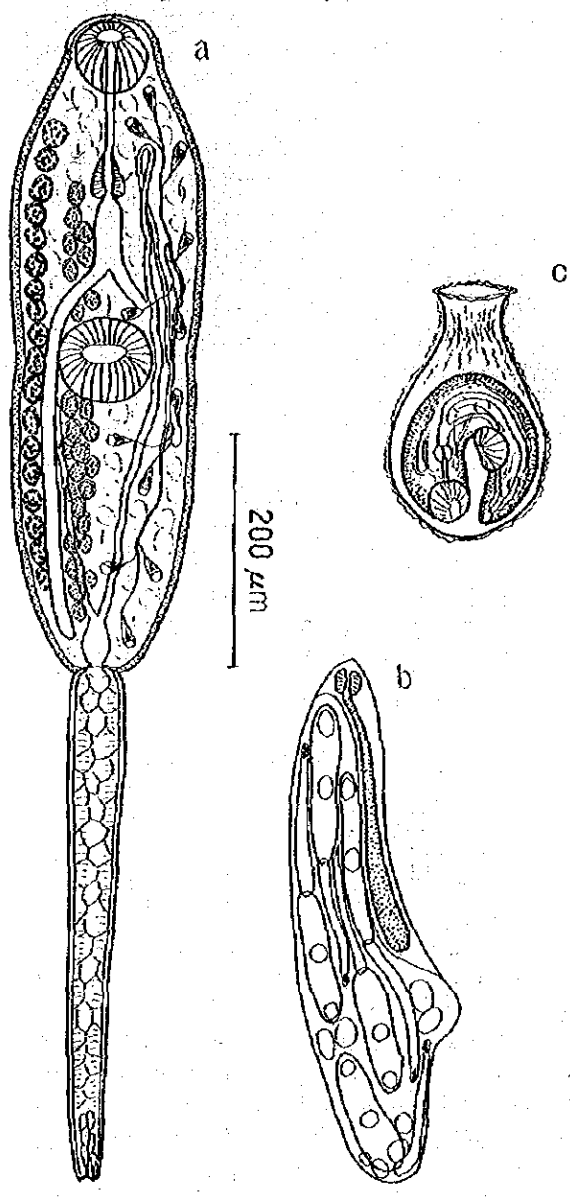
all the 13 species of cercariae found from Thiaridae on Leyte are described and illustrated with some remarks in the following. As noted in Part I of this series, these cercariae are tentatively named *Cercaria leyteensis no. 7* to *Cercaria leyteensis no. 19*. The methodology in the previous report is also followed in this study.

Fig. 7. *Cercaria leyteensis no. 7.*



a. cercaria, b. redia, c. cyst with a metacercaria

Description of cercariae

Cercaria leyteensis no. 7 (=*Cercaria philippindica* Tubangui, 1928)

Presumptive adult form: Philophthalmidae (*Philophthalmus* or *Cloacitrema*)

Snail host: *Meanoides tuberculatus*, *Antemelania dactylus*, and *A. asperata*

Date, locality and infection rate:

On the whole, 10 out of 2704, or 0.37%

Jan. 29, 1976, Dagami (Cabuluran), 1 out of 41, or 2.4%

Feb. 18, 1976, Santa Fe (San Juan), 5 out of 26, or 19.2%

Mar. 1, 1976, Jaro (Malobago), 2 out of 54, or 3.7%

Mar. 15, 1976, Jaro (Malobago), 2 out of 89, or 2.2%

Measurements:

body	570 (409–630) × 156 (140–175) μm
oral sucker	60 (55– 68) × 56 (53– 60) μm
prepharynx	55 (50– 60) μm in diameter
pharynx	36 (35– 40) × 27 (25– 30) μm
acetabulum	72 (70– 75) × 71 (70– 73) μm
tail	436 (380–500) × 36 (32– 40) μm

Specific description: (Fig. 7)

Large cercaria of Megalurous group of Cort (1915). The body is elongated cylindrical, widest at the pre-acetabular region and constricted at the level of acetabulum. The cuticle is 7 μm thick, and not provided with spine or hair. The oral sucker is subglobular in shape. The acetabulum is larger than the oral sucker and situated slightly posterior to the equatorial level. The mouth is followed by a long and narrow prepharynx, and a pear-shaped pharynx. An esophagus is much wider than the prepharynx, and bifurcates into two blind ceca reaching almost the posterior end of the body. Within the body is a thick layer of cystogenous materials obscuring the internal structures except the anterior part and around the acetabulum. In the premature cercariae, on the other hand, these materials are inside of many cystogenous gland cells. These cells are composed of at least two kinds of cells, one of which is coarsely granulated and arranged in the outerside, and the other one is finely granulated and arranged

in the innerside.

The excretory vesicle is bulbous, sending off two main collecting tubes until the side of the prepharynx. These tubes then turn back, making a loop, to the level of acetabulum, where they divide into an anterior and a posterior collecting tubules respectively. Each of these tubules receives 6 flame cells grouped into two. Thus the flame cell formula is 2 [(3+3)+(3+3)]=24. The tail is strongly capable of extension and contraction. It contains vesicular parenchyma along the central axis. Within the posterior one tenth of the tail are found about ten unicellular adhesive glands opening at the terminal invagination.

The redia has a large pharynx and a long intestine which reaches the posterior half of the body. One pair of locomotive appendages is observed. Several cercariae and germ balls are contained in the redia.

The cercariae encyst within 24 hours on any available object. The cyst has a peculiar pyriform or flask-like shape. The cyst wall is consisted of two layers, the outer is thick and brittle, the inner is thin and tough. Inside the cyst wall is an inactivate metacercaria which is bent back upon itself. No remarkable development of metacercaria was observed within a few days.

Remarks: This cercaria seems to be identical with *Cercaria philippindica* Tubangui, 1928. It was found from *Melania* sp. at Los Baños on Luzon Island by Tubangui. His description was rather meagre because of only one case discovered among thousands of snails, the report however was significant for cercarial fauna in the Philippines.

Recently Velasquez (1969) reported one kind of megalurous cercaria with its life history. The report stated that about 3 % of 3,375 snails, a brackish water snail, *Cerithium ornata*, harbored the cercaria in Navotas, Rizal, Philippines. According to her, encysted metacercariae were collected from the slit in finger bowls containing naturally infected snails and fed to laboratory-raised chicks and ducklings. The adults were recovered 12 to 15 days later from the cloaca of the birds. For this fluke a new name, *Cloacitrema philippinum*, was proposed by Velasquez. *Cercaria leyteensis no.* 7 and the cercaria of *Cloacitrema philippinum* resemble each other, but differ in many respects such as the snail host, the flame cell formula, the shape of cyst, etc.

The resemblance is also seen in the genus *Philophthalmus* belonging to the family Philophthalmidae. At present the life cycles of only two species of the genus, *P. gralli* and *P. hegeneri* have been reported in the United States. Both species are well known because the adult worm can be found from the orbit of birds, so that it is called the "eye fluke". It is also possible that the final host of the present cercaria found on Leyte would be a bird. The adult would be obtained from either the cloaca or from the eye of some birds after feeding the birds with this metacercaria.

Cercaria leyteensis no. 8

Presumptive adult form: Echinostomatidae (*Microparyphium* or *Echinochasmus*)

Snail host: *Melanoides tuberculatus*, *Antemelania dactylus*

Date, locality and infection rate:

On the whole, 7 out of 2704, or 0.26%

Jan. 29, 1976, Dagami (Maliwaliw), 1 out of 32, or 3.1 %

Feb. 6, 1976, Santa Fe (San Isidro), 1 out of 45, or 2.2 %

Feb. 9, 1976, Palo (Hubang), 4 out of 35, or 11.4%

Mar. 12, 1976, Tabontabon (Capohu-aw), 1 out of 3, or 33.3%

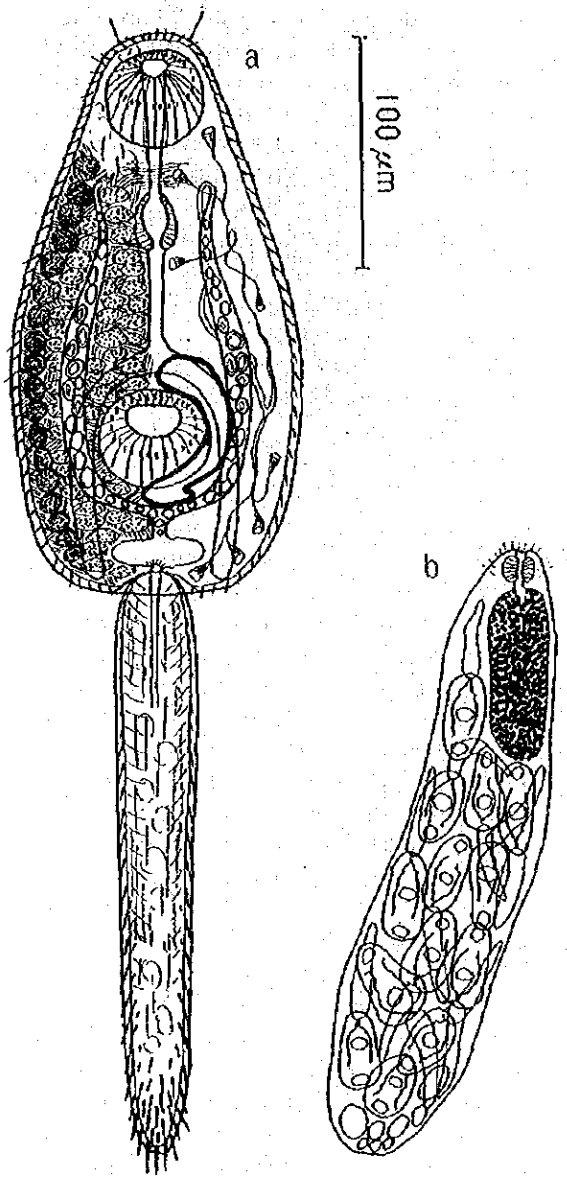
Measurements:

body	242 (200–300) × 124 (110–130) μm
oral sucker	44 (43– 45) × 40 (37– 45) μm
prepharynx	17 (15– 20) μm in length
pharynx	25 (20– 28) × 16 (13– 18) μm
acetabulum	42 (38– 45) × 47 (40– 55) μm
tail	253 (160–290) × 33 (30– 35) μm

Specific description: (Fig. 8)

Echinostome cercaria. The body is elliptical in shape, tapers at the anterior part and broadest at slightly anterior level of the acetabulum. Neither head collar nor spine has differentiated yet. The thick cuticle is covered with many spines which are directed

Fig. 8. *Cercaria leyteensis no. 8.*



a. cercaria, b. redia

backward. The oral sucker is subterminal, and provided with a semicircular transverse row of about 12 toothlets on its dorsal wall, and about 14 on its ventral wall. The acetabulum is about equal to the size of the oral sucker, and situated at or near the junction of middle section with the posterior third of the body. It is provided with a circle of about 31 spinelets around its aperture. A prepharynx, pharynx, and esophagus are well developed. The esophagus bifurcates anterior to the acetabulum. The ceca terminates blindly near the end of the body.

At least two kinds of cystogenous gland cells are found. One is located beneath the thick cuticle, containing the compacted coarse granules. The other one is extended throughout the body except at the cephalic region anterior to the pharynx and contains fine granules. Rod-shaped cystogenous materials are also found scattered in the body. At the right side of the acetabulum, a large cavity corresponding to the genital organ is observed. The nervous commissure is located across the prepharynx. The excretory vesicle is situated at the end of the body. Main collecting tubes which contain more than 20 excretory concretions each, arise from the mid-anterior margin of the vesicle, with a short common stem. These ascend on each side of the pharynx, form a loop, and finally divide at the level of the acetabulum into an anterior and a posterior collecting tubule. Each tubule terminates in two branches with two flame cells each. Thus the flame cell formula is $2[(2+2)+(2+2)]=16$.

The tail is longer than the body, slender, and is provided with backward directed spines mostly distributed at its end. The size of these spines becomes larger toward the distal end of the tail. The caudal excretory tube is detectable only at the anterior one third of the tail.

The redia is cylindrical in shape, with a slightly motile extremity. A collar and locomotive appendages are inconspicuous. The gut contains dark brown granular ingesta. About 20 cercariae in varying degrees of development are contained in one redia.

Remarks: This cercaria belongs apparently to the family Echinostomatidae, though neither head collar nor collar spine is differentiated yet in the cercarial stage. In

Japan, at least four species of echinostome cercariae have been reported from the snail of Thiaridae. Among them the cercaria of *Microparyphium kyushuensis* is much similar to the present cercaria. According to Koga (1952) who completed the life cycle of *M. kyushuensis*, several kinds of fresh water fish serve as the natural and experimental second intermediate host for this fluke. He reported also that cysts obtained from gills of these fish were fed to dogs with positive results, but other animals such as albino rat, mouse, duck and chick gave negative results. The other three species, *Echinochasmus grandis*, *E. milvi* and *E. tobi* have also fishes as their second intermediate host, and some birds and mammals as the final host. So the present cercaria presumably forms cyst in some fresh water fish, and the adult fluke can be obtained from birds or mammals after feeding them with the metacercaria.

Cercaria leyteensis no. 9

Presumptive adult form: Echinostomatidae (*Echinochasmus*)

Snail host: *Melanoides tuberculatus*, *Thiara scabra*, *Antemelania dactylus*, and *A. asperata*

Date, locality and infection rate:

On the whole, 18 out of 2704, or 0.67%
Nov. 18, 1975, Santa Fe (Maslog), 1 out of 38, or 2.6%
Nov. 18, 1975, Palo (Vicob), 8 out of 35, or 22.9%
Nov. 18, 1975, Pastrana (Socsocon), 1 out of 4, or 25.0%
Jan. 12, 1976, Palo (Upper Hubang), 2 out of 50, or 4.0%
Jan. 15, 1976, Palo (Nariwatan), 1 out of 11, or 9.1%
Jan. 19, 1976, Santa Fe (Maslog), 1 out of 29, or 3.4%
Feb. 3, 1976, Palo (Vicob), 2 out of 24, or 8.3%
Feb. 13, 1976, Palo (Nariwatan), 1 out of 32, or 3.1%
Mar. 1, 1976, Jaro (Malobago), 1 out of 145, or 0.7%

Measurements:

body	267 (240–290)	× 105 (90–120)	μm
oral sucker	39 (36– 43)	× 34 (29– 40)	μm
prepharynx	35 (28– 45)	μm in length	
pharynx	24 (20– 30)	× 18 (16– 25)	μm
acetabulum	36 (30– 38)	× 44 (40– 49)	μm
excretory vesicle	20 (17– 25)	× 31 (25– 35)	μm
tail	244 (200–260)	× 31 (28– 35)	μm

Specific description: (Fig. 9)

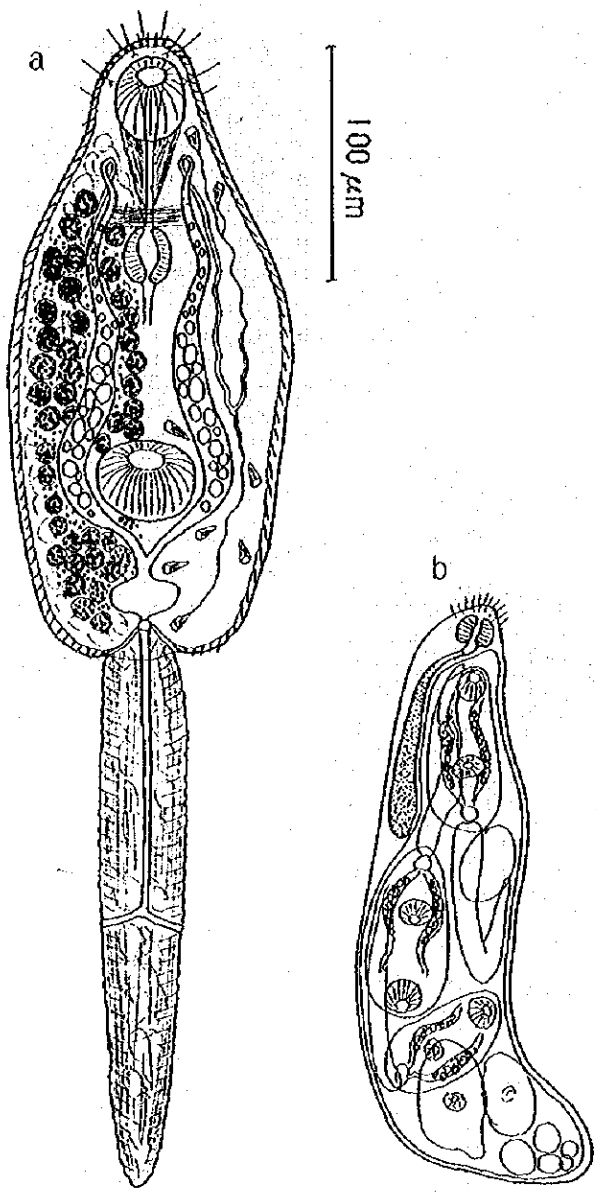
Echinostome cercaria. The body is elliptical in shape, tapers in its anterior portion, and broadest slightly posterior to the pharyngeal level. Neither head collar nor collar spine is differentiated yet. Thick cuticle of the body surface is covered with backward directed many spines. Several pairs of sensory hairs are at the anterior surface of the body. The oral sucker is subterminal. The acetabulum is slightly larger than the oral sucker, at the junction of middle with posterior third of the body. A well developed long prepharynx follows the oral sucker. Oblique muscle fibers from the oral sucker to the pharynx is prominent. Beneath the cuticle are found cystogenous gland cells filled with compact coarse granulated materials. Another kind of cystogenous gland cells with fine granules extends throughout the body except at the cephalic region. The cecum is not differentiated. The nervous commissure is located across the prepharynx.

One chambered excretory vesicle is located at the end of body. From its mid-anterior margin a short stem arises and divides itself into two main excretory tubes each of which contains more than 20 excretory concretions. These ascend on each side of the body until the level of prepharynx, where it forms a loop, and finally divides into an anterior and a posterior collecting tube at the level of anterior margin of the acetabulum. At least 8 pairs of flame cells were observed, but the flame cell formula could not be determined yet. The tail is slightly shorter than the body, slender and aspinose. The tail excretory tube bifurcates a little behind middle of the tail and opens laterally.

The redia is cylindrical, but more blunt in the posterior part. The collar and locomotive appendages are inconspicuous. Many sensory hairs are seen around the mouth opening. A well developed pharynx is followed by a gut containing dark brown granular ingesta. Less than 10 cercariae and germ balls are found in one redia.

Remarks: This cercaria belongs also to the member of Echinostomatidae. In the present study, altogether 9 species of echinostome cercariae were found on Leyte, but this

Fig. 9. *Cercaria leyteensis no. 9.*



a. cercaria, b. redia

cercaria is the only one which lacks an intestinal cecum. Because of this observation, this cercaria seems to resemble that of *Echinochasmus rugosus* or *E. rediduplicatus* which were found from *Viviparus malleatus* in Japan by Yamaguti (1933). He reported that these cercariae form cysts easily within the same snail host, or even in the redia, and the adult worms were obtained from mice after feeding experiments. Studies to clarify the life cycle of this cercaria should be carried on looking after such observations of Yamaguti (1933).

Cercaria leyteensis no. 10

Presumptive adult form: Unknown
Snail host: *Melanoides tuberculatus*
Date, locality and infection rate:
On the whole, 1 out of 2704, or 0.04%
Feb. 23, 1976, Palo (Upper Hubang), 1 out of 6, or 16.7 %

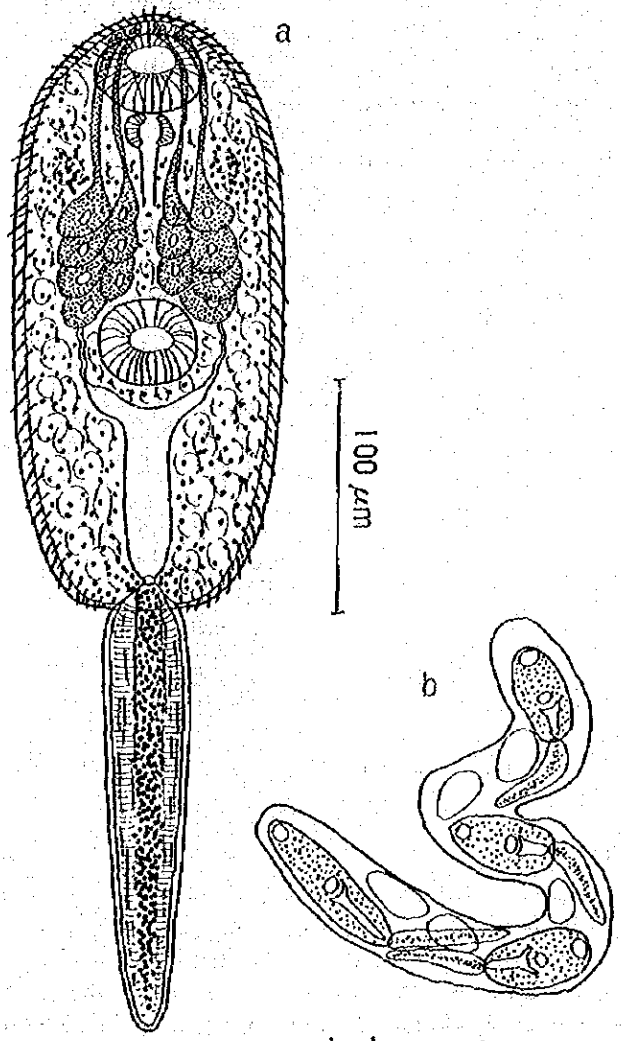
Measurements:

body	292 (260–340) × 114 (100–130) μm
oral sucker	33 (30– 35) × 44 (40– 50) μm
pharynx	16 (15– 17) × 20 (18– 22) μm
acetabulum	38 (35– 40) × 43 (40– 45) μm
tail	195 (150–250) × 37 (35– 50) μm

Specific description: (Fig. 10)

Simple-tailed distomatous, gymnocephalous large cercaria. The body is cylindrical, and covered with 7–10μm long spines all over the surface. The oral sucker is comparatively large, and is followed by a well developed pharynx and a short esophagus, but no cecum is distinguishable. The acetabulum is about the same size as the oral sucker, and situated at the middle of the body. Seven pairs of

Fig. 10. Cercaria leyteensis no. 10.



a. cercaria, b. sporocyst

penetrating gland cells are at the space between pharynx and ventral sucker. Among them three pairs are found inside and four pairs outside. Their ducts run upward and open in front of the oral sucker. Many brownish pigmented granules are distributed throughout the body, but not so dense as to make the body opaque. The excretory vesicle is long Y-shaped, non-epithelial. Neither collecting tube nor flame cell is detectable. The tail is shorter than the body, and aspinose. Brownish pigmented, irregular sized granules are distributed compactly within the axis of the tail. The sporocyst is long, cylindrical, unbranched, non-motile, and bent irregularly. It contains less than five cercariae.

Remarks: This cercaria was found in only one occasion in Leyte. It showed no remarkable characteristics except the tail which is densely filled up with pigmented granules. Up to the present, no similar cercaria has been reported. So It is unpresumable what kind of second host and final host will be necessary to complete the life cycle of this cercaria.

Cercaria leyteensis no. II

Presumptive adult form: Heterophyidae (*Haplorchis pumilio?*)

Snail host: *Melanoides tuberculatus*, *Antemelania dactylus*

Date, locality and infection rate:

On the whole, 82 out of 2704, or 3.03%

Oct. 10, 1975, Dagami (Hinulogan), 4 out of 9, or 44.4%
Nov. 18, 1975, Palo (Vicob), 1 out of 35, or 2.8%
Nov. 25, 1975, Pastrana (Socsocon), 1 out of 45, or 2.2%
Dec. 9, 1975, Limon (Limon), 6 out of 38, or 15.8%
Jan. 8, 1976, Pastrana (Socsocon), 11 out of 69, or 15.9%
Jan. 8, 1976, Santa Fe (Tibak), 5 out of 36, or 13.9%
Jan. 12, 1976, Palo (Upper Hubang), 10 out of 50, or 20.0%
Jan. 12, 1976, Santa Fe (Tibak), 1 out of 14, or 7.1%
Jan. 13, 1976, Santa Fe (Cogon na South), 1 out of 20, or 5.0%
Jan. 13, 1976, Palo (Upper Hubang), 2 out of 20, or 10.0%
Jan. 19, 1976, Santa Fe (Maslog), 1 out of 29, or 3.4%
Jan. 28, 1976, Santa Fe (Maslog), 1 out of 15, or 6.7%
Jan. 28, 1976, Dagami (Maliwaliw), 5 out of 40, or 12.5%
Jan. 29, 1976, Dagami (Maliwaliw), 5 out of 32, or 15.6%
Feb. 9, 1976, Palo (Hubang), 9 out of 35, or 25.7%
Feb. 25, 1976, Palo (Vicob), 1 out of 1, or 100.0%
Feb. 25, 1976, Santa Fe (Maslog), 4 out of 70, or 5.7%
Mar. 3, 1976, Santa Fe (San Juan), 2 out of 72, or 2.8%
Mar. 3, 1976, Pastrana (Socsocon), 12 outof 142, or 8.5%
Mar. 16, 1976, Palo (Upper Hubang), 1 out of 9, or 11.1%

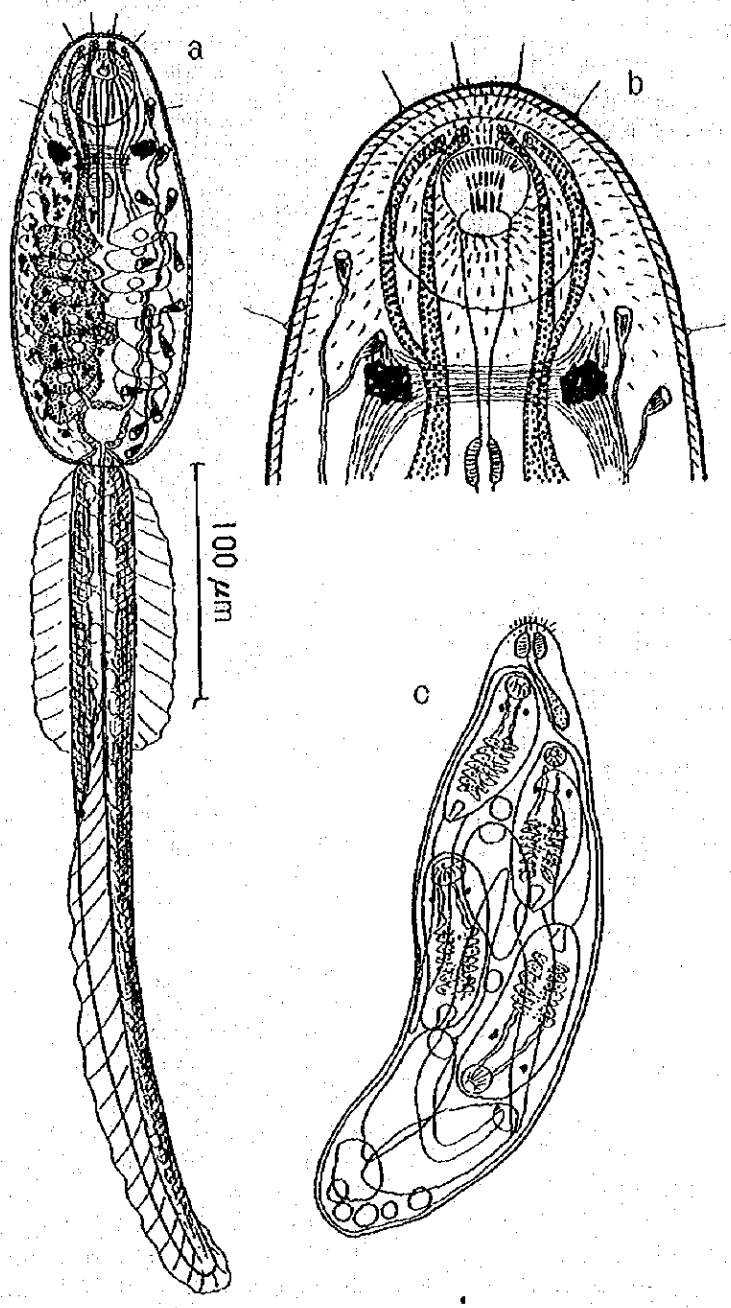
Measurements:

body	180 (150–200)×70 (59–78)μm
oral sucker	32 (28– 38)×26 (24–28)μm
eye spot	10 (7– 14)× 9 (7–10)μm
prepharynx	20 (16– 26)μm in length
pharynx	11 (8– 15)× 8 (7–10)μm
excretory vesicle	21 (19– 25)×24 (19–28)μm
tail	361 (310–410)×26 (25–28)μm
lateral fin	131 (120–160)×21 (15–24)μm

Specific description: (Fig. 11)

Biocellate, pigmented, parapleuro-lophocercous cercaria. The body is elliptical, and covered with fine reverse spines which is more dense at the anterior part. Several pairs of sensory hairs are also found on the anterior surface of the body. Many brownish pigmented granules are scattered throughout the body except in the cephalic region. The oral sucker is well developed. In the mouth cavity are thorn-like spines which is 2–3μm long, and arranged in three transverse rows. The number of spines in each row is about 5 (in lower), 7 (in middle) and 20 (in upper) respectively. A long prepharynx and a small pharynx are found but no cecum is recognizable. A cell mass of acetabulum is faintly observed at the posterior one third of the body if carefully searched for. One pair of prominent, irregularly shaped rectangular eye spots is seen at both sides of the prepharynx. Seven pairs of penetrating gland cells are arranged in longitudinal series, occupying a large part of the body cavity. Their ducts are in two bundles each, run through the dorsal wall of the oral sucker, and open in front of the sucker in 4 groups of 3:4:4:3. The excretory vesicle is epithelial, small and saccular in shape. The main collecting tube is divided into two branches

Fig. 11. *Cercaria leyteensis no. 11.*



a. cercaria, b. anterior part of body, c. redia

at the middle of the body, receiving 6 pairs of flame cells. The flame cell formula is 2[(2+2+2)+(2+2+2)]=24.

The tail is about twice as long as the body, provided with lateral fin-folds on its anterior one third, and dorso-ventral fin-fold on the posterior two thirds, extending around the tail tip to join with each other.

The redia is about 350 μm long and 100 μm wide, fusiform or sausage-shaped, more blunt in the posterior part. Many sensory hairs are around the mouth. The pharynx is followed by a short rhabdocoel gut filling with brownish pigmented ingesta. It contains several mature and maturing cercariae in addition with cercarial embryos.

Remarks: This cercaria was the most commonly found in the thiarid snails during any season in many towns on Leyte Island, especially in Palo, Santa Fe and Dagami. The highest infection rate of 44 % was found from Hinulogan in Dagami on October 10.

This cercaria belongs to Heterophyidae, especially to *Haplorchis* sp. At present in the Philippines, approximately 22 species of Heterophyidae have been reported, covering 15 genera. However their life histories have not been studied yet, except for only two species, *Haplorchis taichui* and *Procerovum calderoni*, both of which were reported by Velasquez (1973 a, b). She found the cercaria of *H. taichui* from *Melania juncea*, and that of *P. calderoni* from *Thiara riquetti*, on Luzon Island. The present cercaria differs from them, but is most similar to the cercaria of *Haplorchis pumilio* in its general feature. Though the high incidence of metacercaria of *H. pumilio* from fresh water fish was reported by Refuerzo and Africa (1936, 1939) and by Africa, Leon and Garcia (1940) in the Philippines, but no report of this cercaria have been made yet in this country.

According to Africa and others (1938), *Haplorchis pumilio*, as well as the other heterophyid trematodes, is the important pathogens for inhabitants who eat raw fish, because of egg embolism in brain, spinal, or cardiac areas. So it is now very obvious that the efforts to prohibit eating raw fish in Leyte should be given much attention, because the high incidence of *Haplorchis* cercaria in this island is revealed by the present study.

Cercaria leyteensis no. 12

(=*Cercaria parvomelania* Tubangui, 1928)

Presumptive adult form: Heterophyidae (*Haplorchis taichui?*)

Snail host: *Antemelania dactylus*

Date, locality and infection rate:

On the whole, 1 out of 2704, or 0.04%

Mar. 1, 1976, Jaro (Malobago), 1 out of 145, or 0.7%

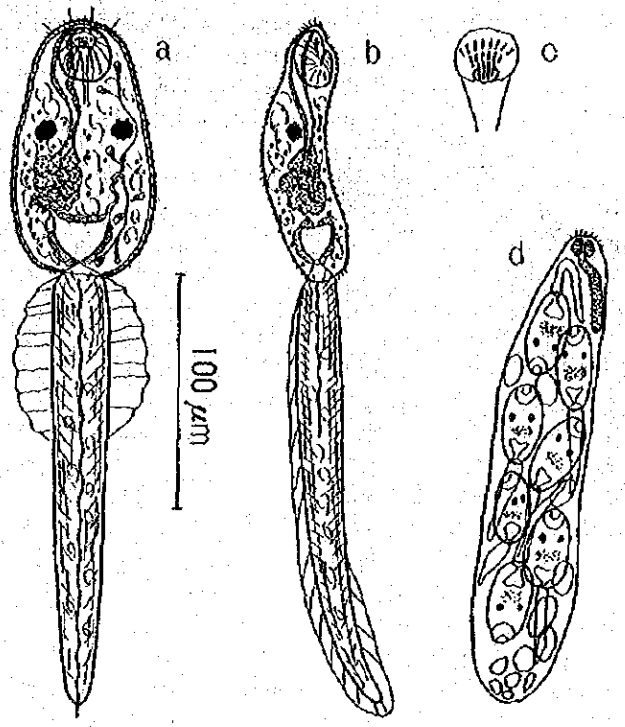
Measurements:

body .....112 (90–120)×60 (55–65) μm
oral sucker..... 23 (22– 25) μm in diameter
eye spot ..... 9 (7– 10) μm in diameter
tail .....190 (170–200)×27 (25–29) μm
lateral fin ..... 65 (60– 70)×16 (15–18) μm

Specific description: (Fig. 12)

Biocellate, pigmented, parapleuro-lophocercous cercaria. The body is elongated oval in shape, covered with fine reverse spines all over the surface, and with a few pairs of sensory hairs on the anterior surface. Brownish pigmented granules are observed throughout the body cavity, but fewer than that of the former species. The oral sucker is spherical in shape. The oral spines along the mouth cavity are arranged in three transverse rows, their number being 4 in the lower, 6 in the middle and 8 in the upper rows respectively. Only a prepharynx is present but neither pharynx nor cecum is distinguished. The primordium of acetabulum can barely be observable. One pair of conspicuous, irregularly rounded eye spots is situated at both sides of the level of anterior two fifths of the body. Seven pairs of penetrating gland cells appear as a mass, and occupies the space between the eye spot and the excretory vesicle. Their ducts run forward in two bundles each, and open in front of the oral sucker in four groups of 3:4:4:3. The excretory vesicle is epithelial and saccular in shape. The main collecting tube from the anterolateral corner of the vesicle is divided into

Fig. 12. *Cercaria leyteensis no. 12.*



a. cercaria, b. lateral view of cercaria, c. oral spine, d. redia

an anterior and a posterior branch at the middle of eye spots and the vesicle. Only seven pairs of flame cells are detected, but the flame cell formula could not be determined.

The tail is less than twice the length of the body, and provided with lateral fin-folds on the anterior one third, and a dorso-ventral fin-fold on the whole length of dorsal side and the posterior one third of ventral side. The dorso-ventral fin-fold is connected with each other at the tip of the tail.

The redia is simple, elongated, sausage-shape, and provided with some sensory hairs on the anterior part. A conspicuous pharynx is followed by a short gut filled with pigmented ingesta. It contains about 10 cercariae at various stages of development in addition with cercarial embryos.

Remarks: As far as the description shows, this cercaria is identifiable with *Cercaria parvomelania* Tubangui, 1928, which was reported from *Melania* sp. on Luzon Island.

A review of references shows that this cercaria is similar to that of *Haplorchis taichui*, the life cycle of which was already completely clarified by Nishigori (1927) in Japan, and by Martin (1958) in Hawaii. The existence of metacercaria of *H. taichui* on Luzon Island was already proven in the fresh water fish, *Ophicephalus striatus* by Africa and Garcia (1935), and by others. Recently Velasquez (1973) made a brief report about the life cycle of *H. taichui*. The report stated that the cercaria from *Melania juncea* on Luzon Island enters into some fresh water fish, and the adult worms were obtained from the small intestines of laboratory raised kitten fed with experimentally infected fish. However the report did not include the morphology of the cercaria.

Although this cercaria was encountered on one occasion only on Leyte Island, it may be presumed that this cercaria is not rare both on Leyte and Luzon Island. It can also be stated that the pathogenecity of *H. taichui*

and of *H. pumilio* was reported in details by Africa and others (1938).

Cercaria leyteensis no. 13

Presumptive adult form: Heterophyidae (*Centrocestus*)

Snail host: *Melanoides tuberculatus, Antemelania dactylus*

Date, locality and infection rate:

On the whole, 7 out of 2704, or 0.26%

Jan. 12, 1976, Palo (Upper Hubang), 3 out of 50, or 6.0%

Feb. 6, 1976, Santa Fe (San Isidro), 2 out of 45, or 4.4%

Feb. 9, 1976, Palo (Hubang), 2 out of 35, or 5.7%

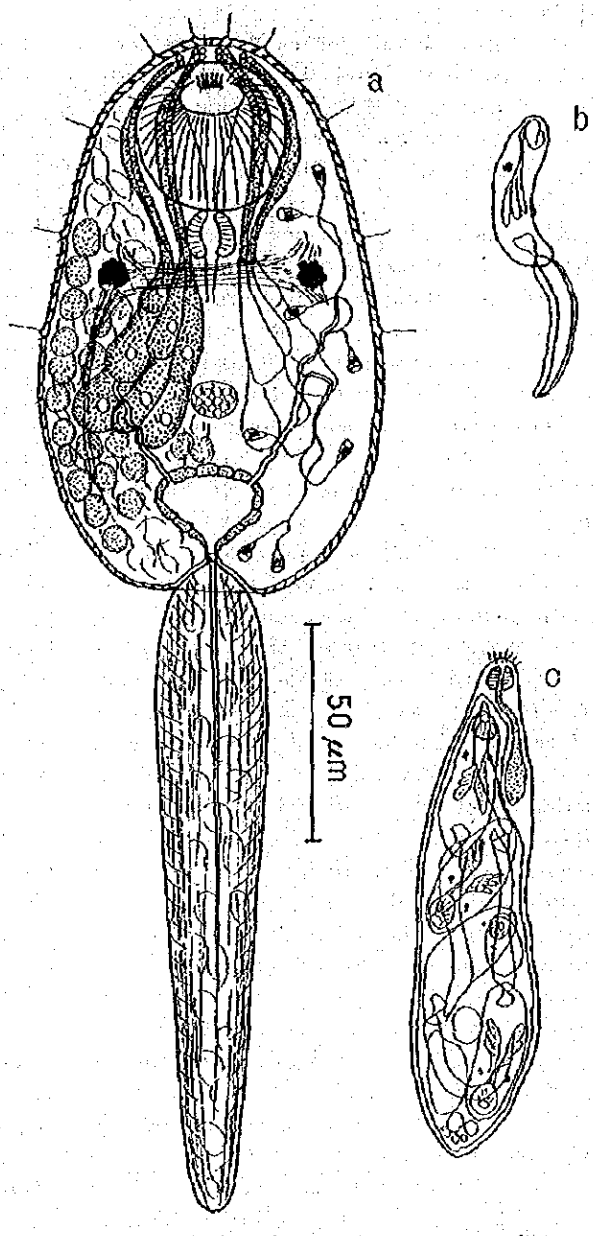
Measurements:

body	120 (105–130) × 74 (70–90) μm
oral sucker	33 (30– 40) × 31 (30–33) μm
pharynx	9 (8– 16) × 12 (10–15) μm
tail	140 (110–165) × 25 (20–28) μm

Specific description: (Fig. 13)

Biocellate, heterophyid cercaria with a simple tail. The body is oval in shape, blunt posteriorly, spinulate and more dense anteriorly. It is also provided with several pairs of sensory hairs on the surface. Five or six minute but prominent oral spines are seen on the dorsal wall of the mouth cavity which are arranged in one transverse row. The oral sucker is well developed. The acetabulum is rudimentary and appears as a small cell mass enclosed in a limiting membrane in front of the excretory vesicle. The pharynx follows the oral sucker directly. The esophagus extends backward for a short distance and then becomes indiscernible. One pair of prominent eye spots is observed at the level of anterior two fifths of the body, consisting of several small masses of dark brown pigmented granules. A transverse nervous commissure is found between two eye spots. Seven pairs of penetrating gland cells are arranged in a mass between the pharynx and the excretory vesicle. Their ducts open in four groups of 3 : 4 : 4 : 3 in front of the oral sucker. Cystogenous gland cells are scattered in the body cavity. Some light brown granules which are not so dense are also found throughout the body. The excretory vesicle is epithelial and saccular in shape. The main collecting tube is divided into an anterior and a posterior collecting tube, receiving four

Fig. 13. *Cercaria leyteensis no. 13.*



a. cercaria, b. lateral view of cercaria, c. redia

flame cells each. The flame cell formula is 2[(2+2)+(2+2)]=16.

The tail is slightly longer than the body, and inserted in the terminal socket of the body. It is slender, and devoid of spine or hair. A slightly developed dorso-ventral fin-fold extends to the whole length of the tail.

The redia is plump or elongated fusiform, and provided with several sensory hairs around the mouth opening. A small pharynx is followed by a comparatively large gut in the young redia, but a smaller one in the mature redia. It contains germ balls and cercariae at different developmental stages.

Remarks: This heterophyid cercaria is closely resembled the cercaria of *Centrocestus*. In the Philippines the presence of this genus has been reported by Vasquez-Colet and Africa (1939, 1940), who found the metacercariae of this genus from four species of fishes obtained from the markets in Manila. The report suggested the existence of more than one species of *Centrocestus* (=*Stamnosoma*). They reported one species of these metacercariae which was identified as *C. formosanus* after feeding laboratory animals with the metacercaria. A review of the descriptions of cercaria of *C. formosanus* reported by Nishigori (1924), Chen (1942) and Martin (1958) in the other countries, shows that the present cercaria is very similar to them, but differ only in the number and the arrangement of the oral spines. Nishigori reported the oral spines arranged in 2 rows but the number was not specified. Chen reported this oral spines as 4 in anterior row and 5 in posterior row. Martin reported 9 oral spines but did not mention the arrangement in rows. On the other hand the present cercaria shows only 5 or 6 in one row. It is difficult therefore to determine if the adult of this cercaria is *C. formosanus* or not at present.

***Cercaria leyteensis* no. 14**

(=*Cercaria maquilingui* Tubangui, 1928)

Presumptive adult form: Lecithodendriidae

Sanil host: *Melanoides tuberculatus*, *Antemelania dactylus*, and *A. asperata*

Date, locality and infection rate:

On the whole, 8 out of 2704, or 0.30%

Oct. 28, 1975, Palo (Gacao), 1 out of 22, or 4.5%
Jan. 20, 1976, Palo (Hubang), 1 out of 25, or 4.0%
Jan. 29, 1976, Dagami (Maliwaliw), 2 out of 32, or 6.3%
Mar. 1, 1976, Jaro (Malobago), 1 out of 54, or 1.9%
Mar. 15, 1976, Jaro (Malobago), 3 out of 149, or 2.0%

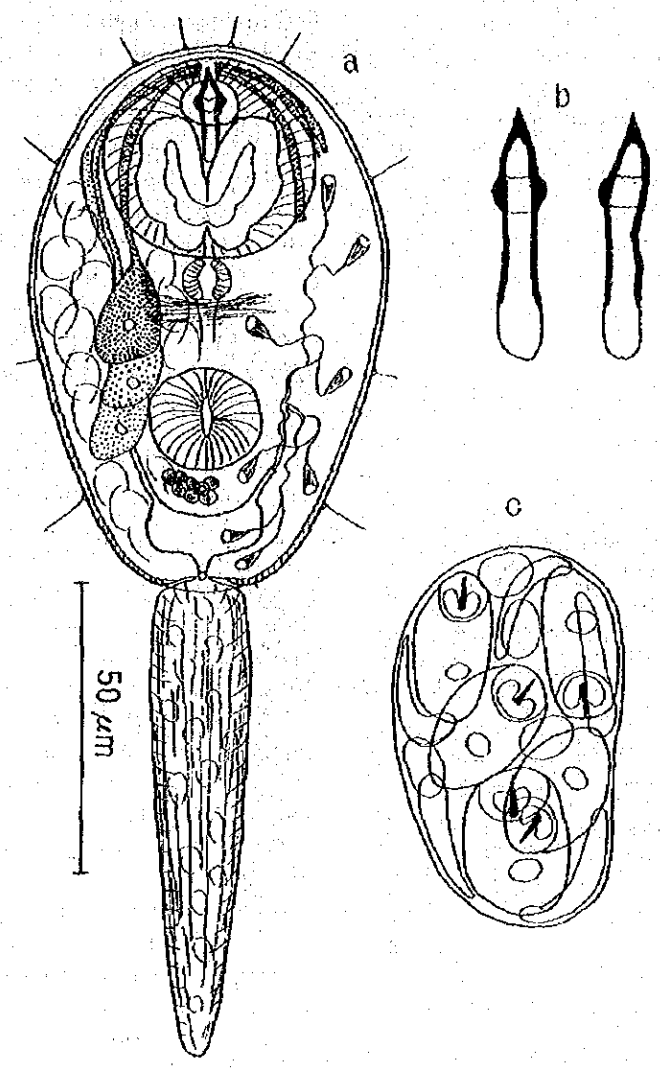
Measurements;

body .....94 (86–102)×61 (52–67)μm
oral sucker.....36 (31– 46)×35 (30–38)μm
stylet .....17 (15– 20)×3 (2.5–3.5)μm
pharynx..... 7 (6– 8)× 9 (7–10)μm
acetabulum ...19 (17–21)μm in diameter
tail .....85 (71–107)×16 (14–19)μm

Specific description: (Fig. 14)

Simple tailed, nonoculate, virgulate xiphidiocercaria. The body is oval in shape, more blunt in the anterior part, and covered with minute spines, more dense toward the posterior part. Five pairs of sensory hairs are on the body surface. A well developed spherical oral sucker contains a big virgula organ, and a shouldered large stylet which is bent slightly ventralward when observed laterally. A spherical acetabulum is about half size of the oral sucker, and located at the level of posterior one third of the body. The mouth leads into a small pharynx without any prepharynx. The esophagus is short and no cecum is distinguishable. Three pairs of penetrating gland cells are situated at both sides of the middle of the body. The first one contains coarse granules, the second one contains slightly coarse granules, and the last one is filled with fine granules. The corresponding ducts of these glands open at the anterior side of stylet, dorsal to the mouth opening. The excretory vesicle is cup-shaped. The main collecting tube divides at the level of acetabulum into an anterior and a posterior tubule, each of which in turn divides into two groups. The flame cell formula is 2[(2+2)+(2+2)]=16. A germinal cell mass is situated between the acetabulum and the excretory vesicle. The tail is slightly shorter than the body, and is not

Fig. 14. *Cercaria leyteensis no. 14.*



a. cercaria, b. stylet, dorsal and lateral view, c. sporocyst

provided with spine, hair and finfold. No excretory tube in the tail is observed.

The sporocyst is roundish oval in shape, 0.2 mm long and 0.15 mm wide. It contains 5–15 cercariae with some germ balls.

Remarks: Tubangui (1928) reported a new cercaria, *Cercaria maquilingui* from *Melania* sp. and *M. asperata philippinensis* on Luzon Island. The present cercaria seems to be similar to *Cercaria maquilingui*, though there are some differences. *Cercaria maquilingui* has a flame cell formula of $2\times6\times1=12$, while this cercaria has that of $2[(2+2)+(2+2)]=16$. The tail of the former species has very minute spines on its posterior half surface, which are not seen in this cercaria. Five pairs of sensory hairs are found on this cercaria, but none on the cercaria reported

by Tubangui. These differences may be due to the accuracy of observation, so the present species was identified as *Cercaria maquilingui*.

Like *Cercaria leyteensis no. 4*, this virgulate xiphidiocercaria is presumed to belong to the family Lecithodendriidae. Therefore, some aqueous larval insects, shrimps or some other arthropods may serve as its second intermediate host. For further details the remarks on *Cercaria leyteensis no. 4* in Part 2 of this series is referred to.

Cercaria leyteensis no. 15

(=*Cercaria melaniasperata* Tubangui, 1928)

Presumptive adult form: Microphallidae (Maritrematinae)

Snail host: *Melanoides tuberculatus*, *Antemelania dactylus*, and *A. asperata*

Date, locality and infection rate:

On the whole, 21 out of 2704, or 0.78%

Oct. 10, 1975, Dagami (Hinulogan), 1 out of 9, or 11.1%
Oct. 27, 1975, Pastrana (Socsocon), 1 out of 38, or 2.6%
Oct. 28, 1975, Palo (Gacao), 1 out of 22, or 4.5%
Nov. 14, 1975, Palo (Nariwatan), 2 out of 24, or 8.3%
Nov. 18, 1975, Santa Fe (Maslog), 4 out of 38, or 10.5%
Dec. 9 1975, Limon (Limon), 2 out of 38, or 5.3%
Jan. 28, 1976, Dagami (Maliwaliw), 1 out of 40, or 2.5%
Feb. 6, 1976, Santa Fe (San Isidro), 1 out of 45, or 2.2%
Feb. 9, 1976, Palo (Hubang), 1 out of 35, or 2.9%
Feb. 25, 1976, Santa Fe (Maslog), 3 out of 70, or 4.3%
Mar. 1, 1976, Jaro (Malobago), 2 out of 199, or 1.0%
Mar. 3, 1976, Santa Fe (San Juan), 1 out of 72, or 1.4%
Mar. 3, 1976, Pastrana (Socsocon), 1 out of 142, or 0.7%

Measurements:

body ..... 100 (85–115)×77 (67–85)μm
oral sucker ... 28 (25– 32)×28 (26–30)μm
stylet ..... 18 (16– 20)×3.5(3– 4)μm
pharynx ..... 7 (6– 8)× 8 (7– 9)μm
acetabulum ... 18 (16– 20)μm in diameter
tail ..... 80 (70– 90)×19 (17–22)μm

Specific description: (Fig. 15)

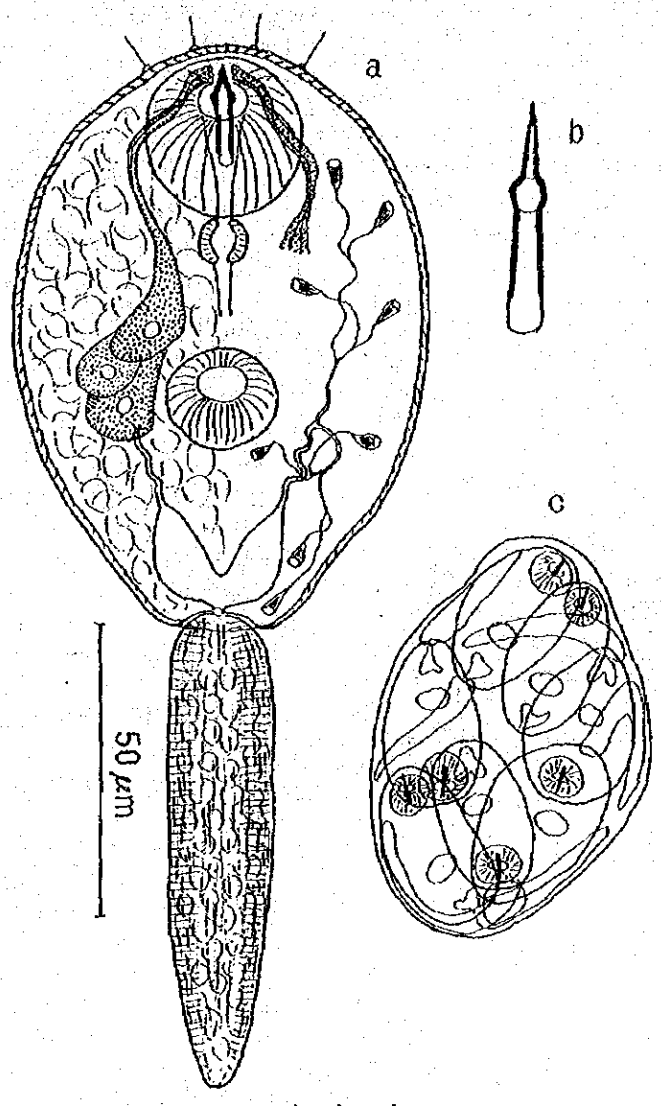
Simple-tailed, nonoculate, nonvirgulate xiphidiocercaria belonging to Microcotyle group of Lühe (1909). The body is oval in shape, coated with a thin cuticle which is provided with many minute spines all over the surface. Two pairs of sensory hairs are on the anterior surface. A well developed oral sucker is at the anterior end, in which a solid, sharply pointed, and markedly shouldered stylet is embedded at its median dorsal side. The acetabulum is slightly behind the middle of the body, smaller than the oral sucker, but well developed. The pharynx follows directly the oral sucker without any prepharynx, and is followed by a faintly recognized short esophagus. No cecum is observed. Three pairs of penetrating gland cells are at the side of the acetabulum. The middle one is filled with fine granules, and the outer two with coarse granules. Their ducts run forward across the lateral side of the oral sucker and open near the apical side of the stylet. The excretory vesicle is V-shape. From its anterolateral corner, one pair of main collecting tubes runs forward, and divides into an anterior and a posterior tubule at the side of the acetabulum. Each tubule receives four flame cells in two groups. The flame cell formula is $2\ [(2+2)+(2+2)]=16$. The tail is shorter than the body, coated with fine cuticular annulations, but without any spine or hair. No caudal excretory tube is recognized.

The sporocyst is irregular sac form in shape, with a thin smooth aspinose wall. It contains a few cercariae at nearly the same stage of development.

Remarks: This cercaria is rather common in Palo, Dagami, Pastrana, Santa Fe, Jaro and Limon. Tubangui (1928) reported a new cercaria, *Cercaria melaniasperata* from *Melania* sp. and *M. asperata philippinensis* on Luzon Island. As far as his description is concerned, the present cercaria is identifiable with that species, though there is only one difference. Tubangui reported a flame cell formula of $2\times6\times1=12$, while the present species shows that of $2\ [(2+2)+(2+2)]=16$. This difference is presumably due to his fail to notice.

This cercaria seems to belong to Microphallidae, particularly to Maritrematinae because of its general appearance. There are many reports on some stages of the life cycle of many species of Maritrematinae, for example from their metacercariae to the adult stage, however there are only a few reports on their

Fig. 15. *Cercaria leyteensis no. 16.*



a. cercaria, b. stylet, c. sporocyst

complete life cycle in the world. According to the references, the second intermediate host covers a wide range of arthropods, such as crabs, shrimps, aqueous larval insects, etc. The final hosts are birds and mammals. An attempt to study the life cycle of this cercaria will be rather difficult because of difficulties in maintaining the experimental arthropod host. The discovery of a natural infection for this metacercaria may serve for this purpose.

Cercariae leyteensis no. 16

Presumptive adult from: Microphallidae (Maritrematinae)

Shail host: *Melanoides tuberculatus, Antemelania dactylus*

Date, locality and infection rate:

On the whole, 7 out of 2704, or 0.26%
Jan. 13, 1976, Palo (Upper Hubang), 1 out of 20, or 5.0%
Jan. 19, 1976, Santa Fe (Maslog), 2 out of 29, or 6.9%
Feb. 2, 1976, Dagami (Maliwaliw), 1 out of 76, or 1.3%
Feb. 25, 1976, Santa Fe (Maslog), 3 out of 70, or 4.3%

Measurements:

body ..... 84 (70–90)×54 (52–60)μm
oral sucker ... 25 (23–27)×23 (20–25)μm
stylet ..... 15 (14–16)× 5 (4– 6)μm
acetabulum ... 15 (13–16)×18 (16–20)μm
tail ..... 53 (50–57)×20 (19–21)μm

Specific description: (Fig. 16)

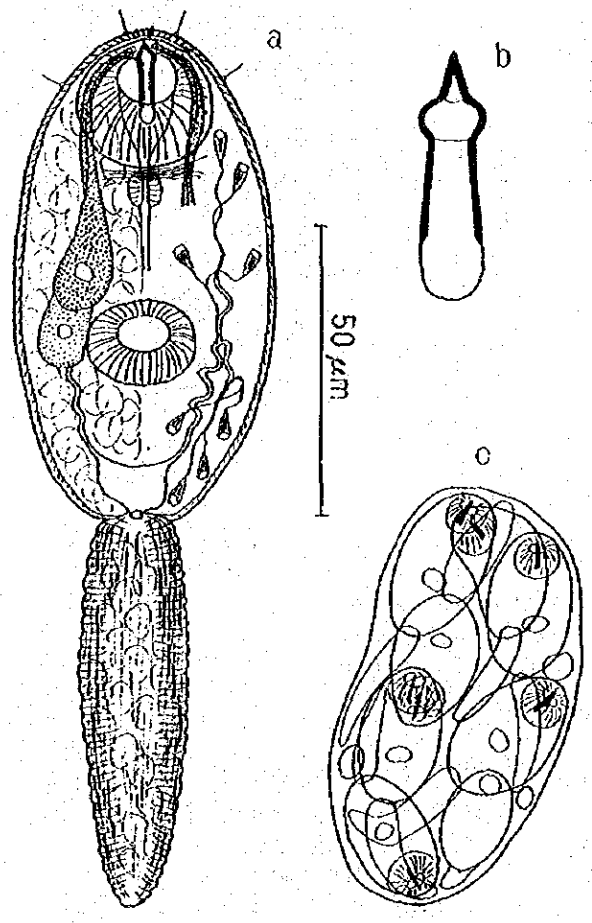
Simple-tailed, nonoculate, nonvirgulate xiphidiocercaria belonging to Microcotyle group of Lühe (1909). The body is ellipsoidal in shape, coated with a thin cuticle and with many minute spines all over the surface. Two pairs of sensory hairs are on the anterior surface. A well developed oral sucker is situated at the anterior end, in which a solid, sharply pointed and strongly shouldered stylet is embedded at its median dorsal side. The acetabulum is smaller than the oral sucker and situates slightly behind the middle of the body. Only a pharynx and a short esophagus are recognized with neither prepharynx nor cecum. Two pairs of penetrating gland cells are found at the side of the acetabulum, the anterior one being filled with coarse granules, and the posterior one with fine granules. Their ducts run forward across the lateral side of the oral sucker, and open near the apical side of the stylet.

The excretory vesicle is cup-shaped, from which one pair of main collecting tubes arises and runs forward, then divides into an anterior and a posterior collecting tubule. Each tubule receives four flame cells. The flame cell formula is 2[(2+2)+(2+2)]=16. The tail is slender and shorter than the body. It is coated with fine cuticular annulations. No caudal excretory tube is observed.

The sporocyst is irregularly ellipsoidal in shape with a thin aspinose smooth wall. It contains less than 10 cercariae with some germ balls.

Remarks: This cercaria resembles closely to the former species, *Cercaria leyteensis no. 15*, but differs in the body size and the number of penetrating gland cells. The present one is smaller, and possesses only two pairs of penetraing glands, while *Cercaria leyteensis no. 15* is slightly larger, and has three penetrating glands. As to its life cycle, the remarks in the former species will be referred to.

Fig. 16. *Cercaria leyteensis no. 16.*



a. cercaria, b. stylet, c. sporocyst

Crrcariae leyteensis no. 17

Presumptive adult form: Microphallidae (Microphallinae)
Snail host: *Melanoides tuberculatus*
Date, locality and infection rate:

On the whole, 1 out of 2704, or 0.04%

Feb. 3, 1976, Palo (Vicob), 1 out of 24, or 4.2%

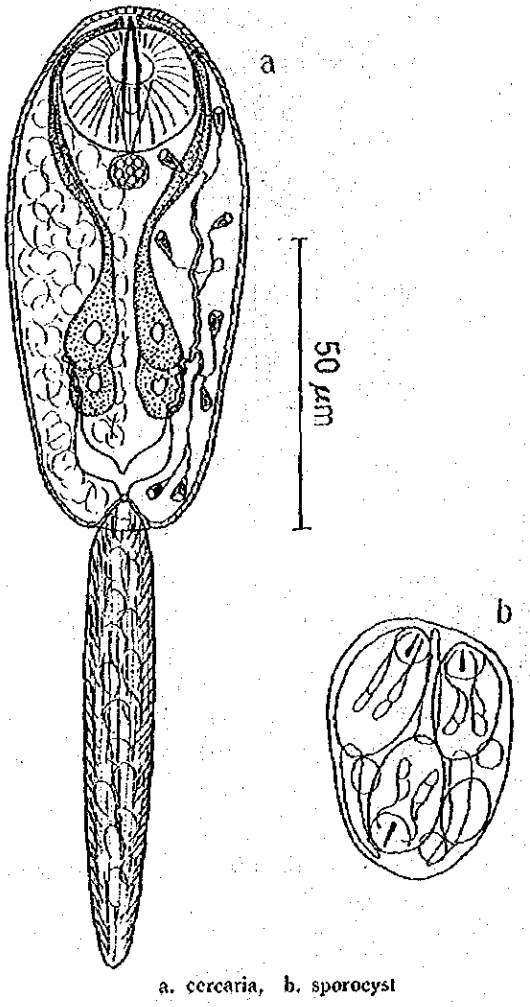
Measurements:

body ..... 85 (80–90) × 37 (36–38) μm
oral sucker ... 23 (21–26) × 22 (21–24) μm
stylet ..... 16 (15–17) × 4 (3– 5) μm
pharynx ..... 6 (5– 7) × 5 (4– 6) μm
tail ..... 85 (76–90) × 13 (12–14) μm

Specific description: (Fig. 17)

Simple-tailed, monostomous, nonvirgulate, small xiphidiocercaria of Ubiquita type belong-to Microcotyle group of Lühe (1909). The body is elongated oval- or tongue-shaped, with scale-like minute spines all over the surface. The oral sucker is well developed, in which a long slender, non-shouldered stylet is embedded. No acetabulum is recognizable. The digestive system consists of only a pharyngeal primordium which is faintly observable as a cell mass just posterior to the oral sucker. Two pairs of penetrating gland cells occupy the posterior part of the middle third of body. Their ducts run upward and open at the side of apical end of stylet. The excretory vesicle is bicornuated and non-epithelial. The main collecting tube divides at the side of penetrating glands into an anterior and a posterior tube, receiving four flame cells each. The flame cell formula is 2 [(2+2)+(2+2)]=16. The tail is as long as the body, slender, without any appendages but with somewhat fine cuticular annulations. It is slightly inserted at the end of body. The tail excretory tube is obliterated.

Fig. 17. *Cercaria leyteensis no. 17.*



a. cercaria, b. sporocyst

The sporocyst is spherical to oval with thin delicate wall and sometimes moves passively due to the activity of cercariae in it. A few cercariae and several germ balls are contained in it.

Remarks: This very minute xiphidiocercaria was found in only one occasion in Vicob, Palo. It belongs to the Ubiquita group of Sewell (1922). Therefore it will develop to the genus *Spelotrema* of Microphallidae because of its general features like for example the absence of an acetabulum in the cercarial stage. Even though the present cercaria is presumed to develop to *Spelotrema* sp., there are still some differences, such as the number of penetrating glands and the flame cell formula. About 10 species of *Spelotrema* cercariae have been reported from various countries at present. Most of them have four pairs of penetrating glands, and a flame cell formula of 2 [(1+1)+(1+1)]=8. However the present one has only two pairs of penetrating glands and the flame cell formula is 2 [(2+2)+(2+2)]=16. It seems that cercaria will encyst in some crustacean intermediate host such as crabs or shrimps. It has been reported also that some of *Spelotrema* cercariae can encyst in the same snail host, or even in

their own sporocyst. After feeding ducklings or laboratory mammals with this cyst, the adult worm would be easily obtainable.

Cercaria leyteensis no. 18

Presumptive adult form: Microphallidae (Microphallinae)

Snail host: *Antemelania asperata*

Date, locality and infection rate:

On the whole, 2 out of 2704, or 0.07%

Mar. 15, 1976, Jaro (Malobago), 2 out of 89, or 2.2%

Measurements:

body .....88 (83-98)×44 (38-55)μm
oral sucker ...23 (20-30)×21 (18-30)μm
stylet .....14 (13-15)× 3 (2- 4)μm
tai .....98 (80-110)×9 (8-10)μm

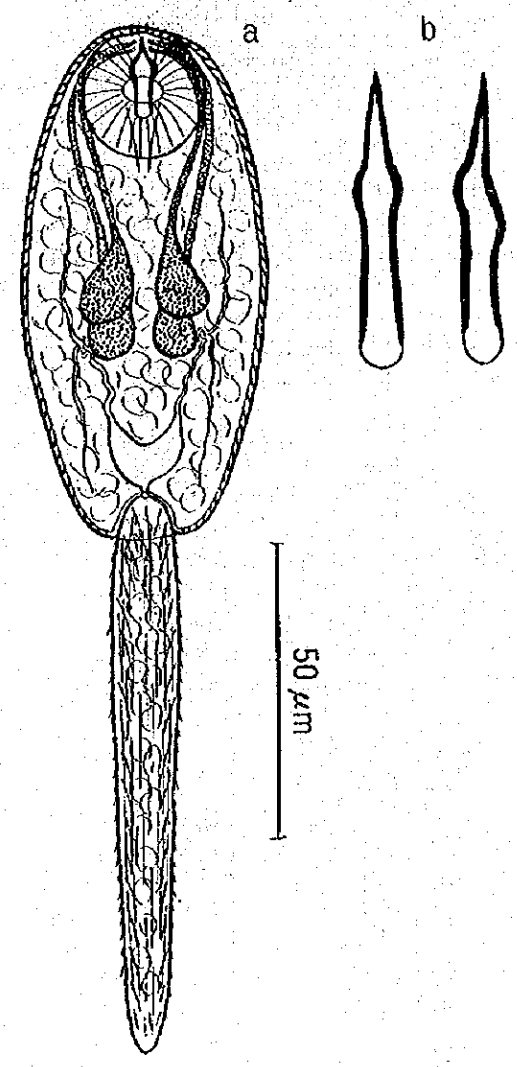
Specific description: (Fig. 18)

Simple-tailed, monostomous, nonvirgulate, small xiphidiocercaria of Ubiquita type belonging to Microcotyle group of Lühe (1909). The body is elongated oval- or tongue-shaped, with scale-like minute spines all over the surface. Within a well developed oral sucker is embedded a solid, and shouldered stylet which is slightly bent ventralward. No digestive system was recognized yet. Two pairs of penetrating gland cells are at the middle of body. Their ducts run forwards and open at the side of the apical end of stylet. The excretory vesicle is cup-shaped. From its antero-lateral corner the main collecting tube arises anteriorly, then divides near the side of penetrating glands into an anterior and a posterior tubules. The flame cell formula could not be determined. The tail is slightly longer than the body. It is slender, and provided with minute scale-like spines directed backward on the surface. The caudal excretoly tubule is obliterated.

The sporocyst is spherical to oval in shape, with thin delicate wall. A few cercariae and several germ balls are contained in it.

Remarks: This cercaria and *Cercaria leyteensis no. 17* are similar, but differ in the shape of the stylet, the presence or absence of a pharynx and spines on the tail. Like *Cercaria leyteensis no. 17*, this cercaria is also expected to develop to Microphallinae. Some crustaceans will serve as its second intermediate host, while birds or mammals will act as the final host.

Fig. 18. *Cercaria leyteensis no. 18.*



a. cercaria, b. stylet, dorsal and lateral view

Cercaria leyteensis no. 19

Presumptive adult form: Paragonimidae (*Paragonimus*)

Snail host: *Antemelania asperata, Antemelania dactylus*

Date, locality and infection rate:

On the whole, 3 out of 2704, or 0.11%
Mar. 1, 1976, Jaro (Malobago), 1 out of 54, or 1.9%
Mar. 15, 1976, Jaro (Marobago), 2 out of 89, or 2.2%

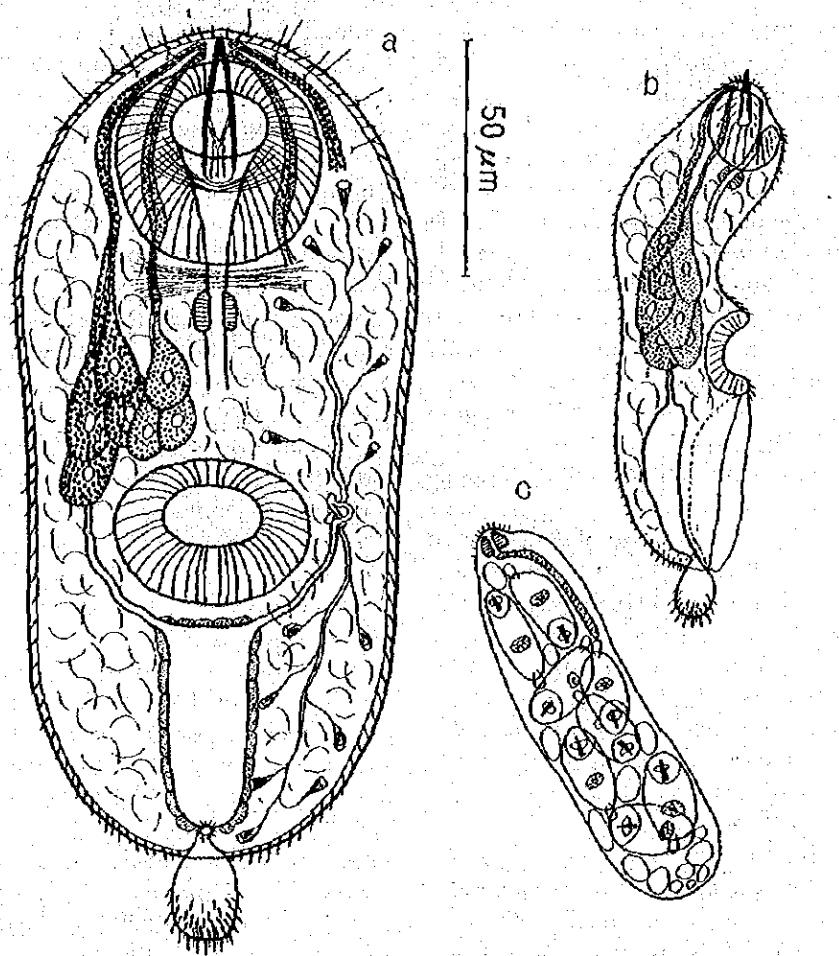
Measurements:

body	179	(150–190) × 85	(77–95) μm
oral sucker	44	(40– 48) × 42	(30–50) μm
stylet	31	(29– 33) × 7	(6– 8) μm
prepharynx	8	(6– 10) × 8	(6–10) μm
pharynx	10	(9– 11) × 10	(9–11) μm
acetabulum	31	(25– 35) × 40	(35–45) μm
tail	20	(15– 24) × 15	(13–18) μm

Specific description: (Fig. 19)

Typical microcercous cercaria belonging to Paragonimidae. The body is ellipsoidal in shape, widest at the level of the prepharynx, and narrowest at the level of the acetabulum. A wide median groove is observed on the ventral side of the posterior half of the body. The body is covered with a somewhat thick cuticle provided with many spines directed backward. On the anterior surface of the body, especially around the mouth opening, there are observed many sensory hairs. The oral sucker is well developed. Within it, a strong, non-shouldered stylet is embedded.

Fig. 19. *Cercaria leyteensis no. 19.*



a. cercaria, b. lateral view of cercaria, c. redia

Several radial muscles coming from the anterior body wall and attaching to the base of the stylet, make the stylet move incessantly. The prepharynx and pharynx follow the oral sucker, but no cecum was recognized except only a short esophagus. The nervous commissure is butterfly-shaped, and situated transversely across the prepharynx. A well developed acetabulum, slightly smaller than the oral sucker, is located posteriorly to the middle of the body. Seven pairs of penetrating gland cells are observed between the pharynx and the acetabulum. The inner three pairs contain fine granules, and the outer four pairs contain coarse granules. Their ducts are grouped in four bundles, and open at both sides of the apical end of the stylet.

A large cylindrical I-shaped excretory vesicle occupies the posterior part of the body. It reaches to the acetabulum, and is lined with a layer of epithelial cells. From its anterolateral corner a main collecting tube arises forward, makes some convolution at the side of the acetabulum, then divides into anterior and posterior tubules, receiving about seven flame cells each. The flame cell formula could not determined, yet. A conical-shaped short tail is inserted into the posterior end of the body. The posterior half of the tail is provided with many spines giving the appearance of shagginess.

The daughter redia is elliptical or cylindrical in shape, and 0.4–0.7×0.2–0.3 mm in size. The pharynx is about 50μm in diameter. This is followed by an intestine extending to the middle of the body. The intestine contains brownish food particles. Many sensory hairs are observed around the mouth opening of the redia. In one redia less than 10 cercariae in various stages of developement and some germ balls are contained.

Remarks: This cercaria will develop undoubtedly into the famous human lung fluke, *Paragonimus* sp. In the Philippines the existence of human paragonimiasis among Filipinos was already reported by Musgrave (1907), but there had been no other reports until 1946 when Tubangui made preliminary notes on its crustacean vector. In order to look for the cercaria and metacercaria of *Paragonimus*, he made studies on the snail, *Melania* spp., the crab, *Potamon* sp., and the prawn, *Palaemon* spp. in Naga, Luzon Island in 1946. He found the metacercaria from *Potamon* sp. with an infection rate of 60%, but no cercaria from *Melania* spp. was found. Tubangui fed rats and cats with these metacercariae and obtained the adults of *Paragonimus*, for which he did not give any specific name. Later on Tubangui, Cabrera and Yogore (1950) made a preliminary report on the life cycle of the human lung fluke in the Philippines. They found metacercariae from a crab, *Parathelphusa* (*Barythelphusa*) *mistio* in Nagaad, Pili, Camarines on Luzon Island. They also discovered the cercaria from *Antemelania asperata* (=*Brotia asperata*) in Sorsogon, Luzon Island with an incidence of 2.0 or 0.10% out of 1986 specimens. However a description of the cercaria was not included in their report.

The first report on the prevalence of paragonimiasis in Leyte Island was made by Yogore in 1958. He revealed that 4.8% of the inhabitants in Jaro was suffering from paragonimiasis. Recently Cabrera and Fevidal (1974) reported again the prevalence of human paragonimiasis in Jaro, Leyte, with a result of 12.5% of infection rate in the barrio of Pitogo and Buri. But both of these reports did not include the study on cercariae of this *Paragonimus*.

After reviewing the above mentioned reports, the discovery of this cercaria from *Antemelania asperata* and *A. dactylus* in Jaro, Leyte seems to be the first report. Consequently there appears several problems to be solved in this endemic area in Jaro; that is, a comprehensive survey of the prevalence of paragonimiasis among the inhabitants, a study on the complete life cycle of this fluke, an epidemiological study on the distribution of the first and the second intermediate hosts, etc. But above all the determination of the scientific name of

this lung fluke is of paramount importance.

At present nearly 23 species of *Paragonimus* have been reported from many countries in the world. Among them only 7 species have been known with regard to their complete life cycles; namely, *Paragonimus westermani*, *P. kellicotti*, *P. iloktsuensis*, *P. ohirai*, *P. miyazakii*, *P. sadoensis* and *P. szechuanensis*. Comparing the present cercaria to these already known cercariae, no identifiable species can be found because of some slight differences, especially because of the smaller size of the present cercaria. So it will be expected that the present cercaria will probably belong to some new species, or at least to some new sub-species of *Paragonimus*. Further study on the life cycle of this cercaria would clarify this problem in the future.

A list of cercariae from Thiaridae in Leyte Island, Philippines.

(Results of 2,704 snail examination during 1975–1976)

Species	Presumptive adult form	Number of snails infected	Body size (μm)	Locality
Cercaria leyteensis no. 7 (*C. philippindica*)	*Philophthalmidae* (*Philophthalmus* or *Cloacitrema*)	10 (0.37%)	570×156	Santa Fe, Dagami, Jaro
Cercaria leyteensis no. 8	Echinostomatidae (*Microparyphyum* or *Echinochasmus*)	7 (0.26%)	242×124	Palo, Santa Fe, Dagami, Tabontabon
Cercaria leyteensis no. 9	Echinostomatidae (*Echinochasmus*)	18 (0.67%)	267×105	Palo, Santa Fe, Pastrana, Jaro
Cercaria leyteensis no. 10	Unknown	1 (0.04%)	292×114	Palo
Cercaria leyteensis no. 11	Heterophyidae (*Haplorchis pumilio* ?)	82 (3.03%)	180×70	Palo, Santa Fe, Pastrana, Dagami, Limon
Cercaria leyteensis no. 12 (*C. parvomelania*)	Heterophyidae (*Haplorchis taichui* ?)	1 (0.04%)	112×60	Jaro
Cercaria leyteensis no. 13	Heterophyidae (*Centrocestus*)	7 (0.26%)	120×74	Palo, Santa Fe
Cercaria leyteensis no. 14 (*C. maquilingui*)	Lecithodendriidae	8 (0.30%)	94×61	Palo, Dagami, Jaro
Cercaria leyteensis no. 15 (*C. melaniasperata*)	Microphallidae (Maritrematinae)	21 (0.78%)	100×77	Palo, Santa Fe, Pastrana, Dagami, Jaro, Limon
Cercaria leyteensis no. 16	Microphallidae (Maritrematinae)	7 (0.26%)	84×54	Palo, Santa Fe, Dagami
Cercaria leyteensis no. 17	Microphallidae (Microphallinae)	1 (0.04%)	85×37	Palo
Cercaria leyteensis no. 18	Microphallidae (Microphallinae)	2 (0.07%)	88×44	Jaro
Cercaria leyteensis no. 19	Paragonimidae (*Paragonimus*)	3 (0.11%)	179×85	Jaro

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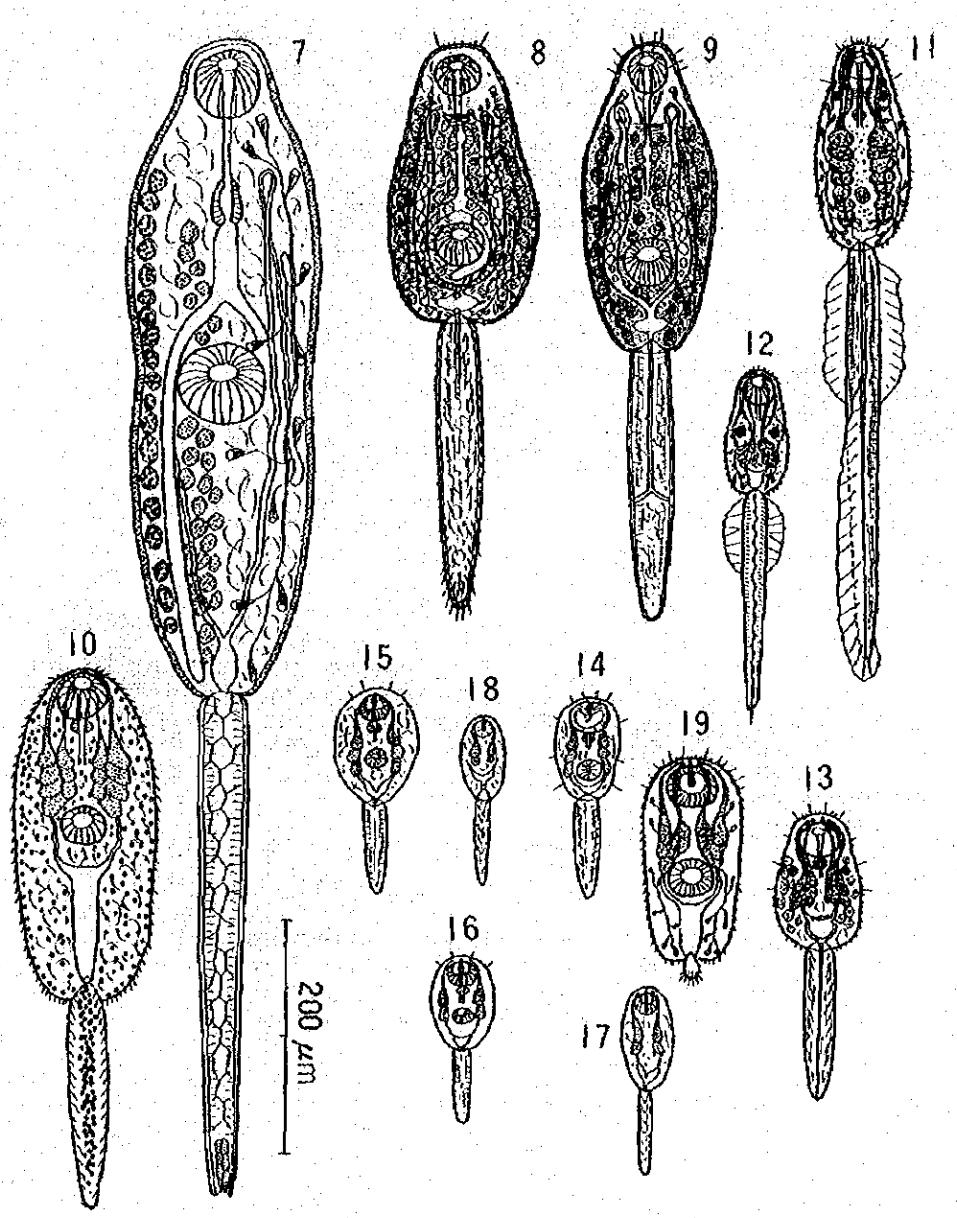
Explanation of Plates

Plate 1, Cercariae from Thiaridae.
(semidiagrammatic drawings by the same scale)

7. *Cercaria leyteensis no. 7* (Philophthalmidae)
8. *Cercaria leyteensis no. 8* (Echinostomatidae)
9. *Cercaria leyteensis no. 9* (Echinostomatidae)
10. *Cercaria leyteensis no. 10* (Unknown)
11. *Cercaria leyteensis no. 11* (Heterophyidae)
12. *Cercaria leyteensis no. 12* (Heterophyidae)
13. *Cercaria leyteensis no. 13* (Heterophyidae)
14. *Cercaria leyteensis no. 14* (Lecithodendriidae)
15. *Cercaria leyteensis no. 15* (Microphallidae)
16. *Cercaria leyteensis no. 16* (Microphallidae)
17. *Cercaria leyteensis no. 17* (Microphallidae)
18. *Cercaria leyteensis no. 18* (Microphallidae)
19. *Cercaria leyteensis no. 19* (Paragonimidae)

Plate 2, Cercariae from Thiaridae.
(photographs by various scales)

C. 7. *Cercaria leyteensis no. 7*
C. 8. *Cercaria leyteensi no. 8*
with the distal end of the tail
C. 9. *Cercaria leyteensis no. 9*
C. 10. *Cercaria leyteensis no. 10*
C. 12. *Cercaria leyteensis no. 12*
C. 13. *Cercaria leyteensis no. 13*
C. 14. *Cercaria leyteensis no. 14*
C. 15. *Cercaria leyteensis no. 15*
C. 17. *Cercaria leyteensis no. 17*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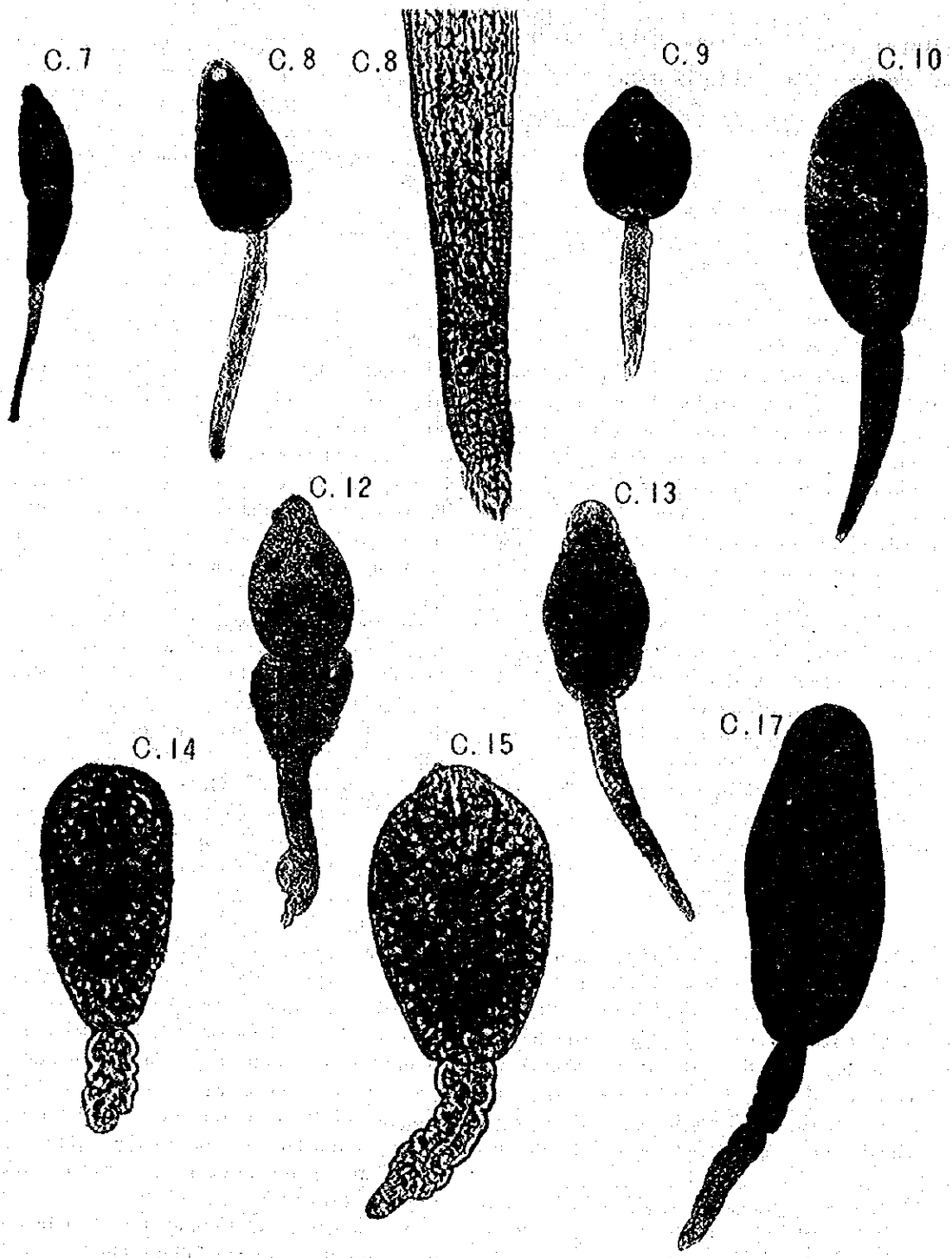
C. 7
C. 8
C. 8
C. 9
C. 10
C. 12
C. 13
C. 14
C. 15
C. 17

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Studies on the Fresh Water Cercariae in Leyte Island, Philippines [1]

4. Cercariae from Viviparidae and Pilidae

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Summary: During the period of 1975 and 1976, about two thousand snails of Viviparidae and two hundred snails of Pilidae were examined for cercarial fauna on Leyte Island, Philippines. From the snails of Viviparidae, 8 species of cercariae were found. These species consisted of two furcocercous cercariae, one monostome cercaria, three echinostome cercariae and two xiphidiocercariae. From the snails of Pilidae three species of cercariae were found; namely, one echinostome cercaria, one xiphidiocercaria, and one unknown cercaria. Among these species, one echinostome cercaria and one xiphidiocercaria were found to be common to snails of both families. In this paper these nine species of cercariae were described and illustrated with some remarks on their presumptive life cycles.

Introduction

During the past two years of 1975 and 1976, a total of 2,075 snails of *Bellamya philippinensis* (Nevill) belonging to Viviparidae and 219 snails of *Pila luzonica* (Reeve) and *Pila ampullacea* (Linnaeus) belonging to Pilidae were examined for the cercarial fauna. These snail specimens were collected from the northeast part of Leyte Island, Philippines. Eight species of cercariae were found from *B. philippinensis*, namely: two furcocercous cercariae, one monostome cercaria, three echinostome cercariae and two xiphidiocercariae. On the other hand, only three species of cercariae were detected from *Pila* spp., among them two species were commonly found from *B. philippinensis*. So only the remaining one was a proper species from *Pila* spp.

To date, in the Philippines, one species of cercaria from Viviparidae and five species from Pilidae have already been reported by Velasquez (1964) and by Tubangui (1928). Velasquez made a report of the life cycle of *Euparyphium paramurinum* which was found from *Vivipara angularis* in Rizal and Quezon. On the other hand Tubangui had reported five new species of cercariae, i.e., *Cercaria redicystica*, *C. lagunaensis*, *C. rarissima*, *C. maitimensis* and *C. dorsocauda* from *Pila luzonica* (=*Ampullaria lagunaensis*) in Los Baños. Thus all species were from Luzon Island only, not yet from Leyte. So the nine species of cercariae discovered by the present study are the first record from Leyte Island. To make use of the results of the inquiry as a basis for future attempts to find out the life cycles of trematodes, these nine species of cercariae are described and illustrated with proper remarks in the following paper. As noted in Part I of this series, these cercariae are tentatively named *Cercaria leyteensis no. 20* to *Cercaria leyteensis no. 28*.

The practice and technic described in the previous report were also followed in the experiments reported in this paper.

[1] This work was performed as a part of the RP-Japan Cooperative Studies supported by the Japan International Cooperation Agency.

伊藤二郎 (静岡大学教育学部衛生学)

DESCRIPTION OF CERCARIAE

Cercaria leyteensis no. 20

Presumptive adult form: Cyathocotylidae

Sanil host: *Bellamya philippinensis*

Date, locality and infection rate:

On the whole, 3 out of 2075, or 0.14%

Nov. 24, 1975, Santa Fe (Maslog), 1 out of 5, or 20.0%
Jan. 19, 1976, Santa Fe (Maslog), 1 out of 28, or 3.6%
Feb. 25, 1976, Palo (Vicob), 1 out of 101, or 1.0%

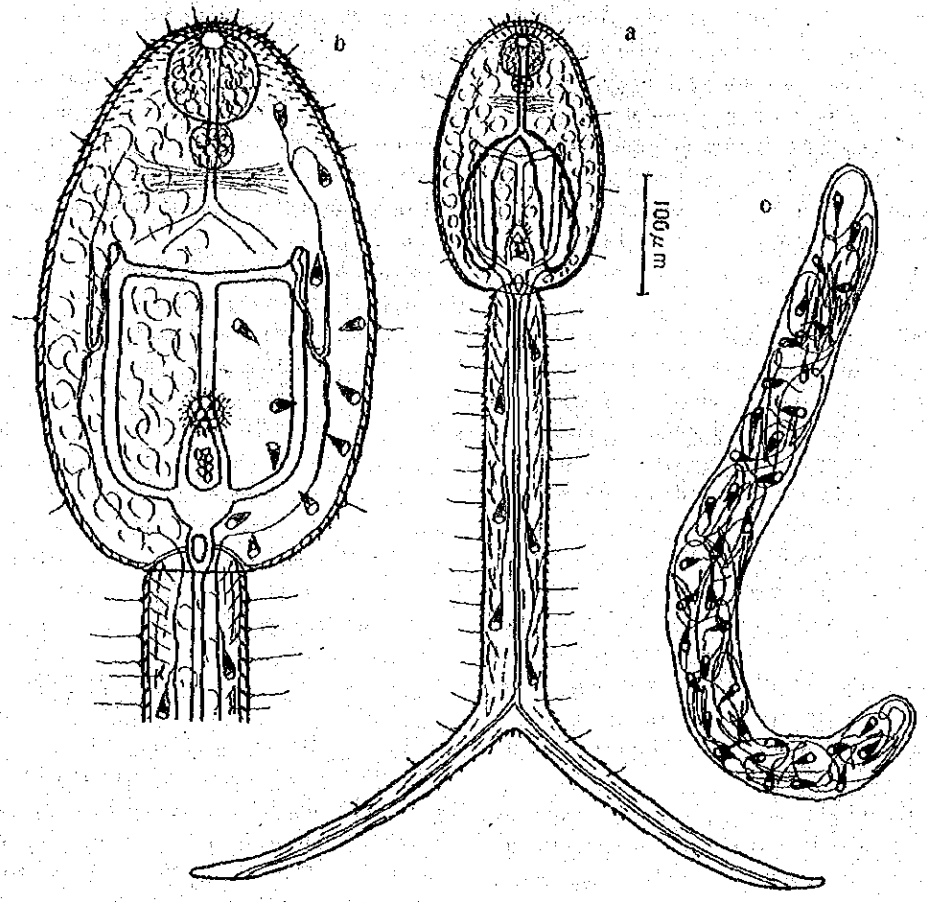
Measurements:

body	232 (220–250)	× 145 (130–165)	μm
oral sucker	40 (35– 45)	× 39 (35– 45)	μm
pharynx	17 (15– 20)	× 19 (18– 20)	μm
tail stem	392 (390–400)	× 45 (40– 50)	μm
tail furca	310 (300–330)	× 19 (18– 20)	μm

Specific description: (Fig. 20)

Nonoculate, pharyngeal longifurcate monostomous furcocercaria belonging to Vivax group of Sewell (1922). The body is flattened elliptical in shape, and is concave ventrally. The body surface is covered with many backward directed spines, more densely at the anterior part. About seven pairs of sensory hairs are also found at right angles on the lateral margin. The oral sucker is spherical in shape, and situated subterminally. A mouth opening is surrounded in quincunx by spines which are a little larger than those covering the rest of body. A pharynx follows directly the oral sucker without

Fig. 20. *Cercaria leyteensis no. 20.*



a. cercaria, b. enlarged body part, c. sporocyst

any prepharynx, and is followed by an esophagus which is divided into broad ceca slightly undulating with sinuous walls, but almost parallel to each other, ending on each side of the excretory vesicle. A transverse nervous commissure is across the esophagus dorsally. A rudimental acetabulum can hardly be observed at the level of the posterior one fourth of the body. A genital primordium is recognized as a compact mass of cells just in front of the excretory vesicle.

The non-epithelial excretory vesicle is situated at the posterior end of the body, giving off two pairs of anterior collecting tubes. The outer pair of collecting tubes runs forward along the cecum, and joins to a transverse anastomosis at the level of the shoulder of cecum. The inner pair runs forward a short distance, then joins with each other at the point of rudimental acetabulum, runs again forward to join to the middle of the anastomosis. From the terminal end of the anastomosis, one pair of collecting tubes runs backward a short distance, then it divides into an anterior and a posterior collecting tubule, respectively. At least 12 pairs of flame cells are observed, but their connection to the tubules could not be traced.

The tail stem is much longer than the body, and attached dorsally to the subterminal end of the body. The tail furca is also longer than the body. The surface of the tail stem, as well as the anterior half of the tail furcal surface, is covered with many backward directed minute spines, and is provided with about 15 pairs of perpendicular long sensory hairs. A tail excretory tube is connected to the excretory vesicle with an islet of Cort, runs backward along the axis of the tail stem and furcae, and opens near the terminal end of the tail. Within the tail stem, three pairs of flame cells are observed. Their capillaries run forward to join with the posterior collecting tubule in the body.

The thread-like sporocyst is 2–5 mm in length. Numerous flame cells are scattered throughout the smooth body wall. One sporocyst contains more than twenty cercariae in the same developmental stage.

Remarks: Tubangui (1928) reported one furcocercous cercaria, *Cercaria dorsocauda*, from *Pila luzonica (=Ampullaria lagunaensis)* on Luzon Island. As far as his description shows, no fundamental differences on the body structure between *C. dorsocauda* and the present species are found, but the former one is much larger than the latter one, and the snail hosts differ from each other. So it is impossible to identify the present species with *C. dorsocauda* at this time.

The general feature of the cercaria shows that this cercaria belongs to some of the genus of Cyathocotylidae, such as *Cyathocotyle, Holostephanus, Prohemistomum,* or *Mesostemphanus.* Thus far, the life cycles of 20 species of these trematodes have been studied by other countries. According to most references, many kinds of fish serve as the second intermediate host for these trematodes, and birds such as *Corvus, Milvus, Gallus, Larus* and *Anas* act as the final host. But there are some exceptions; for example, the second intermediate host of some of the genus *Holostephanus* is the amphibians, and the final host of some of the genus *Prohemistomum* is reptiles or mammals.

In view of the above, the attempt to study the life cycle of this cercaria should begin by letting the cercaria come in contact with the fresh water fish or the amphibian.

Cercaria leyteensis no. 21

Presumptive adult form: Sanguinicolidae?

Sanil host: *Bellamya philippinensis*

Date, locality and infection rate:

On the whole, 1 out of 2075, or 0.05%

Feb. 25, 1976, Palo (Vicob), 1 out of 101, or 1.0%

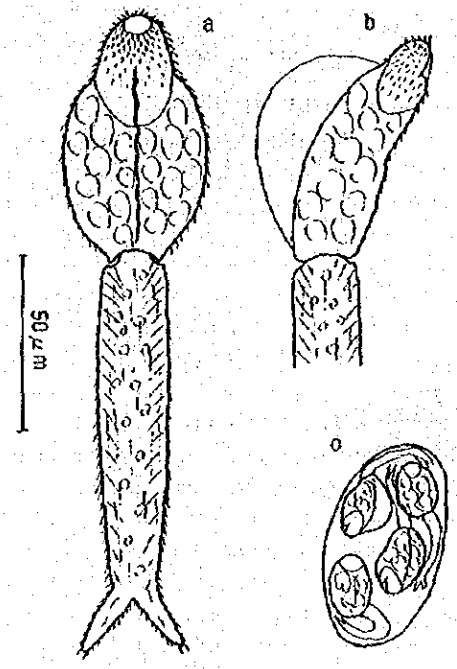
Measurements:

body	70–125 × 40–60 μm
tail stem	90–100 × 18–20 μm
tail furca	30– 40 × 7– 8 μm

Specific description: (Fig. 21)

Small-sized furcocercous cercaria with a dorsal fin-fold on the body. The body is oval in shape, and curved ventrally with a convexed dorsal side. The thin body cuticle is provided with many minute spines which are distributed more densely around the mouth opening. A pro-

Fig. 21. *Cercaria leyteensis no. 21.*



a. cercaria, b. lateral view of body, c. sporocyst

minent fin-fold is observed on the dorso-median line of the body. At a distance 20 μm from the anterior end is a constriction, which separates the conical anterior extremity from the rest of the body. A mouth is located at the subterminal ventral side, but no other digestive system has been differentiated so far. In the body parenchyma there are droplets and granules which give the body a yellowish tinge. Internal structures such as the acetabulum, the glands, the excretory system, etc. could not be recognized, yet.

The tail stem is longer than the body, and is provided with minute spines which beocme dense towards the posterior. It contains a small number of intracellular granules. The tail furca is very short, lanceolate in shape, and is provided with a cup-shaped minute projection at the distal end of the furcae.

The thin-walled, non-motile sporocyst is oval in shape, and about 200 μm in length. It contains a few cercariae of the same stage of development.

Remarks: About twenty species of dorsal fin-fold furcocercous cercariae have been reported in the world. These cercariae belong to the families of Aporocotylidae, Sanguinicolidae, Spirorchiidae, and Clinostomatidae. Their adult worms are found in the blood vessels and other organs of the final host, but not from the intestinal ceca. The first intermediate host of Aporocotylidae is some species of Annelida or Bivalvia in which the rediae and the cercariae develop, and their final hosts are the fish. The members of Sanguinicolidae have as their first intermediate host, the snails such as *Lymnaea* or *Bithynia* and some bivalves. Their final host is the fish into which the cercariae enter directly without the metacercarial stage. The cercariae of Spirorchiidae developing in the snail host of *Helisoma* spp., enter into the blood vessel of turtles to develop in gravid adult worms. As for the member of Clinostomatidae, the cercariae with the redial stage develop in the snails such as *Helisoma, Lymnaea, Eupomatis,* etc. These cercariae enter into many kinds of fish to make the cyst, then develop to the adult worm in the trachea or tongue of some swimming bird.

Comparing the present cercaria to those mentioned above, it seems to resemble the members of Sanguinicolidae because of similarities of the cercarial body structure, although no identifiable species could be found because of the differences of the body size, or because of the snail host. The present cercaria has a marked character of the small body size, being only 70–125 μm in length, whereas the already known cercariae of Sanguinicolidae are all more than 200 μm in length.

As to the life cycle of this group, for example in the case of *Sanguinicola inermis,* it has already been known that the cercaria attacks the fish directly and enters into the blood vessel of the fish. So the attempt to let the cercaria contact with some fish will be successful in getting the adult worm.

Cercaria leyteensis no. 22

Presumptive adult form: Notocotylidae

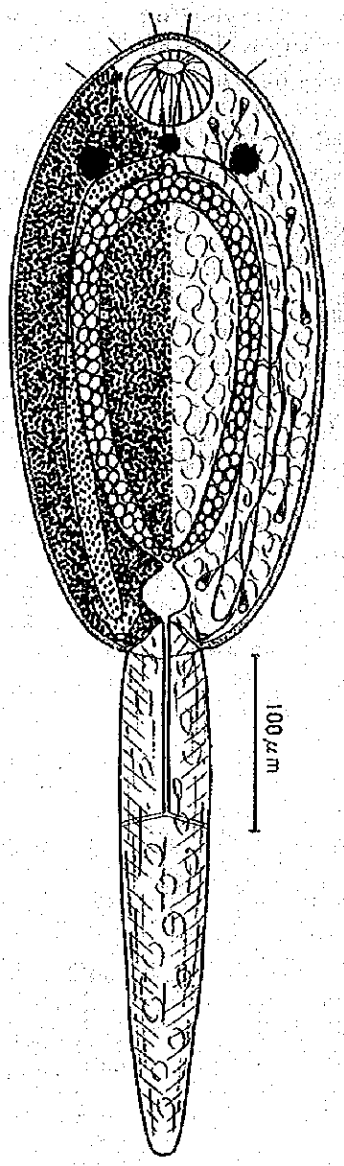
Snail host: *Bellamya philippinensis*

Date, locality and infection rate:

On the whole, 2 out of 2075, or 0.10%

Mar. 8, 1976, Tabontabon (Capohu-aw), 2 out of 81, or 2.5%

Fig. 22. *Cercaria leyteensis no. 22*



Measurements:

body	350 (300–400) × 176 (140–220) μm
oral sucker	43 (40– 48) × 47 (45– 50) μm
tail	310 (270–350) × 48 (45– 50) μm

Specific description: (Fig. 22)

Large, trioculate monostome cercaria belonging to Notocotylidae. The body is ellipsoidal in shape, with one pair of adhesive pockets at the postero-lateral part of the body. The smooth cuticle is aspinose but is provided with several pairs of sensory hairs on the anterior part. A globular oral sucker is followed by a short esophagus which is divided into two ceca terminating near the posterior end of the body. Well developed cystogenous materials and heavily pigmented granules are compacted in the whole body cavity. One pair of lateral eye sopts are prominent, and the median eye spot is smaller than the lateral one. The non-epithelial small excretory vesicle sends off one pair of collecting tubes, which run forward along the ceca, and are connected to each other at the level of intestinal bifurcation, with a very short median vessel anteriorly. Many refractile excretory concretions are compacted in these tubes. From the middle of each main collecting tube, a secondary collecting tube arises to receive 8 pairs of flame cells. The flame cell formula is $2[(2+2)+(2+2)]=16$. The tail is slightly shorter than the body. Its surface is smooth without any spine or hair. A caudal excretory tube divides at about the middle of the tail and opens on the side.

The redia is very similar to that of *Cercaria leyteensis no. 3.*

Remarks: This cercaria is closely related to *Cercaria leyteensis no. 3* which was found from *Oncomelania quadrasi* on Leyte Island, but differs from it in the presence of the adhesive pockets, in its smaller size, and in a different snail host. So this notocotylid cercaria is considered as the third one in the Philippines. That is, the first one is the cercaria of *Paramonostomum philippinensis* Velasquez, 1969, the second one is *Cercaria leyteensis no. 3*, and this third one is *Cercaria leyteensis no. 22.*

As to the life cycle of this group, refer to the

remarks on *Cercaria leyteensis no. 3.*

Cercaria leyteensis no. 23

Presumptive adult form: Echinostomatidae (*Echinostoma* or others)

Snail host: *Bellamya philippinensis*

Date, locality and infection rate:

On the whole, 5 out of 2075, or 0.24%

Nov. 14, 1975, Javier (Javier), 1 out of 10, or 10.0%
Nov. 18, 1975, Burauen (Buri), 1 out of 14, or 7.1%
Feb. 3, 1976, Palo (Vicob), 1 out of 65, or 1.5%
Feb. 25, 1976, Palo (Vicob), 2 out of 101, or 2.0%

Measurements:

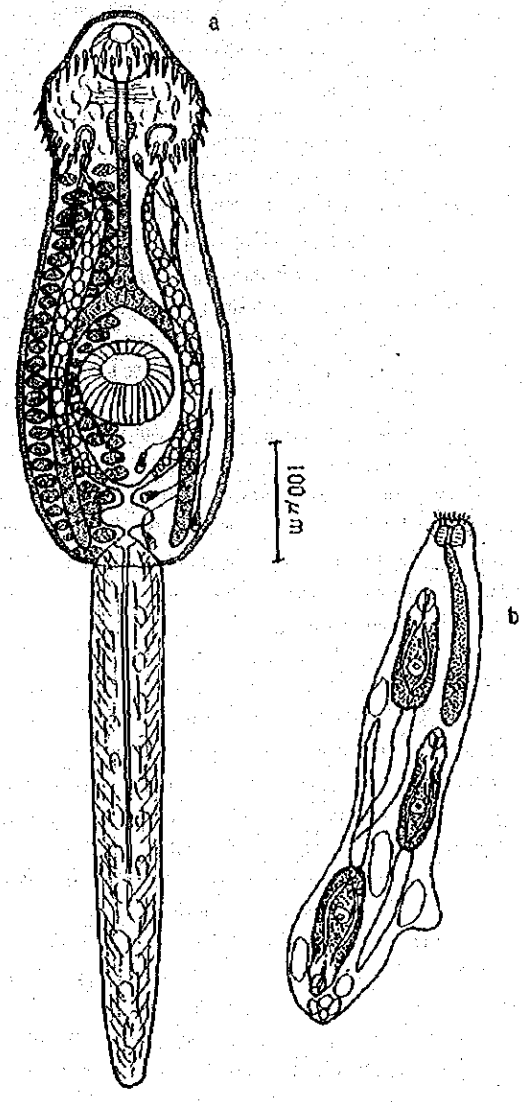
body.....: 477 (400–500) × 182 (170–190)μm
oral sucker..... 53 (50– 60) × 48 (40– 52)μm
prepharynx..... 19 (18– 20)μm long
pharynx..... 33 (33– 38) × 19 (18– 20)μm
acetabulum.... 72 (65– 80) × 76 (70– 80)μm
tail.....478 (450–550) × 57 (50– 65)μm

Specific description: (Fig. 23)

Large echinostome cercaria. The body is proper elogated fusiform in shape, with a prominent head collar and 37 collar spines comprising 10 ventrals, 12 laterals and 15 dorsals in two alternate rows. The body surface is covered with a thick and smooth cuticle without any spine or hair. A well developed spherical oral sucker is subterminal, and is followed by a long prepharynx, a pyriform pharynx, a long esophagus and two intestines reaching to the posterior end of the body. The esophagus and ceca are filled with a linear series of faintly demarcated cells. A well developed subglobular acetabulum is much larger than the oral sucker, situates at the junction of middle section with posterior third of the body. The body cavity is compacted with dense cystogenous gland cells except for the cephalic region. A nervous commissure can be recognized across the prepharynx.

One chambered non-epithelial excretory bladder is located at the end of the body. From its mid-anterior margin a short stem arises and divides itself into two main collecting tubes, which run forward to the level of the pharynx, then turn backward to the level of the acetabulum, and again divide into an anterior and a posterior collecting tubule to receive flame cells. About 40 refractile excretory concretions are easily observed in the ascending main tubes, and several ciliary patches are found in the descending main tubes. Only five pairs of flame cells were observed, but the flame cell formula could not be determined yet. The tail is as long as the body. Its surface is smooth without

Fig. 23. *Cercaria leyteensis no. 23.*



a. cercaria, b. redia

any spine or hair. The caudal excretory tube starts from the posterior margin of the excretory vesicle, with a small chamber at its proximal end, then it runs backward along the axis of the tail and disappears.

The redia is cylindrical, more blunt in the posterior part. The collar and locomotive appendages are inconspicuous. Some yellowish pigments scattering in the cuticle gives the redia a yellowish appearance. Around the mouth opening are located many sensory hairs. A well developed pharynx is followed by a sausage shaped intestine which is compacted by the brownish digesta. A few cercariae with several germ balls are contained in the redia.

Remarks: Tubangui (1947) listed 13 species of Echinostomatidae in the Philippines, and Velasquez (1964) added one more species with its complete life cycle. Among these 14 species, only four have been reported with their life cycles, namely *Echinostoma revolutun* from *Lymnaea*, *Euparyphium ilocanum* from *Gyraulus*, *Euparyphium murinum* from *Lymnaea*, and *Euparyphium paramurinum* from *Vivipara*, all of them are from Luzon Island only.

Comparing the present cercaria with these mentioned above, no identifiable species is found because there is no species possessing 37 collar spines except for one, *Echinostoma revolutum*. Even though *E. revolutum* have the same number of collar spines, it seems to be difficult to identify the present one as *E. revolutum* because the snail host of *E. revolutum* is *Lymnaea* spp., and the cercaria is much smaller than the present one. Even though many species of 37 spined echinostome cercariae have been reported, but no such species was reported from Viviparidae in many countries yet. So the present cercaria is considered as a new species.

An attempt to study the life cycle of this cercaria would be rather easy, because encystation can occur in the same snail host or in other snails, thus many cysts can be gathered easily. Adults will be recovered from birds such as ducks and pigeons, or from the mammals such as guinea pigs, rats, and mice, after feeding the host with the metacercariae.

Cercaria leyteensis no. '24

Presumptive adult form: Echinostomatidae *(Euparyphium paramurinum?)*

Snail host: *Bellamya philippinensis*

Date, locality and infection rate:

On the whole, 4 out of 2075, or 0.19%

Feb. 25, 1976, Palo (Vicob), 4 out of 101, or 4.0%

Measurements:

body	410 (390–430) × 197 (180–230) μm
oral sucker	48 (45– 60) × 55 (50– 63) μm
prepharynx	19 (18– 25) μm in length
pharynx	33 (30– 35) × 23 (20– 25) μm
acetabulum	77 (75– 80) × 80 (70– 85) μm
tail	267 (250–280) × 62 (60– 65) μm

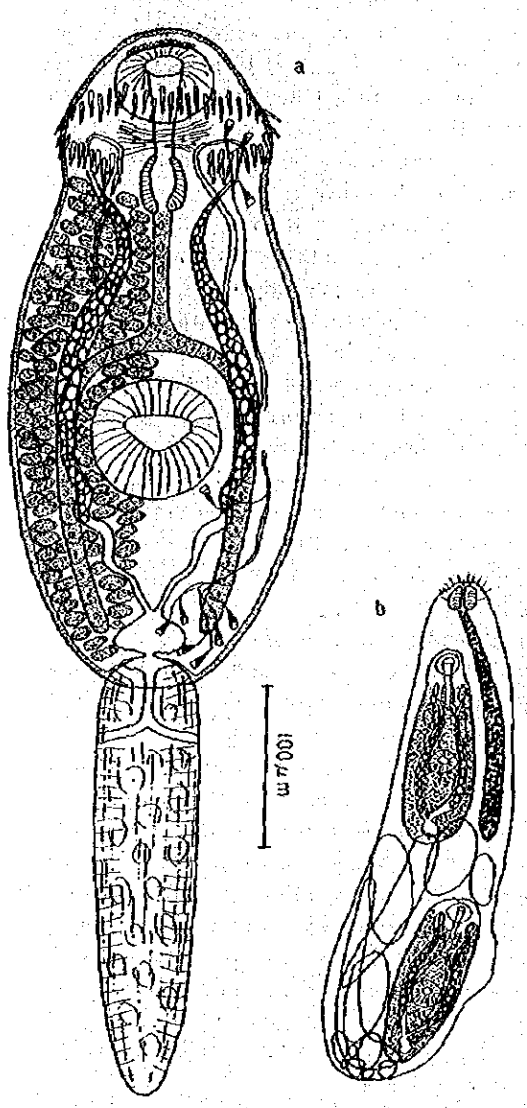
Specific description: (Fig. 24)

Large echinostome cercaria. The body is proper elongated fusiform in shape, with a prominent head collar and 47 collar spines comprising 10 corners, 12 ventrals, 8 laterals and 17 dorsals in two alternate rows. The largest spine measures 15 μm in length. The body surface is covered with thick cuticle without any spine or hair. A subglobular oral sucker is well developed. On the anterior peripheral margin of the sucker, about 14 openings arranged in one transverse row are faintly observed. This is supposed to be the rudimental openings of some gland like the penetrating one. The digestive system is well developed. The subterminal mouth opening is followed by a prepharynx, a pyriform pharynx, a long esophagus and two ceca reaching to the posterior end of the body. The esophagus and ceca are filled with a linear series of faintly demarcated parenchmatous cells. A large globular acetabulum is located at the junction of the middle section with posterior third of the body. Except for the cephalic region the body cavity is filled with densely compacted cystogenous gland cells which make the body very opaque. Across the prepharynx a transverse nervous commissure is observed.

One chambered non-epithelial excretory vesicle is at the posterior end of the body. From its antero-median portion one pair of main collecting tubes arises, runs forward passing through the side of the acetabulum until the level of the pharynx, where it twists into a triangular loop and runs backward to connect

with the secondary collecting tubes. A portion of the main collecting tube between the acetabulum and the pharynx is inflated and filled with about 50 refractile excretory concretions. More than 10 pairs of flame cells are detected, but the flame cell formula could not be determined yet. The tail is much shorter than the body. Its surface is smooth and lacks spine and hair. A caudal excretory tube arises from the posterior margin of the excretory vesicle, with a small chamber at its proximal end, then divides into two side branches to open on lateral sides of the tail at its anterior sixth.

Fig. 24. *Cercaria leyteensis no. 24.*



a. cercaria, b. redia

The redia is much similar to that of the former species, *Cercaria leyteenisis no. 23.*

Remarks: Velasquez (1964) reported one species of echinostome cercaria from *Vivipara angularis* in Rizal, Philippines. She obtained the gravid adults from the small intestines of guinea pigs fed with its metacercariae, for which she designated a new name, *Euparyphium paramurinum.* According to her report, cercaria has well-developed cuticular spines on the body surface, bearing 43–45 collar spines measuring 8–10 μm long, and the flame cell formula of $2[(3+1+1+1+3+3)+(3+3)]=36$. She further stated that this snail is reported for the first time in the Philippines to be an intermediate host of a digenetic trematode.

Comparing the present cercaria with that of *Euparyphium paramurinum,* the present one is slightly larger, but falls within the size range given for *E. paramurinum.* The snail host of both species is closely related one, belonging to the family Viviparidae. Still some differences exist between them. The present cercaria lacks cuticular spine on the body surface, possesses 47 collar spines instead of 43–45 for *E. paramurinum.* Though the flame cell formula could not be determined yet, there still remains some problems of identifying the present cercaria to *E. paramurinum.*

Again according to her report, cysts were easily found from the same snail, and also from the lymnaeid snails, and even within the rediae. She obtained the gravid adult worms from the small intestines of guinea pigs, mice, rats and pigeons fed with snails containing metacercariae, but failed in chicks, and only immature worms from dogs and ducklings. These results will give many suggestions for the future study on the life cycle of this cercaria.

***Cercaria leyteensis* no. 25**

(=*Cercaria redicystica* Tubangui, 1928)

Presumtive adult form: Echinostomatidae (*Echinochasmus* or others)

Snail host: *Bellamya philippinensis*, *Pila ampulacea*

Date, locality and infection rate:

On the whole, 37 out of 2075, or 1.78% in *Bellamya*, and 1 out of 219, or 0.46% in *Pila*

Nov. 18, 1975, Palo (Vicob), 8 out of 45, or 17.7%
Nov. 24, 1975, Santa Fe (Maslog), 2 out of 5, or 40.0%
Nov. 25, 1975, Santa Fe (Maslog), 1 out of 3, or 33.3%
Jan. 8, 1976, Santa Fe (Tibak), 3 out of 33, or 9.1%
*Jan. 8, 1976, Pastrana (Socsocon), 1 out of 2, or 50.0%
Jan. 12, 1976, Santa Fe (Tibak), 3 out of 87, or 3.4%
Jan. 15, 1976, Palo (Nariwatan), 2 out of 29, or 6.9%
Jan. 19, 1976, Santa Fe (Tibak), 5 out of 67, or 7.5%
Jan. 19, 1976, Santa Fe (Maslog), 2 out of 28, or 7.1%
Feb. 3, 1976, Palo (Vicob), 1 out of 65, or 1.5%
Feb. 11, 1976, Santa Fe (Tibak), 1 out of 28, or 3.6%
Feb. 13, 1976, Palo (South Main Canal), 2 out of 45, or 4.4%
Feb. 18, 1976, Santa Fe (Tibak), 2 out of 26, or 7.7%
Feb. 25, 1976, Palo (Vicob), 5 out of 101, or 5.0%
(* mark is from *Pila*, others are from *Bellamya*)

Measurements:

body	167 (150–180)×83 (75–92)μm
oral sucker	40 (38– 42)μm in diameter
prepharynx	15 (10– 20)μm in length
pharynx	18 (16– 20)×16 (15–17)μm
acetabulum	33 (30– 35)μm in diameter
tail	148 (130–170)×31 (25–35)μm

Specific description: (Fig. 25)

Small sized echinostome cercaria. The body is elliptical, attenuates anteriorly and is broadest at the middle of the body. Neither head collar nor collar spine is differentiated yet. The body surface is covered with 5 μm thick cuticle except the anterior surface where the cuticle is thin. No spine, but three pairs of short sensory hairs are observed on the anterior half of the body surface. A well developed spherical oral sucker is subterminal. A semicircular transverse row of 14–16 toothlets is found around the mouth opening. The mouth leads into a long prepharynx which is followed by a oval shaped pharynx, a long esophagus dividing into two ceca terminating near the end of the body. Several pairs of rudimentary glands are faintly recognized between the pharynx and the acetabulum. Their ducts seem to run forward and open at the anterior margin of the oral sucker, but it is very difficult to count the number of the openings. There seems to be 8 or 10. The spherical and well developed acetabulum is situated at the middle of the posterior half part of the body. Around its ventral opening are 40 or 50 toothlets arrenged circularly in one row. A nervous commissure is found across the prepharynx transversely. Some oblique muscle fibers are faintly observed between the oral sucker and the pharynx.

Except for the cephalic region, the whole body cavity is filled with at least two kinds of cystogenous materials. One is rod-like materials and the other one is coarsely granulated materials. In the premature cercaria these materials are compacted in the cells.

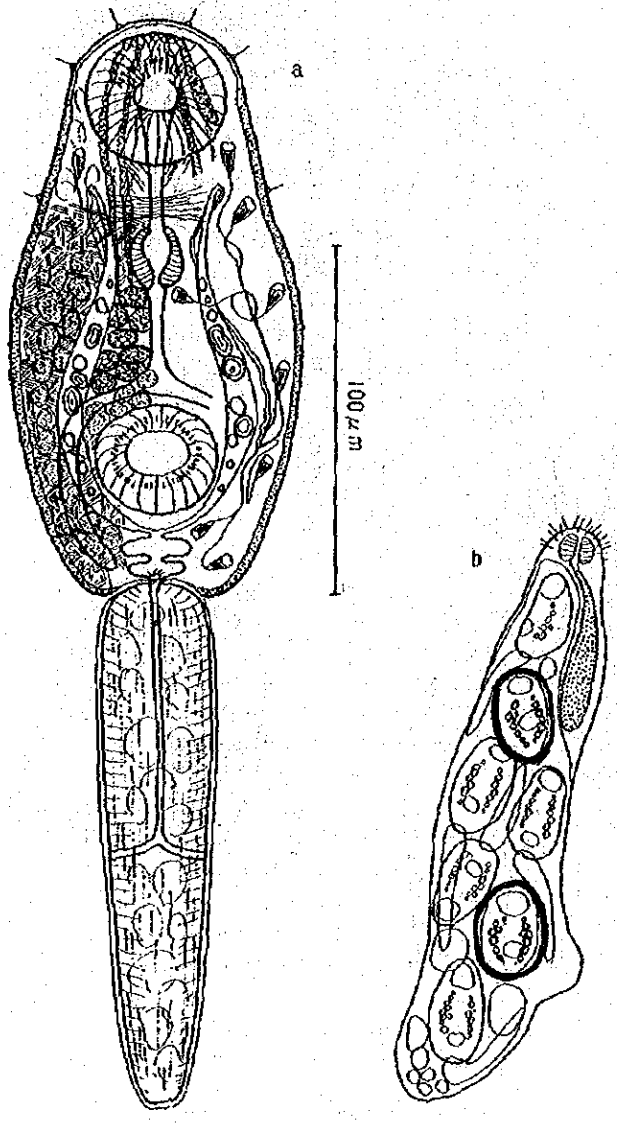
Two chambered, non-epithelial excretory vesicle is at the posterior part of the acetabulum. A pair of the main collecting tubes arises from its antero-median part, runs forward, increasing in size and containing about 10 refractive excretory concretions on the way, until it reaches the side of the prepharynx. Then the tube turns abruptly backward until it reaches the side of the acetabulum and divides into a long anterior and a short posterior branch, each of them receiving four flame cells in two groups. The flame cell formula is therefore constructed as $2[(2+2)+(2+2)]=16$.

The slender and aspinose tail is nearly equl in length to the body. The caudal excretory tube bifurcates at a point a little behind the middle of the tail and opens laterally.

The redia is sausage-shaped and about 1 mm long, being more blunt in the posterior part. The collar and the locomotive appendages are inconspicuous. Many sensory hairs are located around the mouth opening. A well developed pharynx is followed by a gut containing dark brown ingesta. Less than ten cercariae with some germ balls are contained in one redia. Frequently one or two encysted metacercariae of 115–150 μm long and 90–130 μm wide are found in the redia.

Remarks: Tubangui (1928) reported one echinostome cercaria, *Cercaria redicystica*, from *Pila luzonica* (=*Ampullaria lagunaensis*) in Los

Fig. 25. *Cercaria leyteensis no. 25.*



a. cercaria, b. redia

Bañoz, Luzon Island. A comparison of *Cercaria redicystica* with the cercaria, *Cercaria leyteensis no. 25*, shows several differences between them; the former has a larger body size than the latter, which may be caused by measurements of living specimens of the former; the former has only seven pairs of flame cells instead of eight pairs in the latter; circularly arranged toothlets around the mouth opening are reported in the former but it is only a semicircular transverse row in the latter; the bifurcation of the caudal excretory tube was not mentioned in the former. Even though there are some above mentioned differences between the descriptions of both species, the present cercaria seems to be identifiable with *C. redicystica* Tubangui, 1928.

As to the presumable life cycle of this cercaria, it seems most probable that it develops into the genus *Echinochasmus* or other closely related genus of Echinostomatidae. From the Viviparidae in Japan several species of *Echinochasmus*, such as *E. elongatus*, *E. rediduplicatus*, *E. rugosus*, have been reported with their complete life cycles. According to them, cysts were found in the same snail host or even within the redia, or sometimes from the tadpole. The adult worms were easily found from the intestinal ceca of the experimental mammals such as mice, rats, and dogs, which were fed with these metacercariae. So getting specimens of the adult worms of this cercaria will be rather easy.

Cercaria leyteensis no. 26

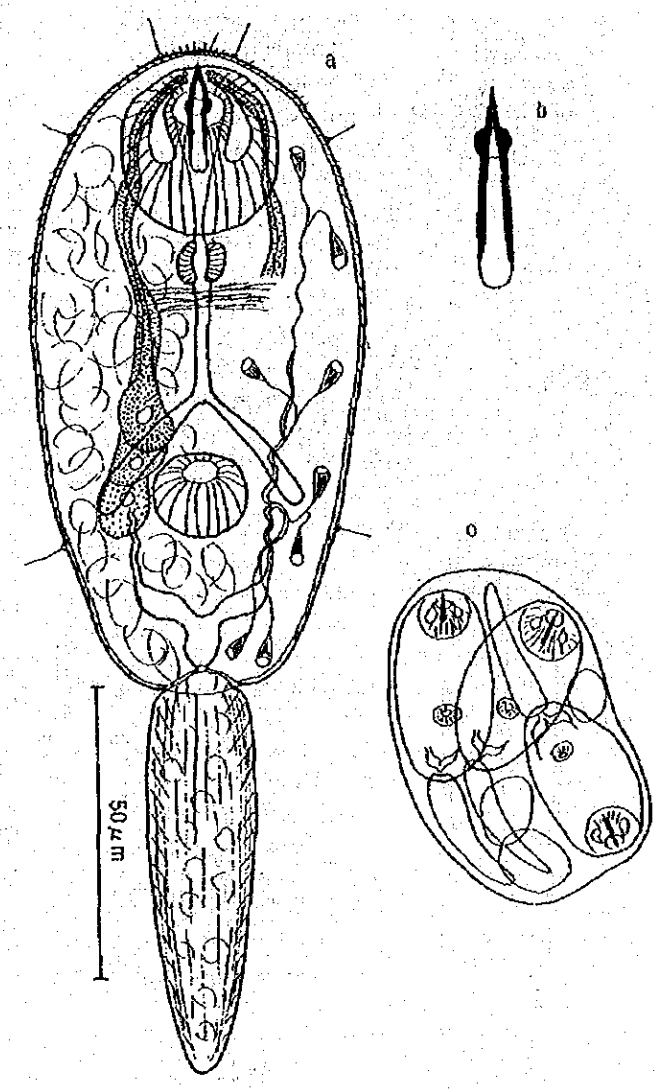
Presumptive adult form:
Microphallidae?
Snail host: *Bellamya philippinensis*
Date, locality and infection rate:
On the whole, 57 out of 2075, or 2.75%
Nov. 18, 1975, Tanauan (Kiling), 1 out of 4, or 25.0%
Nov. 24, 1975, Santa Fe (Maslog), 2 out of 5, or 40.0%
Nov. 25, 1975, Pastrana (Socsocon), 1 out of 22, or 4.5%
Dec. 5, 1975, Pastrana (Socsocon), 1 out of 4, or 25.0%
Jan. 8, 1976, Pastrana (Socsocon), 1 out of 20, or 5.0%
Jan. 13, 1976, Palo (Upper Hubang), 3 out of 56, or 5.4%
Jan. 19, 1976, Santa Fe (Maslog), 2 out of 28, or 7.1%

Jan. 20, 1976, Palo (Hubang), 4 out of 65, or 6.2%
Jan. 26, 1976, Dagami (Dagami), 1 out of 12, or 8.3%
Feb. 2, 1976, Dagami (Maliwaliw), 1 out of 40, or 2.5%
Feb. 3, 1976, Palo (Vicob), 4 out of 65, or 6.2%
Feb. 3, 1976, Santa Fe (Maslog), 1 out of 27, or 3.7%
Feb. 11, 1976, Pastrana (Socsocon), 1 out of 65, or 1.5%
Feb. 18, 1976, Pastrana (Socsocon), 1 out of 31, or 3.2%
Feb. 18, 1976, Santa Fe (Maslog), 5 out of 70, or 7.1%
Feb. 25, 1976, Palo (Vicob), 4 out of 101, or 4.0%
Feb. 25, 1976, Santa Fe (Maslog), 3 out of 54, or 5.6%
Mar. 2, 1976, Tabontabon (Capohuaw), 14 out of 74, or 18.9%
Mar. 3, 1976, Pastrana (Socsocon), 1 out of 80, or 1.3%
Mar. 8, 1976, Tabontabon (Capohuaw), 2 out of 81, or 2.5%
Mar. 10, 1976, Palo (Upper Hubang), 3 out of 58, or 5.2%
Mar. 16, 1976, Palo (Upper Hubang), 1 out of 73, or 1.4%

Measurements:

body	110 (102–117)×55	(48–60)μm
oral sucker	30 (27– 33)×28	(25–30)μm
stylet	17 (15– 18)μm	in length
pharynx	10 (7– 12)μm	in diameter
acetabulum	15 (14– 17)×17	(15–20)μm
tail	70 (50–100)×19	(15–22)μm

Fig. 26. *Cercaria leytensis no. 26.*



a. cercaria, b. stylet, c. sporocyst

Specific description: (Fig. 26)

Simple-tailed, nonoculate, non-virgulate xiphidiocercaria belonging to Microcotyle group of Lühe (1909). The body is elongated oval in shape, being more blunt anteriorly. A thin body cuticle is provided with many minute spines more dense at the anterior. Three pairs of sensory hairs are observed on the surface. A well developed, globular oral sucker is at the anterior part, in which a solid, sharply pointed and strongly shouldered stylet is embedded at its dorsal side. Within the oral sucker one pair of saccular shaped, refractile structure is observed. It looks like a part of convolution of the ducts of penetrating glands, or a sort of cephalic glands. The digestive system is composed of a mouth, a very short prepharynx, a spherical pharynx, a long esophagus and two ceca terminating at the level of acetabulum. A subglobular acetabulum is much smaller than the oral sucker, located at the level of two thirds posteriorly. A transverse nervous commissure is across the esophagus. Three pairs of penetrating gland cells are at the

side of the acetabulum. The anterior one is filled densely with coarse granules, the posterior one with corase but not dense, and the middle one is with fine granules. Their ducts run forward across the lateral side of the oral sucker and open near the apical side of the stylet. The excretory vesicle is Y-shape. From its anterolateral corner one pair of main collecting tubes arises and runs forward, then divides into an anterior and a posterior collecting tube at the both sides of the acetabulum, to receive four flame cells each. The flame cell formula is $2[(2+2)+(2+2)]=16$. The tail is much shorter than the body, coated with fine cuticular annulations, but without any spine nor hair. No caudal excretory tube is recognized.

The sporocyst is an irregular sac-like shape, about 100–200 μm in length. It moves some times passively due to the activity of cercariae in it. A few cercariae and germ balls are contained in the sporocyst.

Remarks: This cercaria was found most commonly from the snails of Viviparidae on Leyte Island. Though a marked character of possessing the peculiar structures in the oral sucker of this cercaria makes some hesitation for presuming its life cycle, but the general appearance of this cercaria seems to develop into the member of Microphallidae. According to the references of the life cycle of Microphallidae, the second intermediate host covers a wide range of arthropods, and the final hosts are birds and mammals. An attempt to study the life cycle of this cercaria will be rather difficult because of difficulties in maintaining the experimental arthropod host. The discovery of a natural infection case of this metacercaria may serve for this purpose.

Cercaria leyteensis no. 27

Presumptive adult form: Microphallidae (Microphallinae)

Snail host: *Pila ampullacea, Pila luzonica, Bellamya philippinensis*

Date, locality and infection rate:

On the whole, 10 out of 219, or 4.57% in *Pila*, and 2 out of 2075, or 0.10% in *Bellamya*

Jan. 28, 1976, Dagami (Maliwaliw), 2 out of 41, or 4.9%

Jan. 29, 1976, Dagami (Maliwaliw), 4 out of 49, or 8.2%

Feb. 2, 1976, Dagami (Maliwaliw), 2 out of 38, or 5.3%

*Feb. 25, 1976, Palo (Vicob), 2 out of 101, or 2.0%

Mar. 8, 1976, Tabontabon (Capohu-aw), 2 out of 6, or 33.3%

(* mark is from *Bellamya*, others are from *Pila*)

Measurements:

body.....83 (70–95)×36 (30–40)μm
oral sucker.....19 (18–20)μm in diameter
stylet.....13 (12–14)μm in length
tail.....75 (60–80)×13 (10–15)μm

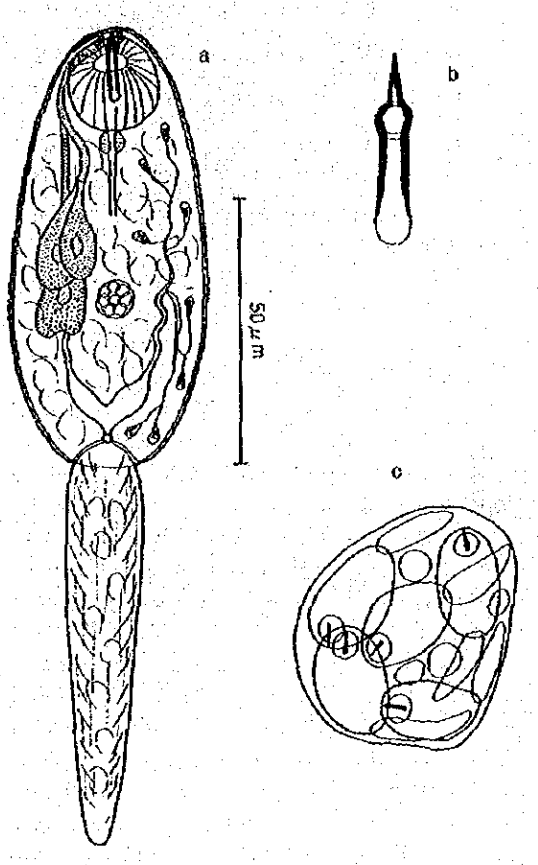
Specific description: (Fig. 27)

Simple-tailed, nonvirgulate, small xiphidiocercaria of Ubiquita type belonging to Microcotyle group of Lühe (1909). The body is elongated oval- or tongue-shaped, with scale-like minute spines all over the body surface. The oral sucker is well developed, in which a sharp pointed and conspicuously shouldered stylet is embedded. A weakly developed rudimentary acetabulum is hardly recognizable at slightly posterior to the middle of the body. A small pharynx is observed attached directly to the oral sucker without a prepharynx. Only a short esophagus but no cecum is recognized. There are three pairs of penetrating gland cells, located at the middle of the body. The first two pairs of these glands are finely granular, while the glands of the third pair are larger and coarsely granular. The ducts of these glands are conspicuous, opening on both sides of the dorsal lip of the mouth.

The excretory system is composed of a V-shaped excretory vesicle, which opens outside through a median dorsal pore; two main collecting tubes, which divide in the middle of the body length into anterior and posterior collecting tubules; and 8 pairs of flame cells, as shown in Fig. 27. The excretory flame cell formula is $2[(2+2)+(2+2)]=16$. The tail is slightly shorter than the body, and attached to the ventral side of the body end. It is slender, without any spine or hair but with somewhat fine cuticular annulations. The tail excretory tube is obliterated.

The cercaria develops in simple, round to oval sporocysts, containing a few cercariae and

Fig. 27. *Cercaria leyteensis no. 27.*



a. cercaria, b. stylet, c. sporocyst

germ balls. The wall of the sporocyst is thin and delicate so that it moves sometimes passively due to the activity of cercariae in it.

Remarks: Tubangui (1928) reported two xiphidiocercariae, *C. lagunaensis* and *C. rarissima* from *Pila luzonica (=Ampullaria lagunaensis)* in Los Baños, Luzon Island. The present cercaria is very similar in appearance to these cercariae of Tubangui, but differs from them in the markedly smaller size of the cercaria and in possessing a primordium of acetabulum instead of the well developed one. So I have decided to consider it as a distinct species. The present cercaria is also very similar to *Cercaria leyteensis no. 17* and *no. 18* which were found from Thiarid snails on Leyte, but differs from them in having three pairs of penetrating gland cells, a primordium of acetabulum, and in developing in different kinds of snail hosts.

As for the expected life cycle of this cercaria, the general appearance shows that the cercaria will develop into the member of Microphallidae, especially into the subfamily Microphallinae. Some aquatic arthropods will act as its second intermediate host, while birds or mammals will serve as the final host.

Cercaria leyteensis no. 28

Presumptive adult form: Unknown

Snail host: *Pila ampullacea*

Date, locality and infection rate:

On the whole, 1 out of 219, or 0.46%

Feb. 3, 1976, Palo (Vicob), 1 out of 5, or 20.0%

Measurements:

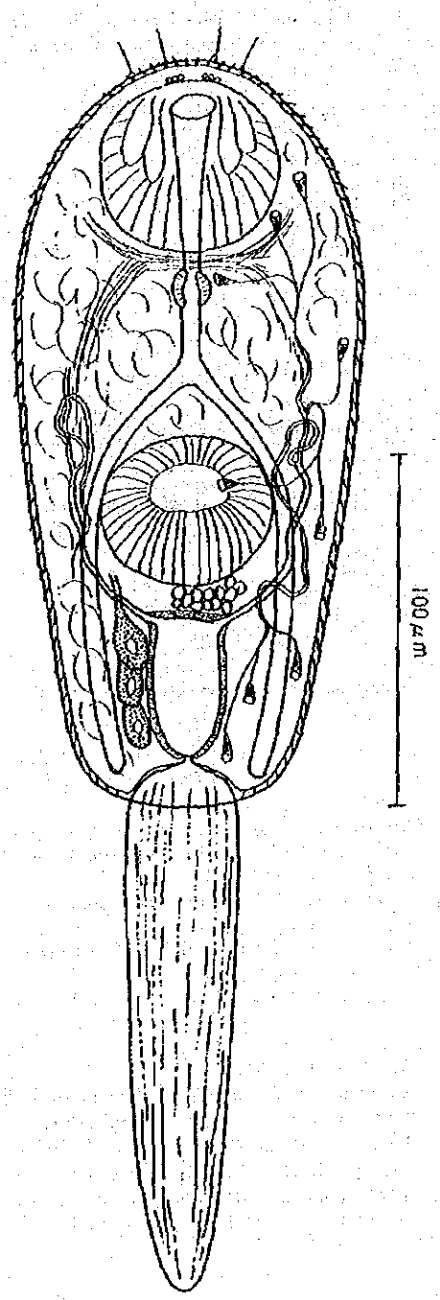
body	216 (210–230)	×	95 (90–100) μm
oral sucker	50 (45– 60)	×	50 (45– 60) μm
acetabulum	43 (37– 48)	×	49 (43– 55) μm

Specific description: (Fig. 28)

Simple-tailed distomatous, gymnocephalous cercaria. The body is ellipsoidal in shape, being more blunt anteriorly, and covered with many backward directed spines all over the surface. Two pairs of sensory hairs are observed on the anterior surface. The oral sucker is large and spherical, in which two pairs of cephalic glands are recognized. The mouth is subterminal, and leads into a short prepharynx, a small pharynx and an esophagus which divides into two long ceca reaching near the end of the body. A well developed acetabulum is a little smaller than the oral sucker, and situated somewhat posteriorly to the middle of the body. A nervous system is composed of a transverse commissure across the prepharynx, a short anterior and long posterior branch along both sides of the body.

A broad I-shaped epithelial excretory vesicle occupies the posterior third space of the body. From its antero-lateral corner one pair of main collecting tubes arises, runs forward in zigzag course along the side of acetabulum, and divides into an anterior and a posterior collecting tubule to receive flame cells. The flame cell formula is constructed as 2[(3+3)+(3)]=18, though it is

Fig. 28. *Cercaria leyteensis no. 28.*



possible that some cells escaped my detection. There are three pairs of brownish, finely granular, pyriform penetrating gland cells at the posterior part of the body, on each side of the excretory vesicle. They are individually distinct when the body is well extended, but when the body is contracted they seem to coalesce. The ducts of these glands are very inconspicuous and hardly recognizable. Only three pairs of openings are faintly observed just in front of the oral sucker. A germinal cell mass is found at the space between the acetabulum and the excretory vesicle. The tail is shorter than the body, and aspinose. No caudal excretory tube could be observed.

Remarks: One of the most outstanding feature of this cercaria is the position of the penetrating glands which are situated on each side of the excretory vesicle. In this point, the present cercaria is much similar to *Cercaria maitimensis* which was reported by Tubangui (1928) from *Pila luzonica (=Ampullaria lagunaensis)* on Luzon Island. Though there found many similarities between these two species, such as the same snail host, the fundamentally same body structures, but a definite difference of the stylet exists between them. Tubangui described and figured a sharply pointed and shouldered stylet measuring 35 × 6 μm in *Cercaria maitimemsis,* while no such one could be found in the present cercaria. So it is impossible to identify *Cercaria leyteensis no. 28* with *Cercaria maitimensis* at present. As to the life cycle of the cercaria, it is unpresumable what kind of second host and final host will be necessary to complete the life cycle of this cercaria. Therefore the presumptive adult form is also unknown at present.

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A List of Cercariae from Viviparidae and Pilidae in Leyte Island, Philippines.
(Results from 2,075 Viviparidae and 219 Pilidae during 1975–1976)

Species	Presumptive adult form	Number of snails infected	Body size (μm)	Locality
Cercaria leyteensis no. 20	Cyathocotylidae	3 (0.14%)	232×145	Palo, Santa Fe
Cercaria leyteensis no. 21	Sanguinicolidae?	1 (0.05%)	100× 50	Palo
Cercaria leyteensis no. 22	Notocotylidae	2 (0.10%)	350×176	Tabontabon
Cercaria leyteensis no. 23	Echinostomatidae (*Echinostoma* or others)	5 (0.24%)	477×182	Palo, Burauen, Javier
Cercaria leyteensis no. 24	Echinostomatidae (*Euparyphium paramurinum*?)	4 (0.19%)	410×197	Palo
Cercaria leyteensis no. 25 (C. rediçystica)	Echinostomatidae (*Echinochasmus* or others)	37 (1.78%) 1 (0.46%)*	167× 83	Palo, Santa Fe, Pastrana
Cercaria leyteensis no. 26	Microphallidae?	57 (2.75%)	110× 55	Palo, Santa Fe, Pastrana, Tanauan, Dagami, Tabontabon
Cercaria leyteensis no. 27	Microphallidae (Microphallinae)	2 (0.10%) 10 (4.57%)*	83× 36	Palo, Dagami, Tabontabon
Cercaria leyteensis no. 28	Unknown	1 (0.46%)*	216× 95	Palo

(* mark is from Pilidae, the others are from Viviparidae)

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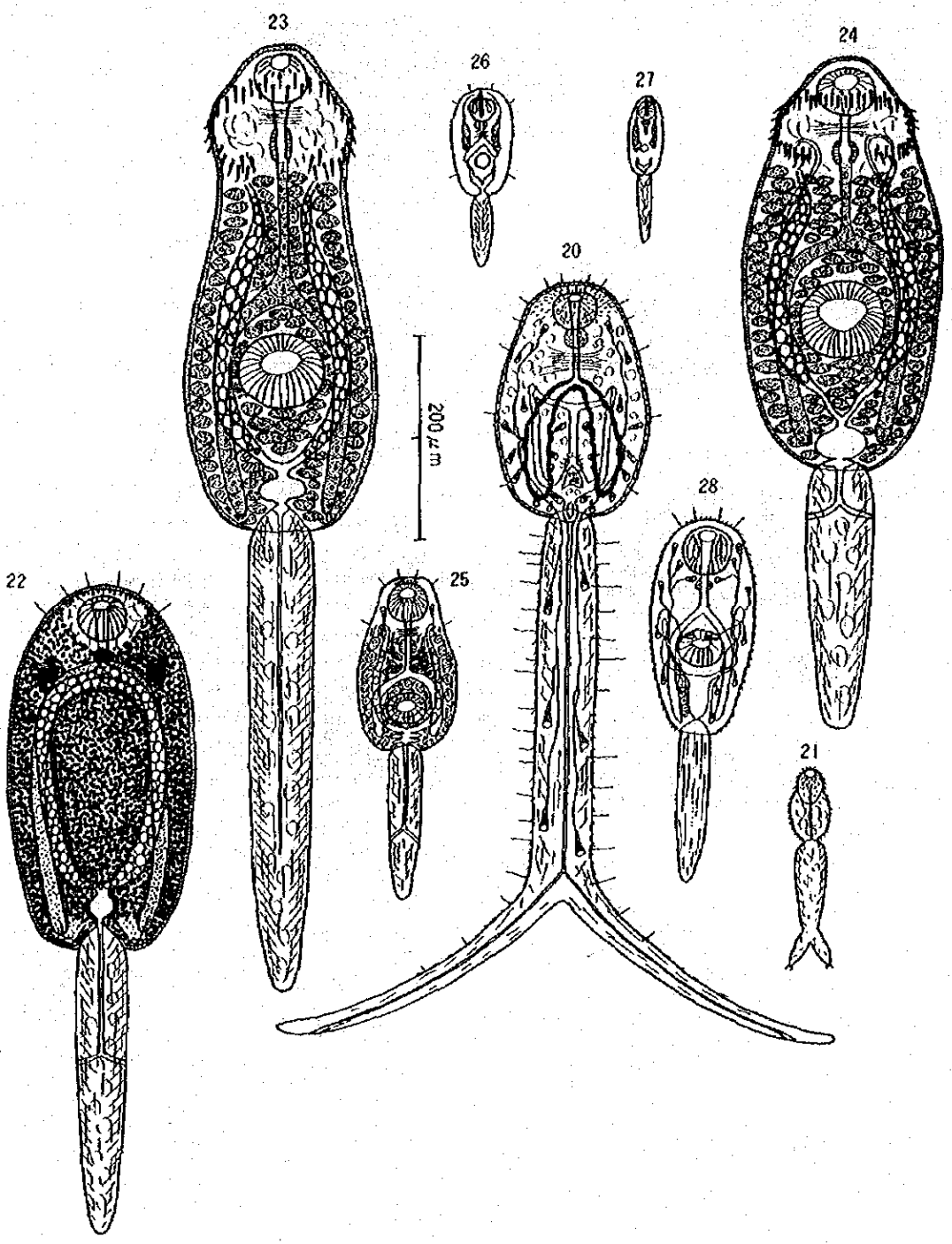
Explanation of Plates

Plate 1. Cercariae from Viviparidae and Pilidae.
(semidiagrammatic drawings by the same scale)

20 *Cercaria leyteensis no. 20* (Cyathocotylidae)
21. *Cercaria leyteensis no. 21* (Sanguinicolidae?)
22. *Cercaria leyteensis no. 22* (Notocotylidae)
23. *Cercaria leyteensis no. 23* (Echinostomatidae)
24. *Cercaria leyteensis no. 24* (Echinostomatidae)
25. *Cercaria leyteensis no. 25* (Echinostomatidae)
26. *Cercaria leyteensis no. 26* (Microphallidae?)
27. *Cercaria leyteensis no. 27* (Microphallidae)
28. *Cercaria leyteensis no. 28* (Unknown)

Plate 2. Cercariae from Viviparidae and Pilidae.
(photographs by various scales)

C. 20. *Cercaria leyteensis no. 20* with the body enlarged
C. 22. *Cercaria leyteensis no. 22*
C. 24. *Cercaria leyteensis no. 24*
C. 25. *Cercaria leyteensis no. 25*
C. 26. *Cercaria leyteensis no. 26*
C. 27 *Cercaria leyteensis no. 27*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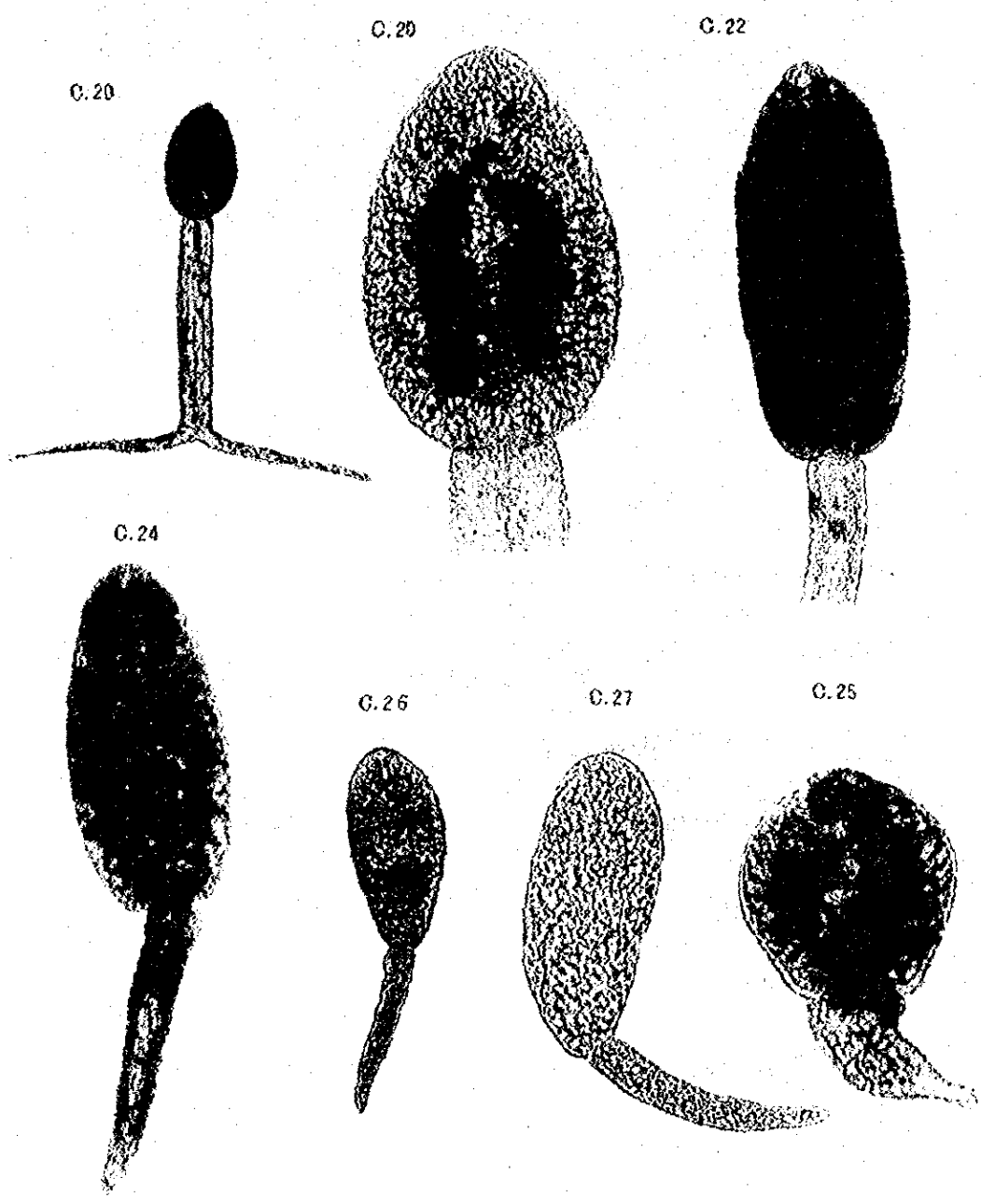
C. 20
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C. 22
C. 24
C. 26
C. 27
C. 25

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