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BIOLOGICAL AND MORPHOLOGICAL STUDIES OF PARACENTROBIA AND01 (ISHII) (HYMENOPTERA : TRICHOGRAMMATIDAE), A PARASITE OF THE GREEN RICE LEAF HOPPER, NEPHOTETTIX CINCTICEPS UHLER (HOMOPTERA : DELTOCEPHALIDAE)

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BIOLOGICAL AND MORPHOLOGICAL STUDIES OF PARACENTROBIA AND01 (ISHII) (HYMENOPTERA : TRICHOGRAMMATIDAE), A PARASITE OF THE GREEN RICE LEAF HOPPER, NEPHOTETTIX CINCTICEPS UHLER (HOMOPTERA : DELTOCEPHALIDAE)[1]

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I. Introduction

The green rice leafhopper, *Nephotettix cincticeps* Uhler, is an important rice insect pest and also the vector of the rice dwarf virus. It widely occurs over Japan (Hokyo, 1971). Its egg parasites are four mymarid wasps and two trichogrammatid species. The former species are ***Ooctonus*** sp. (Esaki and Hashimoto, 1935), *Lymaenon* sp. and ***Anagrus*** sp. (Orita, 1972), and ***Gonatocerus*** sp. (Orita, 1969). The trichogrammatid parasites are ***Chaetostricha*** *aurulenta* Doutt (Orita, 1972) and ***Paracentrobia andoi*** (Ishii). The latter is the dominant parasite. Parasitism by this species was considered to be density-dependent against host densities and the percentage parasitism increased every year in insecticide-free paddy field (Sasaba and Kiritani, 1972). Except for the information given by Sasaba and Kiritani (1972), little is known about the biology and habits of this parasite. From the standpoint of biological control of rice pests, ***Paracentrobia andoi*** is expected to be one of the efficient natural enemies of the green rice leafhopper. Therefore, the studies of biology and habits of this species have been conducted for two years (in 1973 and 1974) in Fukuoka District, Kyushu, Japan. This paper reports the results. Since correct identification of natural enemies is essential to the success of biological control projects (Rosen and DeBach, 1973), the morphological studies of this parasite were also done. These studies, I hope, will serve as basic informations to advanced researches of parasitic Hymenoptera in relation to the biological control of insect pests.

II. Materials and methods

Culture of host and parasite. For laboratory studies the stock of host eggs is necessary. In order to insure the supply of host material for parasite, ***Paracentrobia***

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andoi, adults of *Nephotettix* ***cincticeps*** Uhler were collected from the paddy fields and released in the rearing cage, 70x 50 x 110 cm in size, in which there were rice plants grown in the pots. New rice plants were provided every week. The rearing cages were placed outdoors. After two or three days eggs of *Nepho-****tettix*** were laid in mass in the leaf sheath of rice plants. Then the eggs were removed daily and used for experiments. Some were refrigerated in glass jars, filled with some water, in order to slow down the development of eggs. In such method host eggs in suitable stage for parasitism can be kept for about ten days.

The stock of parasites for experiments was obtained and propagated from the eggs of the green rice leafhopper, N. ***cincticeps,*** in laboratory. Some, in pupal stage, were collected from the paddy fields and kept in test tubes for adults. In laboratory the parasitized host eggs were kept in the cabinet controlled at 25±1°C with 14 hr photoperiod until parasites emerged.

Handling the parasite. To handle small numbers of parasites or transfer a single one from test tube into another, a fine camel's hair brush was used, the tip of which was slightly moistened. By gently touching the parasites' bodies with the tip of the brush, they could be picked up and transferred without injury. For keeping adult parasites as a stock, they were kept in test tubes with concentrated honey streaks applied inside the test tubes, serving as food for adult parasites. They were then kept in the cabinet controlled at 25±1°C. The adults' life span could be prolonged than usual.

Microtechniques and measurements. Dissections of adult wasps were done in order to study the internal morphology. Dissections of parasitized host eggs were made in saline solution for the morphological studies of the immature stage of the parasite. The specimens were dissected with fine needles. The internal reproductive and digestive systems of the adult could be drawn out by this method. Certain microscopic observations were best made of material in saline solution mounted on slide. To differentiate larval stages it was necessary to record the size and shape of the mandibles. They were clearly seen under Nikon microscope (15x ocular, 10 or 20x objective). For higher power magnification the larval specimens were mounted with Neo-shigaral, Japanese product, and covered with slide-cover glass ; then they were observed with high power objectives (15× or 40 x objectives). The measurement of mandibles in microns were done by means of a 15x calibrated ocular micrometer. Several measurements of specimens and structures were taken and an average figure obtained.

III. Classification, distribution and hosts

The genus ***Paracentrobia*** is worldwide in distribution (Doutt, 1968). Systematically it is placed in the hymenopterous family Trichogrammatidae in the super-family Chalcidoidea. ***Paracentrobia andoi*** (Ishii) was originally described as ***Japania andoi*** Ishii (Ishii, 1938). Later the genus of this parasite, ***Japania,*** has been changed to ***Paracentrobia*** by Doutt (1968).

P. ***andoi*** has been found in Japan from the following localities: Shizuoka (Ishii, 1938), in Ishikawa Prefecture at Kanazawa-shi and in Oita (Doutt, 1961), in Shikoku at Kochi (Sasaba and Kiritani, 1972).

This parasite is the dominant species among egg parasites of the green rice leafhopper. The other hosts of *P. andoi* are *Nilaparvata lugens* (Stål), *Nilaparvata oryzae* (Matsumura), and *Delphacodes sameshimai* (Matsumura et Ishihara) (Doutt, 1961).

IV. Morphological studies

A. I. *General description of the species and external morphology*

Adult female. Length, 0.72 mm in average. Head yellowish brown; genae and occiput brown. Head convex above, a little broader than thorax; in frontal view slightly longer than wide, subtriangular. Scrobes distinct, moderately deep ; facial ridge prominent. Toruli oval, nearer to clypeal margin than to each other (6 : 7), equidistant from each other and from eye margin. Occipital margin concave. Frontovertex broad, occupying about two-thirds of width of head. Compound eyes large and red, their longitudinal diameter about as long as width of cheek. Ocelli

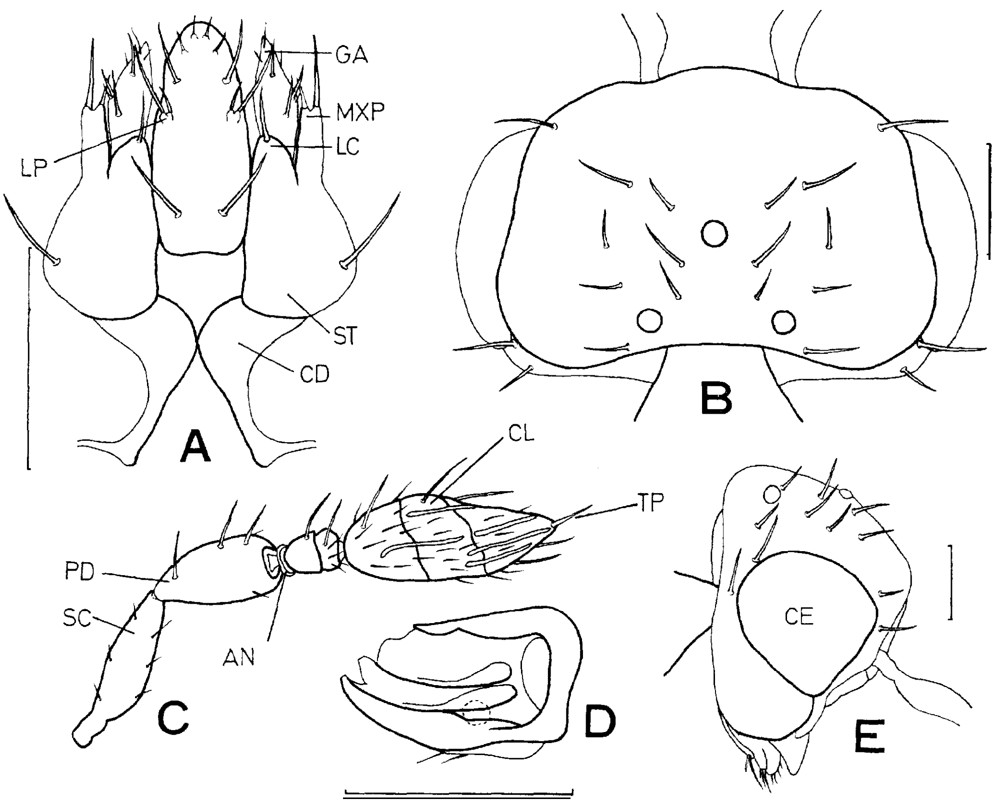

Fig. 1. Head and its appendages of adult of *Paracentrobia andoi*. A, ventral aspect of labium and maxilla ; B, dorsal aspect of head ; C, antenna; D, mandible; E, lateral aspect of head. All scales are 0.05 mm. AN=anelli, CD=cardo, CE=compound eye, CL=clava, GA =galea, LC = lacinia, LP= labial palpus, MXP= maxillary palpus, PD= pedicel, SC = scape, ST= stipes, TP= terminal spine.

red, in obtuse triangular, widely separated from each other; post ocellar distance slightly longer than distance between anterior and posterior ocelli, a little shorter than ocellocular distance; posterior ocelli close to occiput (posterior margin of head) as seen from above (Fig. 1B). Maxillary palpi one-segmented (Fig. IA). Labial palpi short, one-segmented. Mandibles brown, tridentate. Antennae eight-segmented (1, 1, 1, 2, 3), inserted in about the level of lower margin of eyes. Radicle elongate, about one-fourth as long as scape. Scape about three times as long as wide. Pedicel obconical, about twice as long as wide, two-thirds the length of scape. Funicle together with anelli about half the length of pedicel. Club long, three-segmented: third segment bearing a terminal spine.

The tentorium (Fig. 2 B) is Y-shaped sclerotized structure. The dorsal arms of tentorium (Fig. 2, DT) arise from the base of anterior tentorial arms and extend sidewards, not extending upwards. Body of tentorium (Fig. 2 tb) is rather long.

Thorax in dorsal view yellow from pronotum to propodeum; roundly convex from side to side, Pronotum short, with posterior collar covering anterior margin

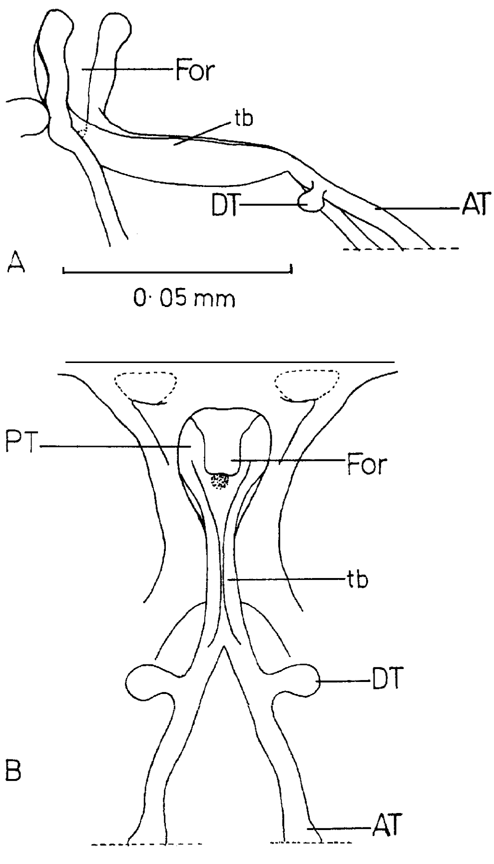

Fig. 2. Tentorium of *Paracentrobia andoi.* A, side view ; B, dorsal view. AT=anterior arm of tentorium, DT=dorsal arm, For=foramen magnum, PT = posterior arm of tentorium, tb= body of tentorium.

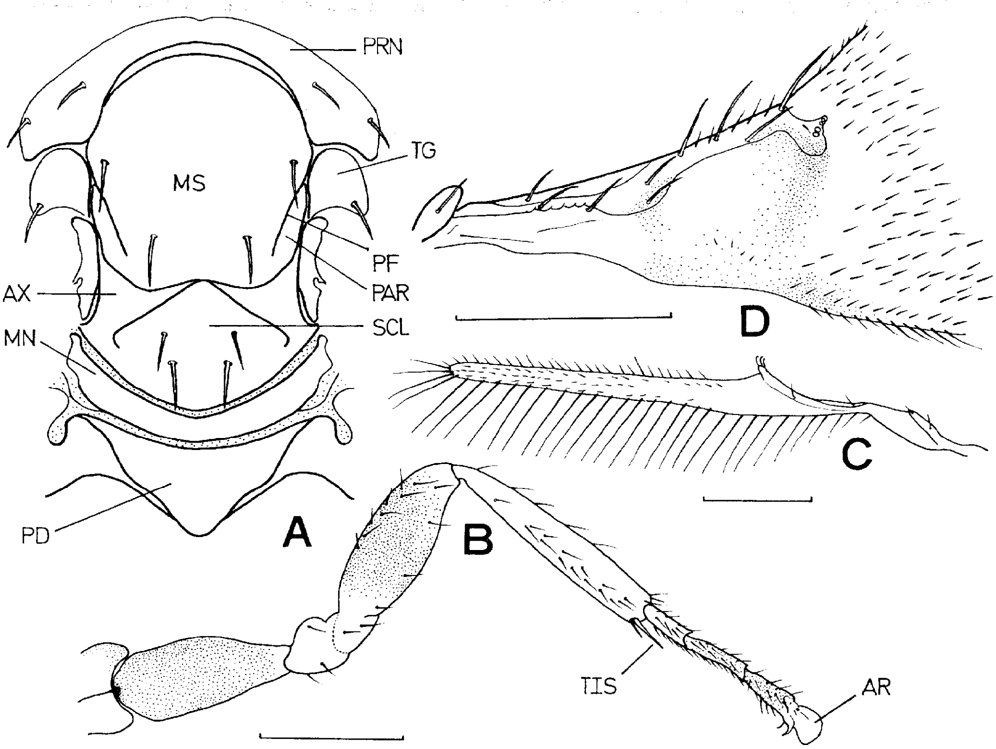

Fig. 3. ***Paracentrobia andoi.*** A, dorsal view of thorax; B, hind leg; C, dorsal view of hind wing; D, dorsal view of fore wing. AR=arolium, AX=axilla, MN=metanotum, MS= mesoscutum, PD = propodeum, PF = parapsidal furrow, PAR= parapsis, PRN = pronotum, SCL =scutellum, TG=tegula, TIS= tibial spur. All scales are 0.1 mm.

of mesoscutum. Mesoscutum (Fig. 3, MS) about as wide as long, bearing two pairs of bristles. Parapsides with conspicuous suture. Scutellum transverse, one and three-fourths times as wide as long, about two-thirds the length of mesoscutum, bearing two pairs of bristles ; anterior bristles a little shorter and more slender than the posterior ones. Axillae triangular, extending antero-laterad of scutellum (Fig. 3, AX). Propodeum rather long, with triangular area pointing caudad.

Abdomen conic-ovate, as long as the head and thorax combined. On the first four segments of abdomen there are dark brown bands around. Dark brown patches present on lateral parts of fifth and seventh segments.

Ovipositor brown. Outer ovipositor plates about eight times as long as wide. Ovipositor not extruded, its base protruding from third abdominal segment.

Legs light brown in general, with third segment of tarsus somewhat dark brown. Hind femur (Fig. 3 B) bigger than the others, dark brown except both ends. Hind coxa dark brown. Legs rather long, all tarsi three-segmented. Hind tibia a little longer than mid tibia; hind tibial spur (Fig. 3, TIS) about three-fourths as long as basi-tarsus.

Forewing (Fig. 3 D) with costal cell long and bearing a single seta. Postmarginal vein absent. Stigmal vein bearing a single seta, two small papillae (circular sensillae) at apex of stigmal vein, with two larger ones next below them. Discal ciliation rather dense, Area from stigmal vein to anal margin (Fig. 3 D) clouded in dark brown, but not uniform in color; it is darker near stigmal vein. A part of submarginal vein connected with marginal vein dusky brown. Hind wing (Fig. 3 C) slender, shorter than forewing, bearing two hooks and one bristle at small projection on hind margin.

A. II. *Digestive system and female reproductive system*

The specimens of digestive and female reproductive systems were stained with

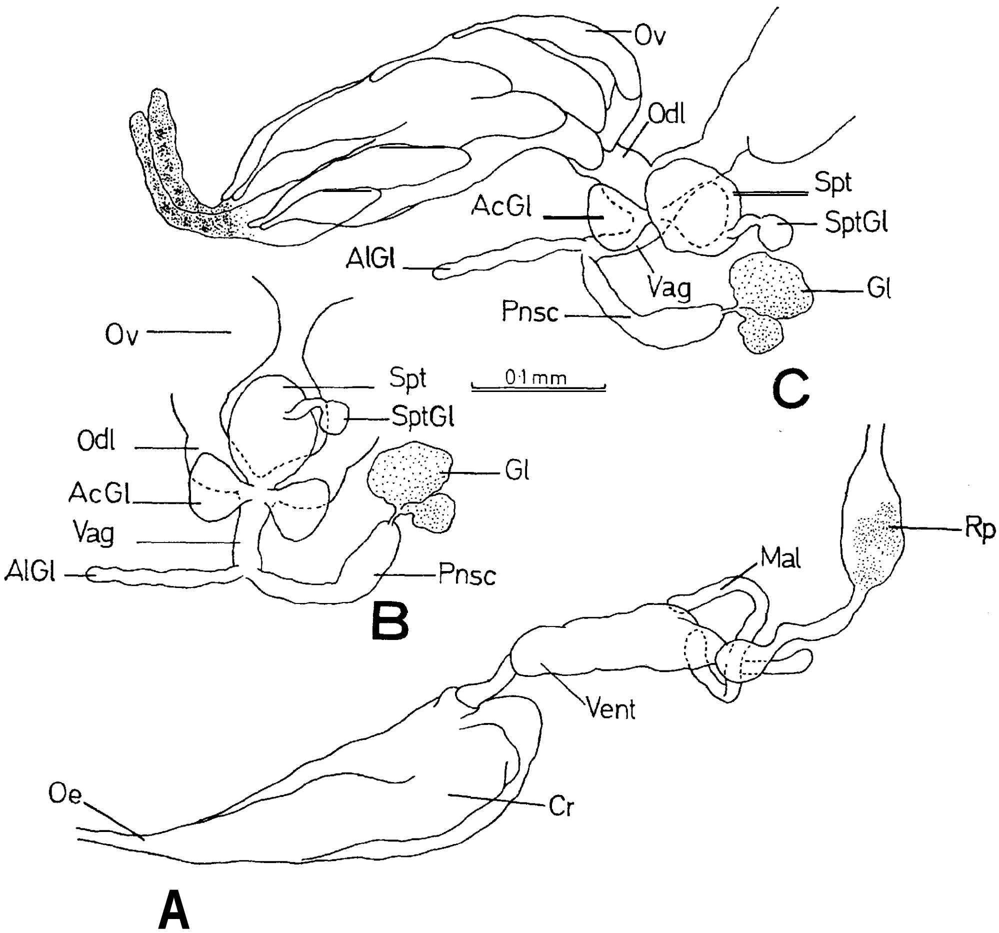

Fig. 4. Digestive and female reproductive systems. A, alimentary canal; B and C, female reproductive system in posterior and lateral view. AcGl = accessory gland, AlGl = alkaline gland, Cr = crop, Gl = acid gland, Mal = malpighian tubule, Odl = lateral oviduct, Oe = esophagus, Ov = ovary, PnSc = poison sac, Pvent = proventriculus, Rp = rectal pad, Spt = spermatheca, SptGl = spermathecal gland, Vag = vagina, Vent = ventriculus.

one or two drops of acetocarmine solution for about one minute and then mounted on slide with saline solution for further microscopic observations and drawings.

Digestive system. Alimentary canal is divided into foregut, midgut, and hindgut. The forgut is divided into esophagus, crop, and proventriculus. The esophagus is thin and narrow, and expands into the crop as it enters the abdomen. The crop is large and thin. The proventriculus is thick and round. Next to proventriculus is an oblong and cylindrical midgut. The hindgut is divided into two regions, anterior and posterior intestines. The region posterior to malpighian tubules is roundly expanded and tapers posteriorly (Fig. 4 A) to be round and moderately long tube, the colon. The posterior intestine is dilated into rectal sac and narrowed posteriorly into a straight tube, the rectum proper. Many chalcidoids possess three malpighian tubules (Hagen, 1964). There is no exception for this species also. There are three malpighian tubules. The third tubule is a little shorter than the others and extends posteriorly.

Female reproductive system. The ovary consists of two pairs of long ovarioles. Each ovariole is in contact with the other ovariole of the pair throughout its length (Fig. 4 C). The posterior end of the combined ovarioles is large before entering

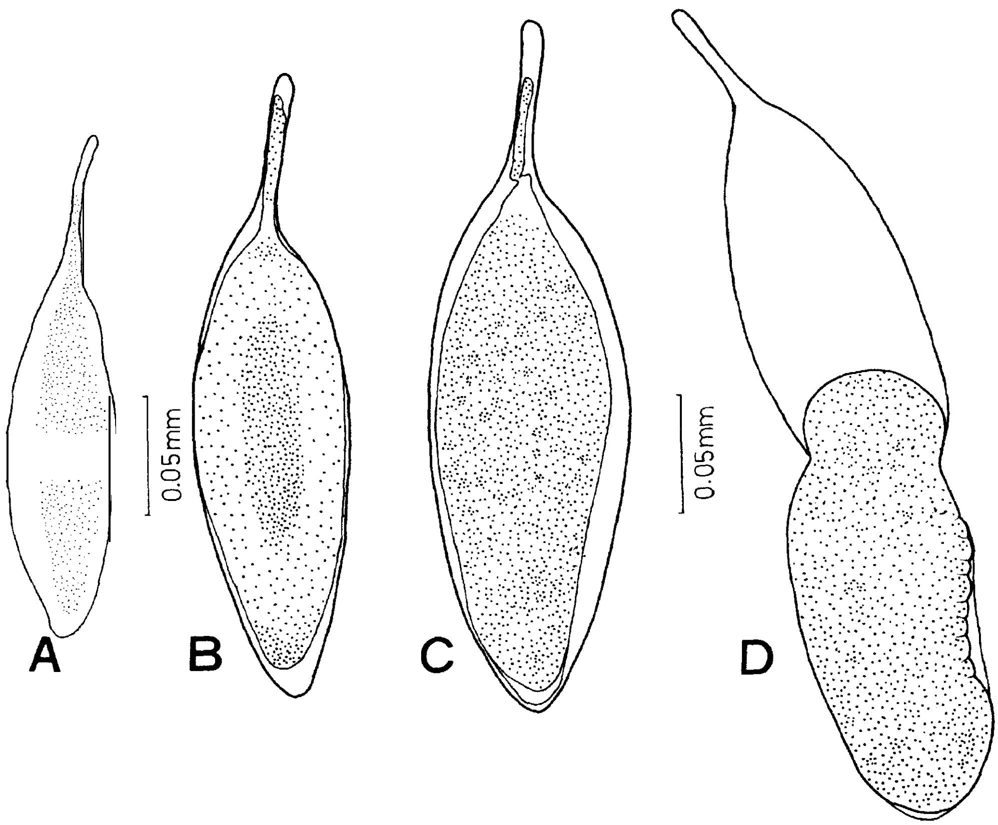

Fig. 5. Egg stage of *Paracentrobia andoi* during development. A, ovarian egg; B, egg after 5 hours; C, egg after 15 hours ; D, egg during hatching.

lateral oviduct. After emergence of adults the ovary contain almost all ripe eggs. Leading to the ovipositor through opening (Fig. 4 B, C) there are the following structures: an alkaline gland (AlGl), an acid gland (Gl), accessory glands and the spermatheca (Spt) with its spermathecal gland (Spt Gl). The spermatheca is round ; the spermathecal grand is also round. Two accessory glands are subspherical in shape and attach closely with spermatheca (Fig. 4 C). The alkaline gland is round, long and tube-like. Acid gland has long and round reservoir sac (PnSc). The opening of acid and alkaline glands seem to be at the base of ovipositor.

B. *Immature stage*

The egg. Ovarian egg (Fig. 5 A) is composed of spindle-shaped egg body with a slender pedicel. The length of egg body is 0.14 mm in average ; the length of pedicel is 0.05 mm in average. The eggs overlap in ovarioles so that the pedicel of one is immediately above the main body of the next (Fig. 4 C). The appearance of deposited eggs are similar to that of the ovarian egg. Newly laid eggs are transparent ; the chorions are smooth and delicate. The deposited egg increased in volume during development. Newly laid egg measures 0.02 mm in length for egg body and 0.08 mm in width. The incubation period ranged from 14 to 20 hours. The head of larva was found developed at the anterior part of egg. The first larval instar was distinctly seen enclosed in the embryonic membrane (Clausen, 1940) before hatching. The chorion was ruptured at the posterior end of egg during hatching.

First **instar** larva. The first instar larva is 0.22 mm in length and 0.07 mm in width (Table 1). The larva is at first inside the embryonic membrane, being scraped out later by wriggling the body especially at the 'posterior end. During this time the spine-like process (Fig. 6 A) can be seen ; it is assumed to use for lacerating the embryonic membrane out. The body wall is membranous and granulated (Fig. 6 E). The segments of body, not certain in numbers, are at first discernible but obliterated later by the expansion of the body. The larva is elongate, nearly parallel-sided and a little tapering posteriorly. The head is cone-shaped. A pair of mandibles are not clearly seen and colorless; they measure 22 microns in average. The alimentary tract cannot be seen in this early stage. The time required for the development of the first instar is short.

Second **instar** larva. The newly emerged second instar larva is firstly the same as the full-grown first instar larva. It measures 0.34 mm in length and 0.10 mm in width in average (Table 1). The body is smooth and translucent. The esophagus and midgut are clearly seen. The midgut is elongated, sac-like and occupies almost all of the body. The mandibles are yellow especially at the tip. The fragment of body wall, assuming to be the first moulting skin, sometimes stick with the larval body. The larva will consume host egg's fluid and the body expands posteriorly in a round shape. There are four segments at the anterior part of larva; now the body become yellow with fat body. The larva is active and will wriggle especially at the anterior part. A pair of mandibles measure 35 microns long, slightly curved (Fig. 6 C) with sharp pointed tip; the base is still narrow. The first and second instar larval stages together last one day.

Third **instar** larva. The second instar larva gradually increases in size ; the

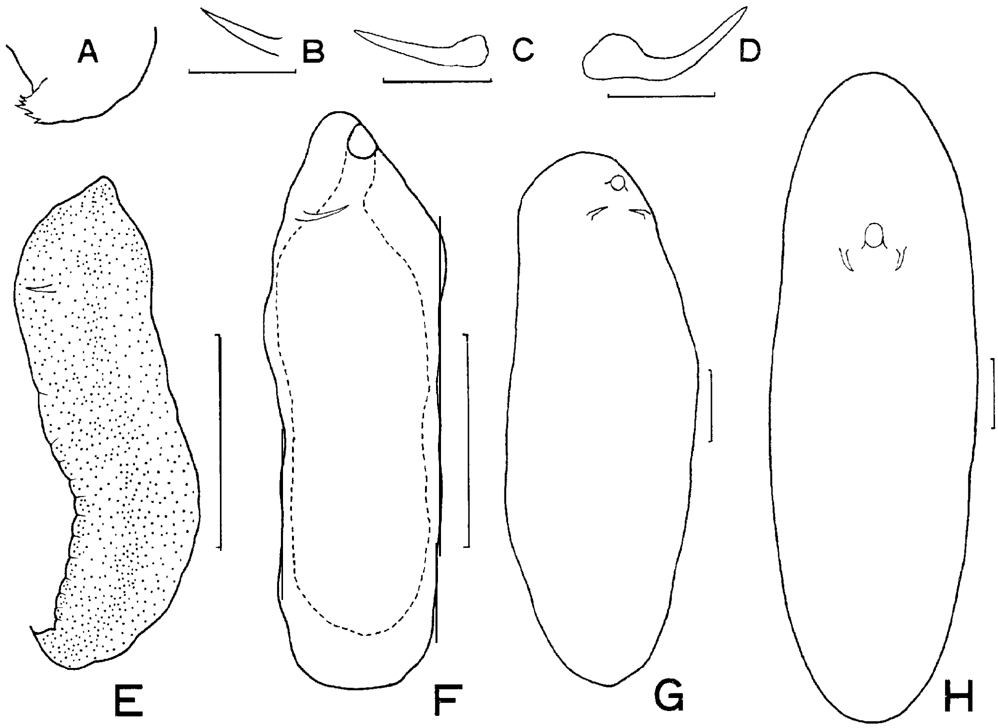

Fig. 6. Immature stages of *Paracentrobia andoi.* A, spine-like process; B, C and D are mandibles of first, second and third instar larvae, respectively; E and F, first and second instar larvae ; G and H, third instar larvae. Scales for larvae are 0.1 mm. Scales for mandibles are 0.025 mm.

body is now yellow. The marked change in the body size and the shape of mandibles during the second instar larva stage suggest the occurrence of moulting. The larvae consume almost all of host egg's fluid and become about as big as host egg's size ; the head or anterior part of larva is now round. The digestive system cannot be seen any more. The mouth part a littte protrudes; later it will be moved a little posteriorly from anterior extremity (Fig. 6 H) in the late third instar. The full-grown larva measures 0.87 mm in length and 0.22 mm in width. The mandibles are now strongly curved and measure 40 microns; their bases are broad (Fig. 6 D).

Prepupa. The third instar larva consumes almost all of the nutrient inside host egg and becomes motionless. The egg shell of host egg now turn into light brown and becomes dark brown or brown in the pupal stage. The prepupal stage lasts about one day.

Pupa. The larva pupates inside the host egg. The pupa is at first whitish yellow. The waste material in dark color inside the food tract can be seen under transmitted light by microscope. As the pupa develops the appendages become visible and closely folded about the body. Initial coloring began with the reddening of the compound eyes and ocelli. Later the characteristic adult color appears. By

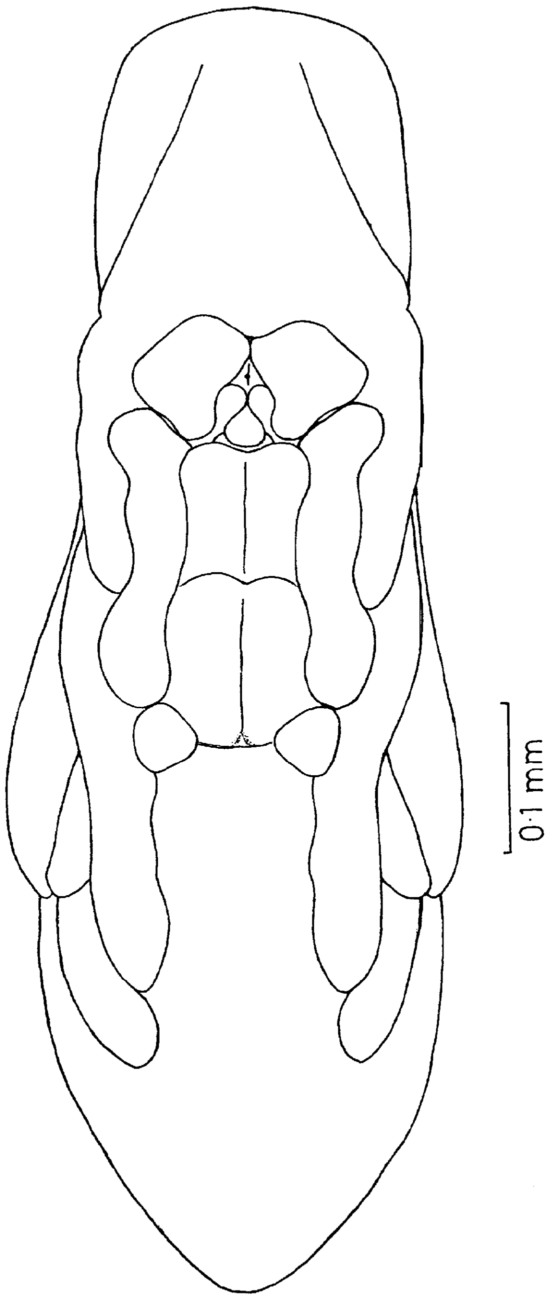

Fig. 7. Pupal stage of *Paracentrobia* ***andoi.***

dissecting of this stage the moulting skin of the third instar larva was found stick at the posterior part of the pupa. The pupa is of naked and exarate type (Fig. 7).

V. Biological studies

A. *Life cycle*

The procedure for studying the life history of ***Paracentrobia andoi*** was as follows: a number of host eggs were confined in a glass vial, 9.5 x **2.3** cm, with one female parasite. Host eggs were exposed for parasitization about 3 hours and then transferred to a temperature-controlled cabinet at 25±1°C with 14 hr photoperiod. They were at first kept in desiccators and one week later were placed in test tubes with cotton wool stoppers which were soaked with water daily for maintaining the moisture inside. Beginning 2 hours after oviposition, parasitized host eggs

Table 1. The measurement and developmental period of immature stages of *Paracentrobia andoi* at constant temperature of 25°C.

Stage	Size in average (mm) Length	Width	Duration**
Egg	0.20”	0.08	14-20
Larva			
1st instar	0.22	0.07	1
2nd instar	0.34	0.10	
3rd instar	0.87	0.22	1-2
Prepupa			1
Pupa	0.82	0.21	13-18
Egg – adult			17-23

* Egg’s pedicel excluded.
** Duration shown in days except egg stage in hours.

were dissected every 2 hours for egg development observation until hatching; 10 parasitized host eggs were dissected each time. The number of instars and duration of larval stages were determined by careful daily measurements of the size and shape of mandibles and larva. The result of the developmental periods required, and body sizes of larvae were shown in Table 1. The incubation period of egg ranged from 14 to 20 hours. The total duration of first and second instars was 1 day. Period of the third larval instar and that of the prepupal stage were 1-2 days and 1 day respectively. The period of pupal stage ranged from 13 to 18 days. Under controlled condition (25±1℃) the developmental period from the egg stage until the adult emergence ranged from **17** to **23** days.

B. *Effect of temperatures to the development*

The experiments were conducted for studying the effect of constant and varying temperatures to the development of immature stage of this parasite. A number of host eggs exposed for a female wasp for three hours were then put in small desiccator and then kept in temperature-controlled cabinets at 15, 20, 25, and 30°C.

Five days later they were transferred to keep inside the test tubes stoppered with cotton wool, being soaked with water every day for maintaining the humidity inside. The relative humidity of the cabinets were all set at 65 % with 14 hour photoperiod. The effect of varying temperatures was also observed in the same procedure stated before except for that parasitized host eggs were kept outdoors. The experiments were conducted during July 12 to October 8, 1974. Average temperatures of each experiment were calculated from daily maximum and minimum recorded from thermometer set near the experimental place. After one week daily counting of emerging parasite adults was started.

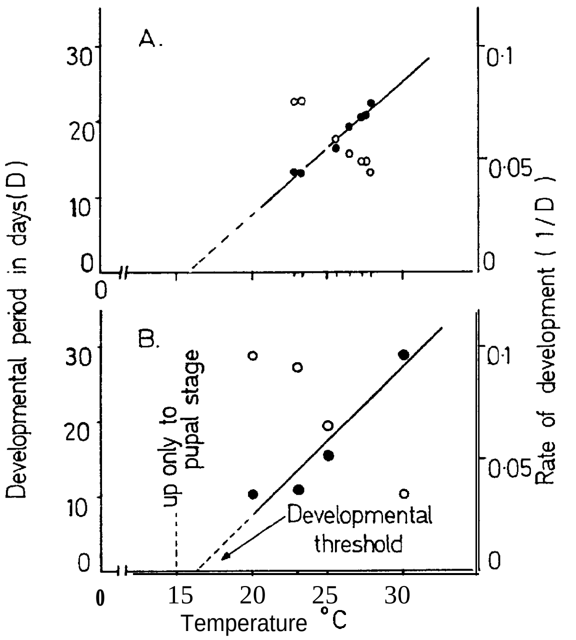

Fig. 8. The relationship between temperatures and rate of development of *Paracentrobia andoi* under constant and varying temperatures. (○), developmental period; (●), rate of development. A, under varying temperatures. B, under constant temperatures.

Results. Under constant temperatures the developmental periods (Table 2) decreased as temperatures increased from 20 to 30°C. At 15°C the parasite larva could not develop beyond pupal stage. The shortest developmental period, 10.31 days, was at 30°C. The threshold of development was calculated by using the method of least square (Bodenheimer, 1926). The threshold of development was 16.3°C for female (Fig. 8). The thermal constant was 149.19 degree-days. The developmental periods under varying temperatures are shown in Table 3. The experiments during August the developmental periods were in the range of 13.36 to 15.5 days in average. In July it was 17.78 days in average. The longest period for development was 22.92 days in September. The experiments done in October the parasite larvae could not develop beyond pupal stage. From Table 3 the

Table 2. Effect of constant temperatures to the developmental period from egg to adult of ***Paracentrobia andoi.***

Tern. ℃	No. of emerging adults observed	Duration of development (days) Mean ± SE.	Range (days)
15	86”	Only to pupal stage	
20	57	28.82 ±0. 29	26-37
23	67	27.38 ± 0.30	25-31
25	84	19.38 io. 15	17-23
30	78	10.31 ±0. 13	9-14

* Numbers of parasitized host eggs.

Table 3. Effect of varying temperatures to the developmental period from egg to adult of ***Paracentrobia andoi.***

Tern. ℃ (Ave.)	Duration of experiment	No. of emerging adults observed	Duration of development (days) Mean ±S.E.	Range (days)
25.44	July 12-31, ‘74	46	17. 78±0. 08	16-19
27.87	Aug. 6-24,	99	13. 36±0. 12	12-18
27. 92	13–Sept. 9	188	14. 56±0. 13	11-19
27.55	19–Sept. 5	41	14. 61±0. 13	13-17
26.98	21 -Sept. 14	46	15. 50±0. 43	12-19
23.52	Sept. 7-Oct. 5	63	22. 92±0. 22	21-28
19. 44*	Oct. 1– 8–	107 ** 155	Developed up only to pupal stage	

* Average of air temperature in October.
** Number of parasitized host eggs.

developmental periods under varying temperatures were just slightly shorter than that under corresponding constant temperatures (Table 2). Heat requirements from egg stage until the emergence of adults under varying temperatures were calculated and compared with thermal constant calculated from constant temperature condition (Table 4). Under varying temperatures the heat units required for whole development are shown in Table 4 and Fig. 9. The number of effective degree-days above 16.3℃ was calculated for each day and accumulated from the date of oviposition to the date of 50 % emergence of adults (the date when the number of adult emerging exceed 50 % of the total emergence). The effective degree-days above the threshold, 16.3℃, is conventionally calculated as follows :

$$\frac{\text{Max.} + \text{Min.}}{2} - \text{developmental threshold.}$$

Table 4. Comparison between laboratory thermal constant and observed heat requirements in outdoor condition.

Date		Heat units* observed (degree-days)	Difference between** observed and calculated value in Heat units	per cent
July	12, '74.	173.55	24.36	16.33
Aug.	6	162.05	12.86	8.62
	13	174.25	25.06	16.77
	19	168.75	19.56	13.11
	21	159.29	10.10	6.77
Sept.	7	166.10	16.61	11.33
	Mean	167.33	18.14	12.55
	S.D.	6.04		
	C.V.	3.61		

* Heat accumulation calculated from the period between ovipositing date and the date of 50 % emergence of adult.

** Difference calculated from laboratory thermal constant, 149.19 degree-days.

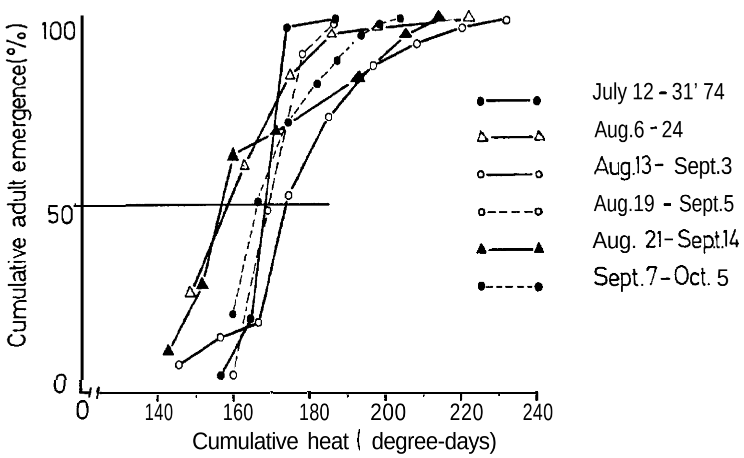

Fig. 9. The relation between cumulative percentage of adult emergence and total heat requirements calculated above the developmental threshold (16.3°C).

During July 12 to October 5, 1974, the daily minimum temperatures were all above the developmental threshold, so the calculations of effective degree-days by using the formula mentioned before are reliable (Arnold, 1960). The heat requirements vary from 159.29 to 174.25 degree-days, the average is 167.33 degree-days. The differences from calculated thermal constant range from 10.10 to 25.06 degree-days (Table 4).

C. *Effect of food and temperatures to adult's longevity*

The longevity of adults in the field is of considerable importance in relation to the parasite's potential rate of increase (DeBach and Sisojevice, 1960) and is one of the factors for effective searching ability (Doutt, 1964). The present study was conducted to find out the effect of food and temperatures to the longevity of adult. Newly emerged female adults were confined in a small vial, 9.5~ 2.3 cm, with cotton wool stopper soaked with water every day and the other tests using concentrated honey streak inside test tubes for the food source. Then they were kept under controlled temperatures of 15, 20, 25, and 30°C. The mortality of them were recorded at 10 a.m. every day. The results (Fig. 10) show that without food, only water provided, the adults has the longest life span at 15°C. When honey was provided as the food source the longevity was 11.10, 11.25, 15.92, and 1.07 days in average at 15, 20, 25 and 30°C, respectively. Under 15°C the parasites were not active.

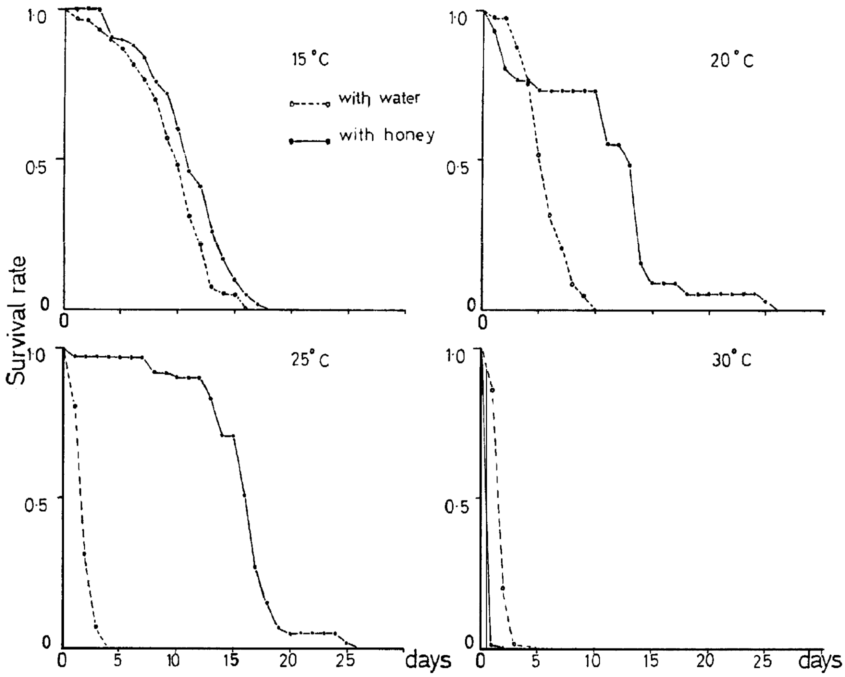

Fig. 10. Effect of food and temperatures to the adult longevity and survival rate.

D. *Over-wintering*

The beginning and the termination of overwintering. A large number of parasitized host eggs were collected from the paddy fields weekly in October. They were put in test tubes and kept outdoors for checking the emergence of adult. Overwintering experiment were conducted in 1973 and 1974. Only in 1973 the termination of overwintering was checked. The result was shown in Fig. 11. After the end of October in 1973 or the beginning of November in 1974 there were no emergence of adults. ***Paracentrobia andoi*** overwinters in the pupal stage.

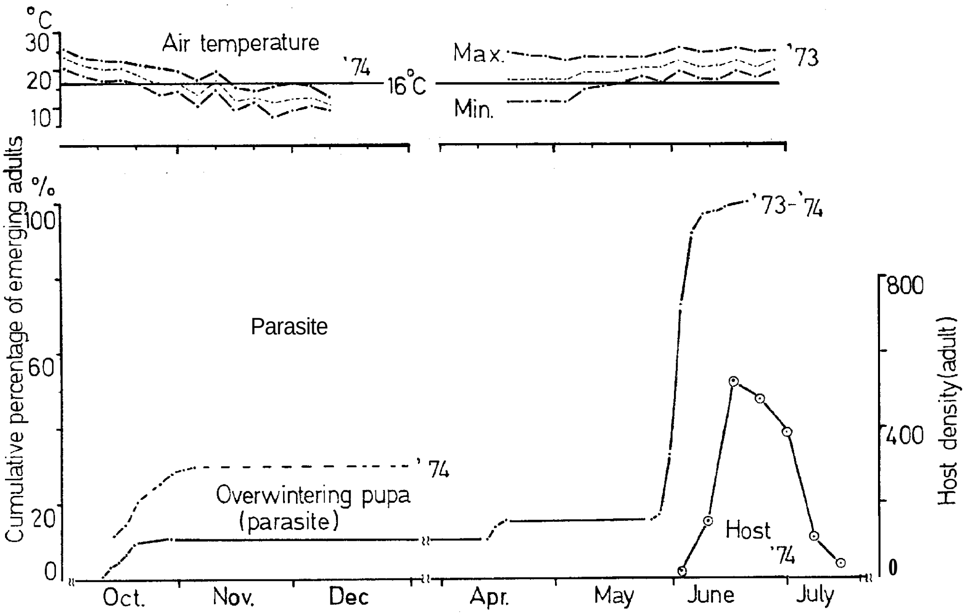

Fig. 11. The beginning and the termination of emerging time of *Paracentrobia andoi.*

In 1974 from November the average of daily temperature was less than 15°C. In 1973 the first emergence of adult was on April 12-17, counting about 5.33 % of total emergence. Emergence of the second time was from May 22; the peak was on June 2. From April 14, 1973, the average of air temperature for five days interval exceeded 17°C.

E. *Effect of parasitism on the host*

About five days after host eggs were parasitized, the color of egg shell became light brown. The black scars of oviposition can be seen under microscope. If the host eggs of early stage are parasitized, their embryos cannot develop any more. But if the host eggs of late stage which have red eyes are parasitized, they can still develop until adult stage.

F. *Adult behavior*

Oviposition. The females of parasite continuously deposited eggs for about one hour and then rested for a while; and they again started depositing eggs. First the female used the antennae tapping the surface of leaf sheath of rice plants. If suitable host eggs were detected they lowered their ovipositors down for exploring the site for oviposition and then thrusted them into the host eggs. The time spent for each oviposition was about two or three minutes. Females never left the batch of host egg to which they were ovipositing to another although there were the other batches nearby. Thus, almost all of the eggs in one batch were parasitized. Newly emerged adults will clean their legs and wings; and a few hours later they are ready for oviposition. In order to know the egg's productivity per one female, one day old female parasite was confined in test tube with two batches of host egg (about 50 eggs) for 1.2 hours for oviposition. Host eggs were changed every day. The experiment was done under constant temperature of 25°C. The average daily laid eggs (Fig. 12) were 35, 3.53, 4.14 eggs on the first, second and third days, respectively. Eggs laid per one female in average was 42.4 eggs, ranging from 28-55 eggs.

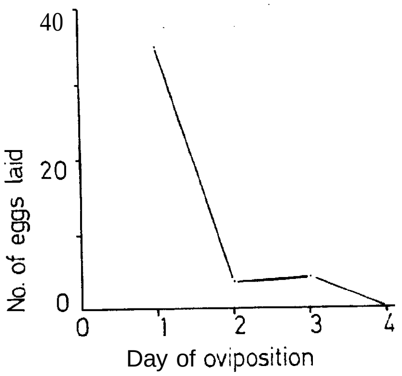

Fig. 12. Average numbers of eggs laid on each day of oviposition.

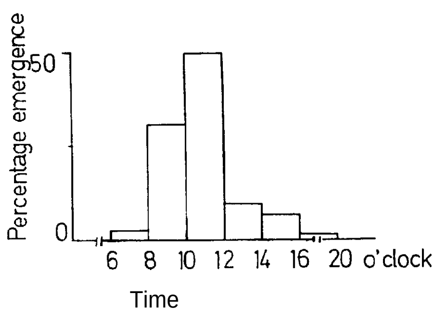

Fig. 13. The emergence of adults of ***Paracentrobia andoi*** at the different time of the day.

Host suitability. The late stage of the host eggs, having red eyes, were not suitable for parasitism. It is assumed that the parasites can discriminate the stage of host eggs.

Superparasitism. The egg of *Paracentrobia andoi* were deposited and floated freely in the fluid of hosts. Usually only one egg was laid per one host egg. But occasionally two or three parasite eggs were found laid in a single host egg both in laboratory and field studies ; but only one larva could develop until adult. Based on this observation and experiment *Paracentrobia andoi* is considered to be a solitary primary egg parasite of *Nephotettix cincticeps.*

Parthenogenesis. From laboratory study it was found that *Paracentrobia andoi* was the thelyotokous parasite. Unmating females could produce progenies and each generation consisted entirely of female.

Emergence. Most of adults emerged in the morning, a few in the afternoon (Fig. 13). The effect of light to adult emergence was studied. The pupae were kept under constant temperature of 25°C with 14 hr photoperiod, the other with no light. Each treatment used 20 parasite pupae with 4 replications. The developmental periods until emergence (Table 5) were 12.49 and 13.23 day in average for

Table 5. Effect of light to the emergence of adult of ***Paracentrobia andoi.***

	Developmental period (days)		% mortality
	Range	Mean	
14 hr. photoperiod	12-14	12.49	10.64
No light	12-15	13.23	58.28

14 hr photoperiod and no light tests, respectively. The mortality was higher in darkness, 58.28 %, than that with light, 10.64 % (Table 5). The dissection showed that pupa could develop until adult; but the adults were mostly found dead inside.

VI. Field experiments

A. *Seasonal fluctuation of host densities and percentage parasitism*

For checking the seasonal fluctuation of host densities and the percentage parasitism, the experiments were conducted in three places. Plot A was set in Kyushu University experimental farm, two others were in farmer's paddy fields. The plot size was about 400 m^2 each. The insecticide was not applied in Kyushu University experimental farm's plot. Farmer's paddy fields in which insecticides were used were at Tatara town and another (Plot B) was near Kyushu University experimental farm. The fluctuation of adult's host density was checked weekly by sweeping 100 times with a sweeping net of 36 cm in diameter per one replication; three replications were made. Host eggs were also weekly collected from ten spots, being chosen at random; each spot had ten hills of rice plants. These ten spots were fixed for next collecting of host eggs. Collected host eggs were brought back to the laboratory for checking the numbers of host and the percentage parasitism. Parasitized host eggs can be distinguished from the healthy ones by the color of their egg shell. Some of eggs which were normal in color were kept in the desiccator for a few days in order to see the changing in color of host egg. The host eggs will be light brown if they were parasitized. The observations were done during August 6 to October 9, 1974. But at Tatara the experimental plot was checked at irregular intervals.

Results. The percentage parasitism (Table 6) ranged from 14.69 to 57.99 % on September 25 and August 22, respectively, in farmer's plot (Plot B) near Plot A. In Plot A the percentage parasitism ranged from 26.05 to 53.59 % on August 29

Table 6. Percentage parasitism of *Paracentrobia andoi* in Plot A and B.

Date	Plot A*		Plot B**	
	Host density	% parasitism	Host density	% parasitism
Aug. 6, '74	274	47.08	1246	32.50
12	451	31.48	2734	37.23
22	237	53.59	626	57.99
29	123	26.05	295	39.66
Sept. 5	146	34.25	137	47.44
11	382	63.87	1551	49.84
18	684	29.53	3219	14.97
25	9472	27.35	7728	14.69
Oct. 2	3002	31.08	5846	19.26
9	773	31.69	3707	26.27

* In Kyushu University experimental farm.
** In farmer's paddy field near Plot A.

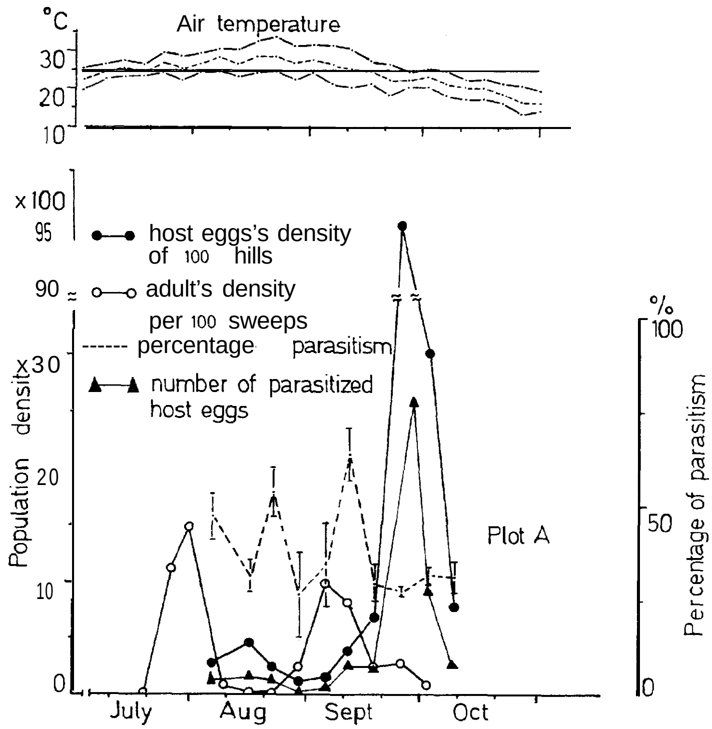

Fig. 14. Seasonal fluctuation of host densities and percentage parasitism in Kyushu University farm (Plot A).

Table 7. Percentage parasitism of *Paracentrobia andoi* at Tatara.

Date	Host density	% parasitism
Aug. 1, '74	251	49.80
Sept. 9	326	56.44
13	480	48.54
20	3531	27.78
27	454	33.32

and 22, respectively. In the farmer's field at Tatara the percentage parasitism (Table 7) ranged from 27.78 (September 20) to 56.44 % (September 9). In Plot B the host eggs' peaks (Fig. 15) were observed on August 12 and September 25 and were parasitized 37.23 and 14.69 %, respectively. In Plot A two peaks of host egg densities were occurred at the same time as that in Plot B and were parasitized 31.48 and 27.35 % (Fig. 14). In Plot A and B there were two peaks of adult densities of *Nephotettix cincticeps* at the end of July and the beginning of September (Fig. 14, 15).

Table 8. Percentage parasitism of *Paracentrobia andoi* and mymarid species.

Date	Plot A*		Plot B**	
	P. andoi	Mymarid species	*P. andoi*	Mymarid species
Sept. 25, '74	26.91	1.56	14.69	0.71
Oct. 2	31.08	0.87	19.26	0.81
9	31.69	1.42	26.27	3.59

* In Kyushu University experimental farm.
** In farmer's paddy field near Plot A.

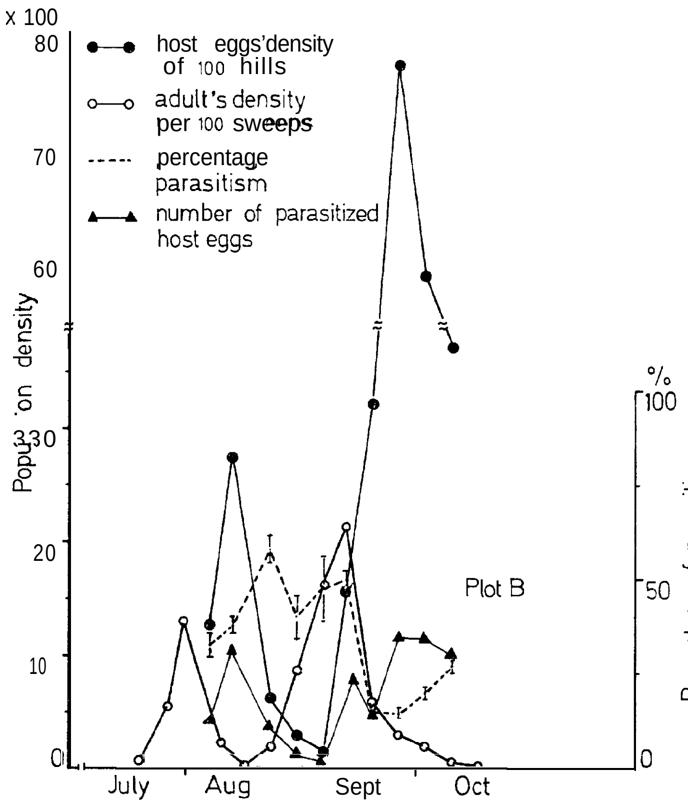

Fig. 15. Seasonal fluctuation of host densities and percentage parasitism in farmer's paddy field (Plot B) near Kyushu University farm.

B. Another parasitic species of the egg of Nephotettix cincticeps

From the field observations in three places, *Paracentrobia andoi* was the dominant egg parasite of *Nephotettix cincticeps*. During this study it was found that mymarid species, unidentified, was also the egg parasite of the green rice leafhopper. The percentage parasitism is shown in comparison with that of ***Paracentrobia andoi*** (Table 8), ranging from 1.42 to 1.56 % in Plot A and 0.71 to 3.59 % in Plot B.

VII. Discussion

Effect of temperatures to development

The thermal constant and rate of development of insects can be used as one kind of index of distribution. The less heat required to complete development the more distribution range should be lied (Messenger, 1959). The thermal constant of ***Paracentrobia andoi*** is rather low, 149.19 degree-days. According to the literature reviewed this parasite distributes from the southern part of Japan, Kyushu Island and Shikoku Island, up to Hokuriku district in Honshu Island (Orita, 1972). ***Paracentrobia andoi*** is an important egg parasite of *Nephotettix cincticeps* in Kochi, Shikoku, and its percentage parasitism was high every year (Sasaba and Kiritani, 1972). Orita (1972) reported that ***Paracentrobia andoi*** in 1967 was dominant in Takada but in 1968 it was replaced by ***Lymaenon*** sp., a mymarid parasite newly found since 1967 in Hokuriku district. From his report ***Lymaenon*** sp. can develop to adult stage even at 15°C but ***Paracentrobia andoi*** cannot. DeBach and Sisojevice (1960) studied the two insect parasites, *Aphytis chrysomphali* and A. ***Zingnanensis,*** and indicated that the former species had exhibited dominance in the field possessed relatively low mean temperature because it possessed a greater relative advantage over A. ***Zingnanensis*** at lower temperature than it does at higher temperature. So the same reason might be used to explain why ***Lymaenon*** sp. was abundant over ***Paracentrobia andoi*** in Takada, Hokuriku district. The calculated thermal constant of ***P. andoi*** is 149.19 degree-days for the whole development. Under varying temperatures or field condition heat requirement is 167.33 $\pm$6.04 degree-days (Table 4). The calculated and observed heat requirements give a considerable agreement. Mean observed heat requirement, about 18.14 degree-days different from calculated one, is not so much different when we consider the other factors affecting to the rate of development. The heat requirement is unstable property (Messenger, 1959). On the basis of heat accumulation of effective degree-days above the developmental threshold this parasite has 6-7 generations per year.

Overwintering

Paracentrobia andoi hibernates in the pupal stage. In an outdoor experiment in October none of parasite's eggs could develop beyond pupal stage. From November the daily mean temperature got down below the developmental threshold. Heat units accumulated above the threshold after late September were not sufficient for completing development. So the parasite's eggs deposited after late September cannot develop beyond pupal stage and they will enter hibernation. The emergence after winter fairly synchronized with the host. The peak of emergence was on June 2. The host's adults were first collected on June 2 and reached its peak on June 16 (Fig. 11) at Tatara town. The host egg density before rice season is important for an increase in parasite population. But the host eggs before rice season were scarce because there were few alternate host plants, ***Leersia sayanuka*** and ***L. japonica.*** It is believed that during this period a large number of female parasites fail to deposit their eggs to the host eggs after emergence.

VIII. **Summary**

Paracentrobia andoi (Ishii) was originally described as ***Japania andoi*** Ishii. This paper presents the results of the morphological studies of adult as well as immature stages of this species. ***Paracentrobia andoi*** is a solitary egg parasite of the green rice leafhopper, *Nephotettix* ***cincticeps*** Uhler. It is thelyotokous species, parthenogenetic parasite; only the females were found. There are three larval instars. Laboratory studies of its biology were conducted at 25 ± 1°C. Under laboratory condition its life cycle lasted 17-23 days : egg, 14-20 hours ; 1st and 2nd instars combined, 1 day; 3rd instar, 1-2 days ; prepupa, 1 day; and pupa, 13 − 18 days. The calculated threshold of development was 16.3°C for completing the whole development, i. e., from the egg stage to adult; thermal constant was 149.19 degree-days to complete development. Under outdoor condition the accumulated degree-days above the developmental threshold were 167.33 degree-days from the egg stage to the adult emergence. In August the development was most rapid. Adults provided with concentrated honey had the longest average life span, 15.9 days in average, under 25°C constant temperature; they had the longest life span at 15°C, 10.87 days in average, if only water was provided. Adult emergence occurred mostly during the morning. Light affected the emergence of adults. The average number of eggs deposited per female was 42.4. The preoviposition period was short. Most eggs were deposited on the first day of oviposition. Oviposition period was 3 days at most. The behavior of adult for oviposition and superparasitism were studied. The parasites hibernate in the pupal stage. They enter in hibernation from the end of October or the beginning of November. The adults from overwintering pupae firstly emerged in April but mostly at the beginning of June. From July to October of 1974 it was found that host densities attained peaks in August and September. The percentage parasitism ranged from 26 to 64 % in the insecticide free plot and from 14-58 % in farmer's paddy fields. The mymarid parasite was also found parasitized eggs of *Nephotettix* ***cincticeps*** but the percentage parasitism was low, ranging from 0.71 to 3.59 %.

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