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Biology of *Cotesia dictyoplocae* Watanabe (Hymenoptera: Braconidae) a parasitoid of *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae)

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Abstract
Cotesia dictyoplocae Watanabe (Hymenoptera: Braconidae) is an endoparasitoid of certain lepidopteran caterpillar. In the present study the endoparasitoid was recorded to parasitize on one of the economically important lepidopteran caterpillar i.e. the caterpillar of muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae).The parasitoid causes considerable damage of muga silkworm crop in Assam. Eggs of the endoparasitoid were observed to deposit inside the body of early instar silkworm caterpillar. Mean incubation period of egg of *C. dictyoplocae* was recorded as 1.17±0.03 days. The larvae after hatching passed their three larval stages within the host caterpillar. Mean larval period, pupal period and adult period of the braconid wasp was recorded as 12.24±0.25 days, 6.14±0.26 days and 8.46±0.22days respectively. Mean developmental time from egg to adult was recorded as 28.06±0.51 days at mean temperature of 22.12±0.25 °C and mean relative humidity of 67.27±0.34%. The sex ratio of male and female was 1:1.4.

Keywords: *Cotesia dictyoplocae*; parasitoid; *Antheraea assamensis*; biology

Introduction
The genus *Cotesia* has been gaining much attention to the biologist as an applied biological control agent of some injurious pest especially of the lepidopteran pests. However, comparatively little attention has been paid to *Cotesia* sp. as destructive one from the point of infestation incurred by them against beneficial lepidopteran insects like silkworm. In present studies one of the *Cotesia* species namely *Cotesia dictyoplocae* has been identified as threat to muga silkworm *Antheraea assamensis* Helfer which causes considerable damage to the successful rearing of the muga silkworm crop. Earlier *Cotesia dictyoplocae* was reported to infest *Dictyoploca japonica* (Moore) Butler (Lepidoptera: Saturniidae) in Japan (Watanabe, 1940) [1]. The muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) is a sericigenous multivoltine insect which is endemic to Assam, India and produces unique golden yellow silk fiber of high repute (Phukan, 2012) [2]. Assam, a state of northeastern part of India alone produces 158 MT Muga Raw Silk during 2014-15 and muga silk export has been contributing a good support to national economy (Anonymous, 2015) [3]. However the production of muga silk is not enough to meet the growing demand at both national and international market. Owing to the semi domestic nature of this commercial silkworm variety, there are many constrains for its successful rearing like infestation by pathogens (NPV, *Pseudomonas* sp. etc.), parasitoids (uzi fly etc.), predators (bat, birds) and other natural enemies and direct stress of adverse weather. Some of the *Apanteles* species viz. *Apanteles stantoni*, *Apanteles glomeratus* were reported to cause more than 40% infestation of muga silkworm and demonstrated as one of the serious threat to sericulture industry (Thangavelu and Rao, 1982 [4]; Anonymous, 2007 [5]; Barman, 2010 [6]). In present studies *Cotesia dictyoplocae* was identified as one of the major brachonid parasitoid infesting muga silkworm. To understand host –parasitoid interaction and to adopt control measures it is essential to know the biology of both host and parasitoid. In this context biology of the silkworm *A. assamensis* is already well established (Tikader *et al*., 2013 [7]; Ghosal *et al*., 2009 [8]). But there is no systematic work on the biology of *C. dictyoplocae* infesting on *A.assamensis*. Therefore in the present investigation attempt was made to study the biology of *C. dictyoplocae* infesting muga silkworm *A. assamensis* with special emphasis on its life history.

Materials & methods

Cocoons of the parasitoid were collected from the infested muga silkworms which were reared outdoor on Som plants (*Persea bombycina* Kost) (Magnoliaces: Lauraceae) in government sericulture farm, Assam, India. Samples of *C. dictyoplocae* were observed under stereomicroscopic. The sample was finally identified at NBAIR, Bangalore (Registered no. NBAIR/14515).

To study the biology of the *Cotesia dictyoplocae*, culture of the parasitoid was maintained in the laboratory following the method of Sethe and Jadhave (2001) [9] with little modifications. Three culture of *C.dictyoplocae* were studied each year in the Laboratory from February to May and ten replications were maintained for each culture. Such studies were made for five years from 2010 to 2014. Initially the mean average of each life cycle was calculated. Then average of the mean of three life cycles was determined for each year and finally average of the mean for five years was calculated. One cocoon cluster of *C. dictyoplocae* emerged out from a single larva were kept in a large beaker (1000 ml) covered with muslin cloth in laboratory to observe emergence of adult flies. Cotton soaked in honey solution (1:1 of honey and water) were kept on the muslin cloth as food for adult parasitoid. 10 numbers of beakers with equal cocoon clusters were set for observation. When adult flies in the beaker emerged completely (After 5 days), 10 numbers of early instar *Antheraea assamensis* larvae with some fresh green leaves of som plant were introduced in each beaker for parasitization to occur by the parasitoid. After successful parasitization, the infested silkworm larvae were transferred to host plants planted on tub for feeding leaves.

To study the incubation and larval period, parasitized host larvae containing the eggs or larva of *C. dictyoplocae* were dissected batch wise in insect saline solution. Eggs were observed under binocular stereo microscope till hatching. Dissection was performed till emergence of the III instar larvae of *C. dictyoplocae*. The date, time, temperature, humidity, behavior, sex ratio and morphological pattern of the emerging maggots from the host silk worm, cocoon formation, egg laying, incubation period, larval period, pupal period and emergence of adult flies from cocoons were recorded. Sex ratio of adult C. *dictyoplocae* was observed by keeping one cluster of cocoons in one test tube (20 ml). A total of 10 replicas were maintained for the experiment. After emergence of adult flies, the flies were allowed to die without providing food for two days. Finally the numbers of male and female were counted in order to determine the sex ratio in each of the test tube.

Results and Discussions

C. dictyoplocae infestation on *A. assamensis* was recorded to predominantly occur from mid of February to May of each year. Therefore three culture of *C. dictyoplocae* were maintained each year from Feb to May by allowing the parasitoid to parasitize on host larvae for five years (from 2010 to 2014). During culture it was observed that mating of the male and female flies took place after an average of 5.1±2.10 minutes of emergence of adult wasp from cocoons in the laboratory during day hours. After mating, female parasitoid approached the host larvae and the ovipositor of female flies were observed to insert into the host silkworm body by bending her body at a right angle and last for about 10.2±2.1 seconds. She laid eggs in haemocoel of the host larvae. The host larvae were found to exhibit defensive behavior by jerking the raised head and thorax during oviposition time by the parasitoid. Female parasitoid was found to select mostly second instar silkworm caterpillar for oviposition. The newly deposited eggs were white, thin walled, transparent, stalked and tapered toward posterior end. Mean incubation period of *C. dictyoplocae* for five years was calculated as 1.17±0.03 days (Minimum mean 1.06±0.04 days, Maximum mean 1.26±0.03 days). The parasitoid was found to undergo two larval moltings and having three instars. The mean larval period was recorded as 12.24±0.25 days (Minimum mean 11.66±0.35days, Maximum mean 12.86±0.20 days) (Table: 6). The first instar larvae were vermiform, body opaque. The second instar larvae were cylindrical, milky white while the third instar larvae were creamy white and grub like. The length of the larvae were found about 0.5 cm. The maggots came out of the host body and started to spin yellowish cocoon around the host body (Fig.1). Pupa was formed inside the yellow cocoons. Length of the pupa was recorded about 0.22 cm. It was free type. The colour of the pupa was initially light yellow except blackish eyes and gradually became brownish. The mean pupal period was calculated as 6.14±0.26 days (Minimum 5.66±0.31days, Maximum 6.6±0.20 days) (Table:6). The adult body length of male and female was recorded as 2.86±0.21mm and 3.10±0.70mm respectively. Antennae were filiform, 3.16±0.89 mm (female) and 3.12±0.08 mm (male) long. Male adult abdomen was smaller in size than female (fig.1). Male and female head is black. Ovipositor (1.05±0.05 mm) of female was prominent. The mean adult period was recorded as 8.46±0.22 days (Minimum 8.33±0.21 days, Maximum 8.73±0.20 days). The male and female sex ratio was calculated as 1:1.4 (Table.7).

Table 1: Life cycle (mean±SE) of *C. dictyoplocae* recorded in the year 2010.

No. of culture each year	Time of study	Incubation period* (days)	Larval period* (days)	Pupal period* (days)	Adult Period*(days)	Life duration * (days)	Temp. * ºC	RH* %
Culture I	28/2/10 to 30/3/10	1.20±0.07	13±0.33	6±0.33	8±0.25	28.2±0.98	21±0.25	67±0.25
Culture II	18/3/10 To 13/4/10	1.07±0.05	12±0.47	6±0.36	8±0.33	27.07±1.21	22±0.25	68±0.25
Culture III	7/4/10 To 5/5/10	1.11±0.05	11±0.32	5±0.25	9.1±0.23	26.21±0.85	22±0.33	69±0.25
Mean of three culture **		1.12±0.05	12±0.37	5.66±0.31	8.36±0.31	27.16±1.01	21.66±0.27	68±0.25

*->Mean of 10 replications; **-> average of the three culture.

Table 2: Life cycle (mean±SE) of *C. dictyoplocae* recorded in the year 2011

No. of Culture each year	Time of study	Incubation period (days)	Larval period (days)	Pupal period (days)	Adult Period (days)	Life duration (days)	Temp. ºC	RH %
Culture I	2/3/11 to 30/3/11	1.5±0.07	13±0.25	7±0.25	8±0.25	29.5±0.82	20±0.25	68±0.25
Culture II	23/3/11 to 20/4/11	1.1±0.02	11±0.25	6±0.25	8±0.36	26.1±0.88	22±0.25	70±0.25
Culture III	13/4/11 to 12/5/11	1.03±0.03	13±0.25	5±0.25	9.1±0.02	28.13±0.55	24±0.25	65±0.25
Mean of three culture **	Mean	1.21±0.04	12.33±0.25	6±0.57	8.33±0.21	27.91±0.75	22±0.25	67.66±0.25

*->Mean of 10 replications; **-> average of the three culture

Table 3: Life cycle (mean±SE) of *C. dictyoplocae* recorded in the year 2012

No. of Culture each year	Time of study	Incubation period (days)	Larval period (days)	Pupal period (days)	Adult Period (days)	Life duration (days)	Temp. ºC	RH %
Culture I	12/3/12 to 10/4/12	0.9±0.02	13.1±0.02	7.7±0.02	8±0.25	29.7±0.31	20±0.25	68±0.55
Culture II	3/4/12 to 1/5/12	1.6±0.02	12±0.25	6.2±0.02	8.6±0.02	28.4±0.31	22±0.25	70±0.25
Culture III	23/4/12 to 20/5/12	1.2±0.02	12±0.02	5.14±0.04	9.1±0.02	27.44±0.1	24±0.25	65±0.36
Mean of three culture **	Mean	1.23±0.02	12.36±0.09	6.34±0.02	8.56±0.09	28.51±0.24	22±0.25	67.6±0.38

*->Mean of 10 replications; **-> average of the three culture

Table4: Life cycle (mean±SE) of *C. dictyoplocae* recorded in the year 2013

No. of Culture each year	Time of study	Incubation period (days)	Larval period (days)	Pupal period (days)	Adult Period (days)	Life duration (days)	Temp. ºC	RH %
Culture I	2/3/13 to 30/3/13	1.0±0.05	12±0.25	8±0.55	9±0.55	30±1.4	21±0.25	63±0.55
Culture II	23/3/13 to 20/4/13	1.2±0.02	10±0.55	6.6±0.02	8.1±0.02	25.9±0.61	23±0.55	71±0.25
Culture III	13/4/13 to 12/5/13	1.0±0.05	13±0.25	5.2±0.04	9.1±0.05	28.3±0.39	24±0.36	62±0.36
Mean of three culture **	Mean	1.06±0.04	11.66±0.35	6.6±0.20	8.73±0.20	28.06±0.8	22.6±0.38	65.3±0.38

*->Mean of 10 replications; **-> average of the three culture

Table 5: Life cycle (mean±SE) of *C. dictyoplocae* recorded in the year 2014

No. of Culture each year	Time of study	Incubation period (days)	Larval period (days)	Pupal period (days)	Adult Period (days)	Life duration (days)	Temp. ºC	RH %
Culture I	14/2/14 to 13/3/14	1.5±0.02	13±0.55	7±0.55	8±0.55	29.5±1.67	20±0.36	68±0.25
Culture II	8/3/14 to 6/4/14	1.2±0.05	11.8±0.02	6.2±0.05	8±0.36	27.2±0.48	22.2±0.02	70±0.55
Culture III	1/4/14 to 27/5/14	1.1±0.03	13.8±0.05	5.3±0.02	9.1±0.02	29.3±0.12	24.9±0.05	65.5±0.55
Mean of three culture **	Mean	1.26±0.03	12.86±0.2	6.1±0.2	8.33±0.31	28.66±0.75	22.36±0.14	67.83±0.45

*->Mean of 10 replications; **-> average of the three culture

Table 6: Mean duration of total life cycle (mean±SE) of *C. dictyoplocae* recorded from 2010 to 2014

Time of study	Incubation period (days)**	Larval period (days)**	Pupal period (days)**	Adult Period (days)**	Life duration (days)**	Temp. ˚C**	RH %**
2010	1.12±0.05	12±0.37	5.66±0.31	8.36±0.31	27.16±1.01	21.66±0.27	68±0.25
2011	1.21±0.04	12.33±0.25	6±0.57	8.33±0.21	27.91±0.75	22±0.25	67.66±0.25
2012	1.23±0.02	12.36±0.09	6.34±0.02	8.56±0.09	28.51±0.24	22±0.25	67.6±0.38
2013	1.06±0.04	11.66±0.35	6.6±0.20	8.73±0.20	28.06±0.8	22.6±0.38	65.3±0.38
2014	1.26±0.03	12.86±0.2	6.1±0.2	8.33±0.31	28.66±0.75	22.36±0.14	67.83±0.45
Mean***	1.17±0.03	12.24±0.25	6.14±0.26	8.46±0.22	28.06±0.51	22.12±0.25	67.27±0.34

-> average of the three culture of each year; *-> mean for five years

Table 7: Sex ratio of *C. dictyoplocae*

Time of study (Year)	Male (No.)	Female (No.)	Total numbers
2010	13	17	30
2011	15	21	36
2012	16	19	35
2013	14	20	34
2014	12	21	31
Mean	14	19.6	33.2
S.E.	±0.70	±0.74	±1.15
Ratio	1	1.4	

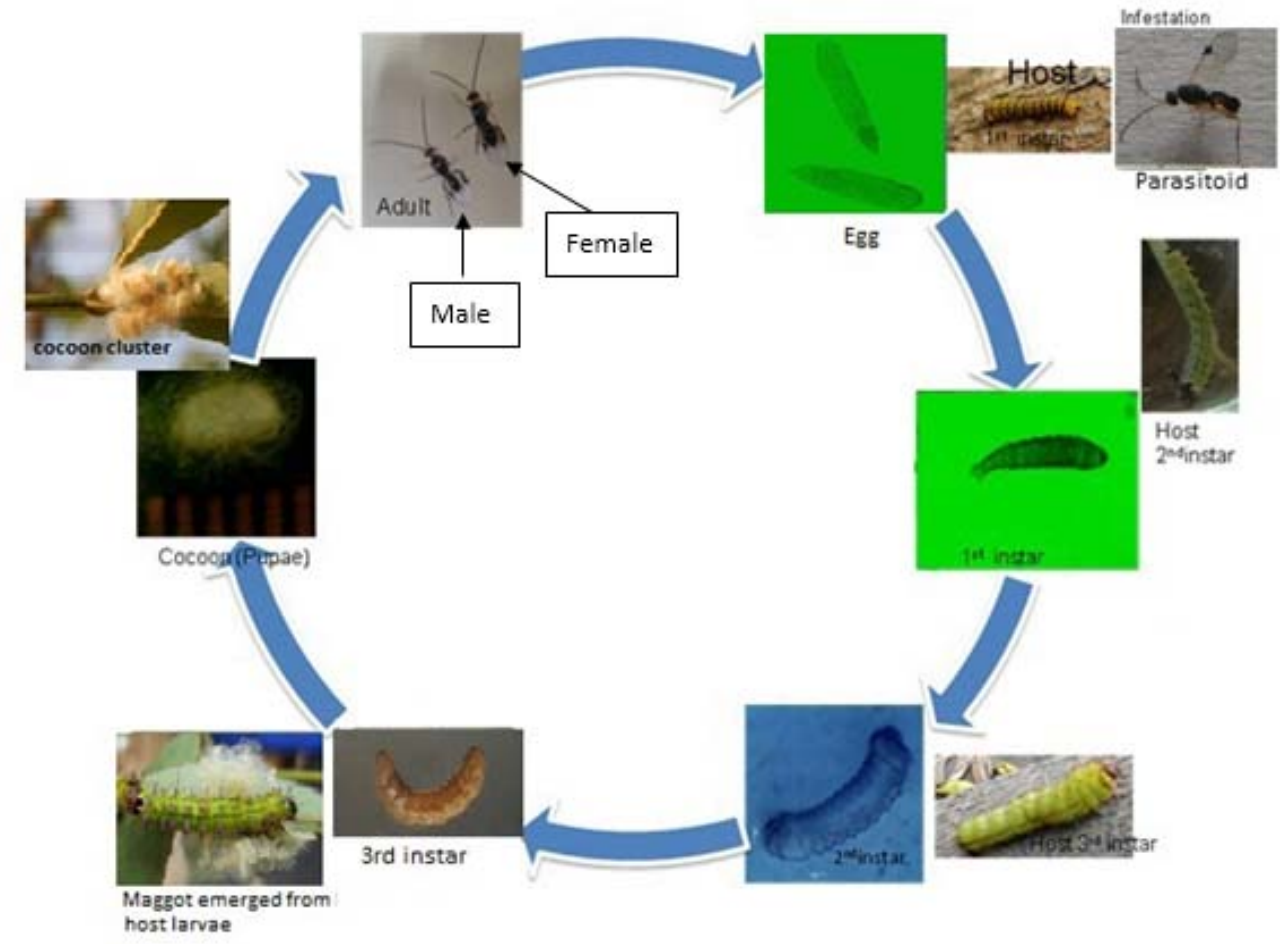

Fig. 1: Life cycle of Cotesia dictyoplocae parasitoid.

The mean life period of parasitoid was calculated as 28.06±0.51 days (Minimum mean 27.16±1.01 days, Maximum mean 28.66±0.75days). The average temperature and humidity under which the experiments performed in the laboratory were recorded as 22.12±0.25 °C (Minimum mean 21.66±0.27 °C & Maximum mean 22.6±0.38 °C) and 67.27±0.34 RH (Minimum mean 65.3±0.38 RH & Maximum mean 68±0.25RH) respectively (Table:6). A good number of studies related to biology of *Apanteles* or *Cotesia* sp. infesting different species of lepidopteran host caterpillars have already been reported. Peter and David (1999) [10] studied the biology of endoparasite *Apanteles machaeralis* Wilkinson (Hymenoptera: Braconidae) infesting caterpillar of *Diaphania indica* (Lepidoptera: Pyralidae) where they reported mean developmental time from egg to adult as 12·63 days at 29·25±1·82 °C and 59-66% RH and sex ratio as 1:1.22 (males/females). Wiedemann *et al*., (1992) [11] studied laboratory rearing and biology of *Cotesia flavipes* (Hymenoptera: Braconidae) by using *Diatraea saccharalis* (Lepidoptera: Pyralidae) as host while McCutcheon *et al*., (1983) [12] studied the biology of *Apanteles ruficrus* parasitoid which uses *Pseudoplusia includens*, *Trichoplusia ni*, and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) as its host. Farahani *et al*., (2012) [13] and Caballero *et al*., (1993) [14] studied the biology of *Apanteles myeloenta* and *Cotesia* (= *Apanteles) telengai* on the host of *Ectomyelais ceratoniae* (Lepidoptera: Pyralidae) and *Agrotis segetum* (Lepidoptera: Noctuidae), respectively. However study on the biology of *Cotesia* sp. that uses silkworm as host, most particularly muga silkworm as host is limited. In the present study the biology of *Cotesia dictyoplocae* infesting muga silkworm was made and the results are described above. The similarity of some aspect of biology of the parasitoids including *C.dictyoplocae* with others might due to similar mode of life and the differences that obtained might influence by the type of host they exploit to complete there life cycle and the prevailing temperature, humidity and other factors. Earlier a few number of researchers reported about the infestation of muga silkworm by different *Apanteles* sp. (Thangavelu and Rao, 1982 [4]; Das and Das, 2008 [15], Barman, 2010) [6] but their biology were not found to be systematically studied. Sathe and Jadhav (2001) [9] studied the life cycle of *Apanteles glomeratus* on the host larvae of *Bombyx mori* and reported that the parasitoid completed its life cycle in 21.1 days in 25±1 ºC and 75±3 RH. The results of the present study thus contribute to the knowledge of biology of *Cotesia dictyoplocae* infesting muga silkworm. The results will help to understand *Antheraea assamensis*– *Cotesia dictyolpocae* interaction and to adopt preventive or control measures if necessary during culture of the silkworm in outdoor condition. This will also throw light for mass rearing of the *Cotesia* by using silkworm as a host.

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