

# CULTURE DEVELOPMENT OF AN INDIGENOUS SPECIES OF *TRICHOGRAMMA* FROM PUNJAB AND ITS LABORATORY TESTING AGAINST POPLAR DEFOLIATOR

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## ABSTRACT

An indigenous species of *Trichogramma*: *T. chilonis* Ishii have been collected from Punjab, India. Morphometrics and diagnostic characters (Body length, head length & width; eye width & malar space; setae in RS1, RS2 & r-m vein tracks and setae between RS1 & r-m vein tracks; female & male genitalia) were measured in detail. The body length of male *T. chilonis* was found smaller than the female. The genital length and width of male *T. chilonis* were ranges from 0.117 to 0.138 mm and 0.05 to 0.08 mm respectively. Ovipositor length were ranges from 0.150 to 0.198 mm. The average ratio of fore wings length and width for female and male were 2.06 and 1.89 respectively. The setae in RS1, RS2, r-m and between RS2 & r-m were almost similar for both males and females. The *T. chilonis* have also been tested against Poplar defoliator, *Clostera fulgurita* in one pair and 5 pairs and shows the parasitization rate of 18.80% and 58.90% respectively.

## INTRODUCTION

Westwood (1833) erected the genus *Trichogramma*, with the type species *Trichogramma evanescens*. This is the most familiar genus among entomologists, as its several species have been widely utilized for biological control of key insect pests of agricultural crops, commercial cash crops, vegetables and forest insect pests (Debach and Rosen, 1991). In India, different exotic *Trichogramma* spp. have been utilized in biological control of many insect pests of agricultural importance including cotton, rice, sugarcane etc., but indigenous species of *Trichogramma* have neither been taken in culture nor applied in biological control of insect pests including poplar defoliator. Therefore, for the first time culture of indigenous *Trichogramma* spp. has been taken from Punjab which was finally identified as *Trichogramma chilonis*. Culture was maintained in laboratory on the eggs of *Corcyra cephalonica*. The detailed morphometric and taxonomic characters have been studied for the proper identification up to species level. Also the *Trichogramma chilonis* has been tested against the eggs of Poplar defoliator (*Clostera fulgurita*). In India, record on release of *Trichogramma chilonis* in forestry goes as early as 1937 (Beeson, 1941). The *Trichogramma* spp. are reared in the laboratory on the eggs of *Corcyra cephalonica*. Yousuf (2005) gave the complete technique of the rearing of *Corcyra cephalonica*. He has also tested *T. chilonis* against teak defoliators, *Eutectona machaeralis* and *Hyblaea puera*. Yousuf et al. (2012) have given the pre-treatment of *Corcyra cephalonica* eggs for maximum

production of the wasps of *Trichogramma* spp. This technique was also utilized for taking the culture of *Trichogramma* spp. from the fields (Yousuf, 2008). The cultures taken from the field is being reared following the same methodology. Proper conditions for the better growth of *Corcyra cephalonica* and eventually *Trichogramma* spp. have been suggested from different workers. Nadeem et al. (2009) reared the *T. chilonis* on different temperature regime. Similarly, Wadaskar et al. (2015) worked on the effect of temperature on the biology of *Corcyra cephalonica* which is being used in the maintenance of *Trichogramma* spp. in laboratory. The objective of the present work is to study the detailed morphometric characters so that the culture of same species can be identified easily by subsequent worker of the field and can authentically be applied in biological control of insect pests. So to identify the *Trichogramma* at microscopic level we go for morphometrics analysis. Choudhury et al. (2011) have worked on the morphometrics aspects for distinguishing the different species of genus *Puntius*. Similarly, Burks and Heraty (2002) have done morphometrics studies of four species of *Trichogramma* namely, *T. californicum*, *T. exiguum*, *T. minutum* and *T. platneri*. Querino and Zucchi (2002) worked on *Trichogramma bruni* to study morphological variations in the Genital Capsule. Nagararkatti and Nagaraja were given brief description of *T. australicum* (Girault, 1912) and re-described as *T. australicum* (Nagararkatti and Nagaraja, 1971). Later, Nagararkatti and Nagaraja (1977) concluded that their *T. australicum* was in fact *T. chilonis* Ishii (Nagararkatti and Nagaraja, 1979) but details

of genitalia structure and other morphometric characters of *T. chilonis* which play an important role in identification were not given. Present study has been carried out for identification, maintenance of culture and morphometric studies of *Trichogramma chilonis* in details and Testing against the Poplar defoliator.

## MATERIALS AND METHODS

### Culture extraction and development of indigenous species of *Trichogramma*

Culture was taken from Punjab by fixing blank *Corcyra cephalonica* eggs strips on the plantation area of Eucalyptus with full of grasses as ground flora following the methodology of Yousuf (2008). The egg strips were fixed in the evening at Rajpura, Patiala, Punjab, India on 14.xi.2012 and recollected in the early morning of the next day and blackening was observed in the eggs after 3 days (Yousuf and Hassan, 2007). After 10 days the adult *Trichogramma* were emerged out which were multiplied was being maintained on *Corcyra cephalonica* eggs.

### Culture maintenance of indigenous *Trichogramma* species

Cultures of *Trichogramma chilonis* was maintained on the eggs of *Corcyra cephalonica* (Rice meal moth) and other insect's eggs (Yousuf, 2005 and Wadaskar *et al.*, 2015). The eggs of *Corcyra cephalonica* are oval and creamish in colour. Chilling for 3 hours of *Corcyra cephalonica* eggs gives better results of mass multiplication (Yousuf, 2005). 50% diluted honey solution soaked in small cotton rolls, was also provided, as adult *Trichogramma* spp. Food, for improving their survival, longevity and egg laying potential. This culture was regularly maintained for three years for different studies and experimental purposes.

### Identification and confirmation of indigenous *Trichogramma* species on the basis of detailed morphometric characters

Following the morphometric technique of Burks and Heraty, (2002) the *T. chilonis* male and female was studied for morphometric analysis. For detailed studies, normal process of dehydration was adopted and specimens were cleared and dissected in clove oil medium. The dissected parts were placed on micro slides in a drop of Canada balsam, oriented to the required position and covered with cover slips (Wirth and Marston, 1968). Photography of important structures were taken with the help of Leitz Labor Luxs Microscope. Measurements of body parts were taken with the help of an ocular micrometer. Observations on various morphometric characters of ten males and ten females were taken after dissection (Burks and Heraty, 2002; Hassan and Yousuf, 2007 and Yousuf *et al.*, 2008). Later on, the species was identified as *Trichogramma chilonis*. The detailed morphometric studies were carried out for this species at maximum level of accuracy. 15 parameters of female and 19 parameters of male *Trichogramma chilonis* were measured and their ratio parameters were also calculated (Burks and Heraty, 2002; Chan and Chau, 2000; Hassan and Yousuf, 2007; Honda *et al.*, 2006 and Querino, *et al.*, 2002 and Yousuf *et al.*, 2008).

### (iv) Testing of indigenous species against poplar defoliators

Following the method of testing of *Trichogramma* spp. against

insect pest of forestry importance suggested by Ahmad (1990) and Ahmad (1992), the *Trichogramma chilonis* culture was tested against the defoliator of Poplar (*Populus deltoides*) trees. The Poplar defoliator (*Clostera fulgurita*) was reared in the laboratory and their eggs were collected and tested against *Trichogramma chilonis*. One pair (one male and one female) and 5 pairs (five males and five females) of *T. chilonis* was exposed to the 100 eggs of *Clostera fulgurita* in each case.

## RESULTS AND DISCUSSION

Till date no biological control agents have been used against *Clostera fulgurita* in such study design. On an average one female can parasitize 20 eggs of host insects. So keeping this in mind 1 pair and 5 pairs design was constructed and tested against *C. fulgurita* in five replications each. The *Trichogramma chilonis* in one pair parasitized 14-23 eggs out of 100 eggs of *C. fulgurita*. This shows the parasitization rate of 18.80% for one pair of *T. chilonis* against *C. fulgurita*. Similarly, in five pairs *T. chilonis* parasitized 52-65 eggs out of 100 eggs of *C. fulgurita* with 58.90% parasitization rate. This finding is somewhat approximate to the work of Kumar *et al.* (2008) who have tested the *C. fulgurita* with *T. chilonis* and *T. poliae*. So keeping this in mind that *T. chilonis* attack successfully on *C. fulgurita* and can be used in future for poplar defoliator in Haryana, Punjab and Uttarakhand.

Important taxonomic characters (Male antennae, forewings, hind wings, genitalia, hind tibia, female antennae, fore wings, hind wings, Ovipositor) were studied and ascertained the status of collected species of culture as *Trichogramma chilonis*. Details are as under:

### Diagnosis

The *Trichogramma* species has been examined for morphological characteristics. Females and males of this *Trichogramma* shows following features:

#### Female

Body length 0.42 mm, Head yellow, wider than long in facial view; ocelli orange, arranged in obtuse triangle; eyes dark red. Antennae yellow; scape four times as long as wide; pedicel about two times as long as wide; single ring segment present; funicle two segmented, both segments together slightly longer than wide; club single segmented, about two and a half times as long as wide. Thorax honey yellow. Fore wing hyaline except area beneath venation lightly infuscated; disc densely setose, setae arranged in rows; costal cell broad; vein track RS1 with 5 setae; marginal fringe about one-sixth the wing width. Abdomen yellow, slightly longer than thorax; ovipositor hidden, arising from basal one third of abdominal venter, about as long as long as or slightly longer than hind tibia.

#### Male

Body length 0.37 mm and color same as female. Antennae yellow, scape about three times as long as wide; pedicel about two times as long as wide; pedicel slightly less than two times as long as wide; flagellum about five times as long as wide; with long and tapering 35-44 flagellar hairs, longest of which is slightly more than 2 times the maximum width of flagellum. Male genitalia with DEG triangular with prominent lateral lobes, CS slightly below the level of GF. Aedeagus as long as or

**Table 1: Morphometrics of important taxonomic characters of *T. chilonis*.**

S.N.	Characters	MEASUREMENTS IN mm $\pm$ SD	
		<i>T. chilonis</i> female	<i>T. chilonis</i> male
1.	Body length	0.42 $\pm$ 0.029(0.38 – 0.45)	0.37 $\pm$ 0.048(0.27 – 0.45)
2.	Head Length	0.172 $\pm$ 0.011(0.146 – 0.187)	0.150 $\pm$ 0.009(0.13 – 0.16)
3.	Head width	0.207 $\pm$ 0.015(0.176 – 0.223)	0.205 $\pm$ 0.014(0.176 – 0.234)
4.	Eye width	0.091 $\pm$ 0.008(0.073 – 0.099)	0.085 $\pm$ 0.005(0.08 – 0.09)
5.	Malar space	0.061 $\pm$ 0.004(0.055 – 0.066)	0.063 $\pm$ 0.005(0.05 – 0.06)
6.	Flagellar length (male)	-	0.162 $\pm$ 0.016(0.124 – 0.183)
1.	Club length (Female)	0.083 $\pm$ 0.006(0.073 – 0.089)	-
7.	Flagellar width	-	0.035 $\pm$ 0.0020(0.029 – 0.040)
1.	Club width (Female)	0.031 $\pm$ 0.005(0.022 – 0.036)	-
8.	Flagellar hair length	-	0.07 $\pm$ 0.009(0.05 – 0.08)
9.	Fore wing length	0.503 $\pm$ 0.029(0.437 – 0.531)	0.503 $\pm$ 0.028(0.446 – 0.539)
10.	Fore wing width	0.244 $\pm$ 0.02(0.195 – 0.261)	0.256 $\pm$ 0.11(0.232 – 0.289)
11.	Fore wing; marginal fringe	0.038 $\pm$ 0.003(0.033 – 0.044)	0.038 $\pm$ 0.11(0.03 – 0.04)
12.	Hind wing length	0.369 $\pm$ 0.041(0.293 – 0.418)	0.389 $\pm$ 0.02(0.362 – 0.446)
13.	Hind wing width	0.039 $\pm$ 0.006(0.27 – 0.046)	0.042 $\pm$ 0.004(0.037 – 0.046)
14.	Length of hind tibia	0.175 $\pm$ 0.008(0.09 – 0.19)	0.17 $\pm$ 0.019(0.13 – 0.20)
15.	Width of hind tibia	0.026 $\pm$ 0.002(0.025 – 0.032)	0.027 $\pm$ 0.003(0.025 – 0.033)
16.	Genital capsule length	-	0.13 $\pm$ 0.006(0.117 – 0.138)
17.	Genital capsule width	-	0.064 $\pm$ 0.008(0.05 – 0.08)
18.	Distance from CS to GF	-	0.012 $\pm$ 0.002(0.010 – 0.018)
19.	Aedeagus length	-	0.132 $\pm$ 0.007(0.11 – 0.14)
20.	Ovipositor length	0.178 $\pm$ 0.014(0.150 – 0.198)	-

(Range is given in parenthesis)

**Table 2: Ratio of important taxonomic characters and setae of fore wings**

S. N.	Characters (Ratio)	<i>T. chilonis</i> female	<i>T. chilonis</i> male
1.	Flagellar hairs/ Flagellar width	-	2.002 $\pm$ 0.28(1.58-2.5)
2.	Flagellar width/ Flagellar length	-	0.215 $\pm$ 0.015(0.196-0.235)
3.	Flagellar Length/ Hind tibia length	-	0.960 $\pm$ 0.099(0.78-1.14)
4.	Antennal club width/ antennal club length	0.380 $\pm$ 0.067(0.255-0.493)	-
5.	Antennal club length/Ovipositor length	0.467 $\pm$ 0.041(0.408-0.518)	-
6.	Antennal club length/ Hind tibia length	0.493 $\pm$ 0.077(0.392-0.633)	-
7.	Fore wing length/ Fore wing width	2.06 $\pm$ 0.103(1.89-2.24)	1.89 $\pm$ 0.072(1.78 – 1.87)
8.	Longest marginal fringe/ Fore wing width	0.146 $\pm$ 0.015(0.129 – 0.172)	0.160 $\pm$ 0.020(0.126 – 0.205)
9.	Genital capsule width/ Genital capsule length	-	0.490 $\pm$ 0.047(0.427 – 0.594)
10.	Genital capsule length/ Hind tibia	-	0.773 $\pm$ 0.071(0.632 – 0.887)
11.	Ovipositor length/ Hind tibia	1.02 $\pm$ 0.071(0.91-1.13)	-
12.	Fore wings setae in RS1	3 - 4	3 - 5
13.	Fore wings setae in RS2	11 - 15	11 - 14
14.	Fore wings setae in r-m	18 - 26	18 - 26
15.	Fore wings setae in b/w RS2& r-m	40 - 80	45 - 60

(Range is given in parenthesis)

slightly longer than apodemes, together slightly shorter than entire genitalia and about 2/3 the length of hind tibia. Males and females of this *Trichogramma* species resembles the characteristics of *Trichogramma chilonis* reported by Chiriach (2005); Honda *et al.* (2006); Ishii, (1941); Nagarkatti and Nagaraja (1971); Nagarkatti and Nagaraja (1979). Hence this *Trichogramma* has been successfully identified as *Trichogramma chilonis* Ishii.

#### Material examined

INDIA: Punjab; Patiala; Rajpura, 14.xi.2012, culture taken in *Corcyra cephalonica* eggs by Mohsin Ikram.

#### Morphometric characters

During the course of present study, observation on various morphometric characters of ten males and ten females were taken and these are summarized in Table 1 & Table 2.

*Trichogramma chilonis* Ishii is characterized with body length of female 0.42  $\pm$  0.029 mm & male 0.37  $\pm$  0.047 mm respectively; male antennae having 32 – 44 long tapering hairs, longest of which about 2.002  $\pm$  0.28 times the maximum width of flagellum; fore wing with RS1 having 3-5 setae, RS2 having 11-14 setae; r-m 18-26 setae; setae between RS2 & r-m with 45-60 setae; length of fringe on tornus is 0.146  $\pm$  0.015 in female & 0.160  $\pm$  0.020 in male as long as wing width. Similar type of study has been conducted by Hassan and Yousuf (2007) for *T. plasseyensis* but average RS1, RS2, r-m and setae between RS2 & r-m were 3, 8, 20 and 38 respectively which can also separate *T. chilonis* with *T. plasseyensis*. Yousuf *et al.* (2008) have also reported the measurement of morphometric character for *T. raoi* including the setae counts, Genitalia, flagellar measurements etc. and RS1, RS2, r-m and setae between RS2 & r-m were reported as 4, 10, 20 and 32

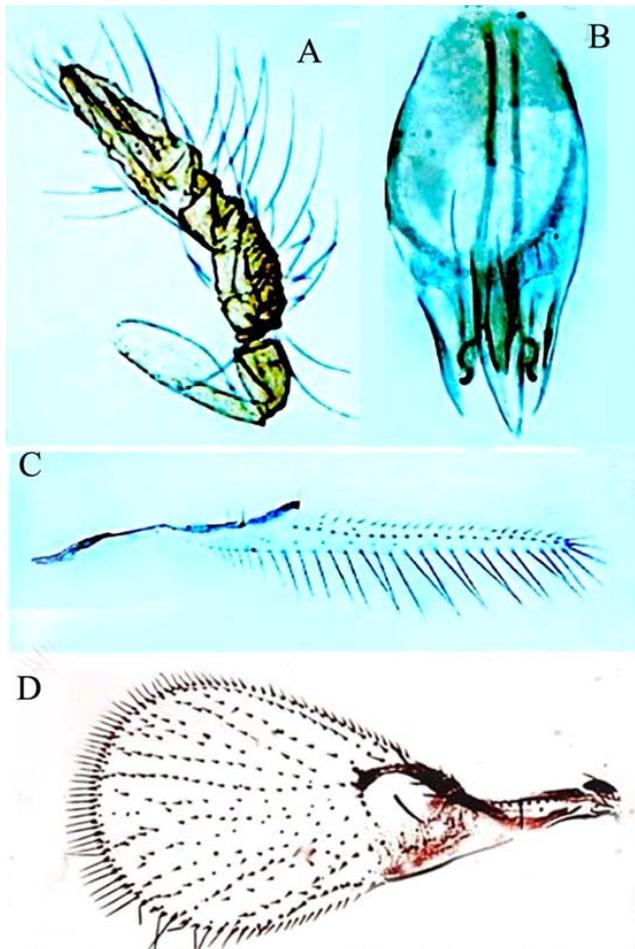


Figure 1: (A-D) *Trichogramma chilonis* (Male): A, Antenna; B, Genitalia; C, Hind wing; D, fore Wing.

respectively which is also different from the setae of *T. chilonis*. Other characters were also found measured values.

The average length and width of male genitalia in *T. chilonis* was measured as 0.13 and 0.064 mm respectively. This result is complying with the results of Burks and Heraty (2002) in which *T. californicum* shows similar genitalia length but not genitalia width. They have analyzed four different species of *Trichogramma* morpho-metrically. Similarly, the ratio of width and length of male genitalia were measured as 0.490 which in compare smaller than *T. bruni* (0.364) reported by Querino and Zucchi (2002). The average length of ovipositor in females were measured as 0.178 mm. Burks and Heraty (2002) reported approximately similar ovipositor length for *T. exiguum* but other three species of *Trichogramma* were far beyond this value.

In both, males and females of *T. chilonis* the average fore wings length was 0.503 mm. But the fore wing width in females was 0.244 mm and in males it was measured as 0.256 mm respectively. It shows similar trends reported by Burks and Heraty (2002) for *T. exiguum* and *T. californicum*. In This study the additional ratio character ratio of fore wings length and width were also calculated as 2.06 and 1.89 for females and males respectively and that makes the study more relevant to

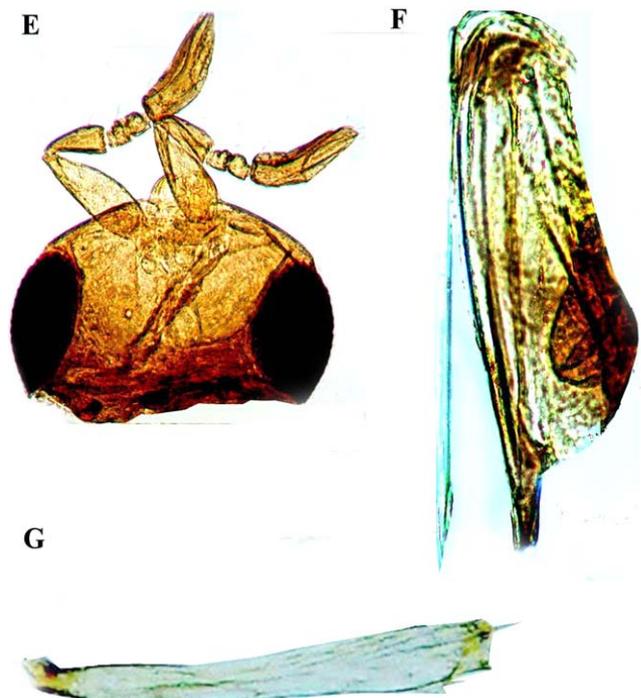


Figure 2: (E-G) *Trichogramma chilonis* (Female): E, Head & Antenna; F, Genitalia; G, Hind tibia

taxonomy of this micro-hymenopteran. The similar ratio of fore wings length and width were also measured by Querino and Zucchi (2002) for *T. bruni*.

In conclusion the *Trichogramma* collected from Punjab has been identified as *Trichogramma chilonis* and successfully tested against *Clostera fulgurita* eggs. As *C. fulgurita* is a major pests of Poplar and cause large level destruction of the leaves of the poplar. So it is being suggested to use *Trichogramma chilonis* for controlling the pests of Poplar by biological control.

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