



MORPHOMETRICS OF *TRICHOGRAMMA* SPP. RELEASED IN SUGARCANE

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ABSTRACT

Trichogramma populations were collected from sugarcane fields from four districts of Punjab. Important taxonomic characters such as body length, head length, head width, female antennae, fore wings, hind wings, male antennae and genitalia were studied and a morphometric analysis was performed. Descriptive analysis of ratios of characters (flagellar width/ length, club width/ length, fore wing length/ width, genital capsule length/ width) was also undertaken. Results revealed that females were bigger than males, and head was wider than long. Despite slight variations in size, all specimens were found to belong to *T. chilonis*.

Key words: *Trichogramma*, *T. chilonis*, sugarcane, taxonomic characters, ratios, morphometrics, genitalia, female, male

Trichogramma chilonis (Ishii) (Hymenoptera: Trichogrammatidae) is of great importance to biological control against many lepidopterous pests of crops and vegetables. The small size of trichogrammatids and the relatively uniform morphology of species lead to difficulties in taxonomy, resulting in many nomenclatural problems. There had been many vague species descriptions frequently based on one sex. Around 239 species of *Trichogramma* are known of which 37 are from India and 6 from Punjab (Jalali, 2013; Pinto, 2006; Noyes 2018). Various researchers distinguish the species of *Trichogramma* by morphometrics. Burks and Heraty (2002) analysed morphometrics of *T. californicum* Nagaraja & Nagarkatti, *T. exiguum* Pinto & Platner, *T. minutum* Riley and *T. platneri* Nagarkatti. Querino and Zucchi (2002) studied *T. bruni* for variations in the genital capsule.

In India, Nagaraja and Mohanraj (2010) described *T. rabindrai*, *T. pteridis* and *T. giriensis* from south India. Nagarkatti and Nagaraja (1971) gave brief description of *T. australicum* Girault and redescribed it as *T. australicum* (Nagarkatti and Nagaraja 1971). Later, Nagarkatti and Nagaraja (1977) concluded that their *T. australicum* was in fact *T. chilonis* Ishii based on male genitalia, pigmentation, wing and antennal trichation (Nagarkatti and Nagaraja 1979). As *T. chilonis* is being widely used as a biocontrol agent, there is a need to establish the species composition as occurring in the fields, and morphologically (male genitalia) define it, through modern techniques for differentiating among the species/strains.

T. chilonis and *T. japonicum* have been released

for the management of sugarcane borers i.e early shoot borer (*Chilo infuscatellus* Snellen), top borer (*Scirpophaga excerptalis* Walker) and stalk borer (*Chilo auricilius* Dudgeon) in northwest India since 2000. The trichocards were being used for inundative releases. Need was felt to evaluate the species status in the field under different agroclimatic zones of Punjab. Therefore, present study on the morphometrics of *Trichogramma* spp.

MATERIALS AND METHODS

The nucleus culture of *T. chilonis* in the form of trichocard was procured from Biological Control Laboratory, Department of Entomology, PAU, Ludhiana. The eggs of *Corcyra cephalonica* (Stainton) [Lepidoptera: Pyralidae] were used for the preparation of trichocards. Cleaned eggs of *Corcyra* were deep frozen for 12-14 hr to prevent hatching. These eggs were glued on white cards of size 15 x 12 cm to prepare sentinel *Corcyra* cards.

Sentinal cards with eggs of *C. cephalonica* were used for collection of *Trichogramma* spp. from four locations in Punjab (Gurdaspur, Shaheed Bhagat Singh Nagar, Patiala and Fazilka). These cards were applied in the field where trichocards had been applied during the previous ten years under the augmentative biological control of sugarcane borers conducted by the Punjab Agricultural University, Ludhiana. These cards were cut into 40 strips, each having approximately 100 eggs. These strips were stapled on the lower surface of the leaves uniformly at 100 spots/ ha in sugarcane fields. A minimum distance of 10 m was maintained between

two spots. These sentinel card strips were removed after 24 hr and brought to the laboratory. These were then kept separately in the glass vials for adult emergence. The emerging adults were used for morphometrics. The laboratory reared *T. chilonis* [supplied by NBAIR, Bangalore for rejuvenation] was used as a standard.

For examination of antennae, wings and genitalia methodology given by Noyes (1982) with some modifications. In males, flagellum characteristics were taken, and in females, the antennal club was used. For maceration, specimens were heated in 10% KOH at 80°C for 10 min. Following neutralization with glacial acetic acid for 10 min, specimens were washed in distilled water for 1 hr and were then dehydrated for 5 min in graded alcohols of the following concentration: 35, 70, 85 and 100%. The specimens were cleared and dissected in clove oil medium. The dissected antennae, genitalia (males only) and wings were placed on slides in a drop of Canada Balsam, oriented to the required position and covered with cover slips. Specimens were dissected and slides were prepared under stereozoom microscope. Observations on various morphometrics (n= 10 males and females each) were taken after dissection. A total of 11 characters of males and 9 characters of females were examined. Descriptive analysis of ratio of characters was also done. Morphometrics was done with Nikon SMZ 25 stereomicroscope, and mean \pm SD computed.

RESULTS AND DISCUSSION

Important taxonomic characters such as body length, head length, head width, female antennae, fore wings, hind wings, male antennae and genitalia were studied and a comparative morphological analysis obtained as below:

Gurdaspur: The body length of Gurdaspur population varied from 0.361 to 0.406 mm in females and 0.349 to 0.393 mm in males. Males were smaller in size in comparison to the females (Table 1). Head length and head width were nearly similar in both the sexes. Antennal club length and width in female measured 0.07 and 0.03 mm, respectively. The flagellar length and width were measured as 0.16 mm and 0.02 mm, respectively while in males, fore wing length and width were measured as 0.49 and 0.25 mm, respectively. Fore wing length in females was found to be 0.48 mm (range= 0.457-0.526 mm) while width was 0.24 mm (range= 0.228 mm to 0.260 mm). The hind wing length and width measured 0.36 and 0.038 mm, respectively. Similarly, in males the hind wing length varied from 0.342 to 0.419 mm with a mean length of 0.39 mm

whereas the width varied from 0.025 to 0.038 mm with width of 0.03 mm, respectively. The genital capsule length varied from 0.12 to 0.17 mm with a mean of 0.13 mm. The width varied from 0.044 to 0.082 mm with mean width of 0.05 mm. Descriptive analysis of ratio of characters revealed that the ratio of antennal club width and length was 0.42 but in males, the ratio of flagellar width and length was measured as 0.12. The ratio of fore wing length and its width was calculated as 2.00 in females and 1.96 in males. The ratio of genitalia capsule width and length in males was calculated as 0.38

SBS Nagar: The body length of SBS Nagar population was found to be 0.412 to 0.507 mm in females and 0.380 to 0.476 mm in males. The head length in females and males was 0.19 mm and 0.17 mm, respectively. Antennal club length and width measured 0.08 and 0.03 mm, respectively. The flagellar length and width in males were measured as 0.17 mm and 0.03 mm, respectively. Fore wing was found to be similar in length in both sexes with very small variation in width. In females, the hind wing length and width measured 0.36 and 0.03 mm, respectively while, in males the hind wing length varied from 0.361 to 0.393 mm with a mean value of 0.37 mm. The width ranged from 0.031 to 0.038 mm with a mean width of 0.03 mm, respectively. The genital capsule length was observed to be 0.12 mm and width as 0.04 mm. The ratios revealed that the club length was 0.37x the club width. The ratio of fore wing length and its width was calculated as 2.04 in females and 1.96 in males. The ratio of genitalia capsule width and length in males was calculated as 0.33.

Patiala: The body length was 0.390 mm in females and 0.370 mm in males. Head width and head length was found to be nearly similar in both the sexes. Antennal club length and width measured 0.08 mm and 0.03 mm, respectively. The flagellar length and width in males were measured as 0.16 and 0.02 mm, respectively. Club length and width in females measured 0.07 and 0.02 mm, respectively. Measurement range of fore wing length and width; hind wing width were relatively similar for both the sexes. The hind wing length varied from 0.317 to 0.368 mm in females while it ranged 0.380 to 0.406 mm in males. The genital capsule length was 0.12 mm and width was 0.04 mm. The ratio of fore wing length and its width was observed to be 2.00 in females and 1.95 in males. The ratio of genitalia capsule width and length in males was 0.33.

Fazilka: The body length varied from 0.330 to 0.393 mm in females and 0.285 to 0.380 mm in males. Males were smaller in size as compared to the females. Head

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

Gurdaspur

S. No.	Characters	Female		Male	
		Mean±SD (mm)	Range (mm)	Mean±SD (mm)	Range (mm)
1.	Body length	0.39±0.016	(0.361-0.406)	0.37±0.016	(0.349-0.393)
2.	Head length	0.17±0.015	(0.152-0.209)	0.16±0.016	(0.133-0.184)
3.	Head width	0.19±0.017	(0.171-0.228)	0.20±0.018	(0.177-0.247)
4.	Flagellar length	-	-	0.162±0.016	(0.133-0.184)
5.	Flagellar width	-	-	0.02±0.005	(0.019-0.038)
6.	Club length	0.07±0.007	(0.057-0.082)	-	-
7.	Club width	0.03±0.003	(0.025-0.038)	-	-
8.	Fore wing length	0.48±0.025	(0.457-0.526)	0.49±0.018	(0.457-0.52)
9.	Fore wing width	0.24±0.010	(0.228-0.26)	0.25±0.013	(0.234-0.273)
10.	Hind wing length	0.39±0.014	(0.361-0.406)	0.39±0.026	(0.342-0.419)
11.	Hind wing width	0.03±0.003	(0.025-0.038)	0.03±0.004	(0.025-0.038)
12.	Genital capsule length	-	-	0.13±0.022	(0.12-0.171)
13.	Genital capsule width	-	-	0.05±0.011	(0.044-0.082)

Ratios

S. No.	Characters (Ratio)	Female	Male
1.	Flagellar width/ Flagellar length	-	0.12
2.	Club width/Club length	0.42	-
3.	Fore wing length/ Fore wing width	2.00	1.96
4.	Genital capsule width/ Genital capsule length	-	0.38

SBS Nagar

S. No.	Characters	Female		Male	
		Mean ± SD (mm)	Range (mm)	Mean ± SD (mm)	Range (mm)
1.	Body length	0.45±0.033	(0.412-0.507)	0.43±0.040	(0.38-0.476)
2.	Head length	0.19±0.009	(0.171-0.203)	0.17±0.013	(0.158-0.190)
3.	Head width	0.24±0.022	(0.209-0.279)	0.22±0.014	(0.203-0.247)
4.	Flagellar length	-	-	0.17±0.009	(0.158-0.184)
5.	Flagellar width	-	-	0.03±0.005	(0.025-0.038)
6.	Club length	0.08±0.004	(0.076-0.088)	-	-
7.	Club width	0.03±0.005	(0.031-0.038)	-	-
8.	Fore wing length	0.49±0.020	(0.463-0.52)	0.49±0.017	(0.476-0.533)
9.	Fore wing width	0.24±0.009	(0.234-0.266)	0.25±0.010	(0.241-0.279)
10.	Hind wing length	0.36±0.021	(0.349-0.393)	0.37±0.014	(0.361-0.393)
11.	Hind wing width	0.03±0.005	(0.025-0.038)	0.03±0.003	(0.031-0.038)
12.	Genital capsule length	-	-	0.12±0.017	(0.095-0.146)
13.	Genital capsule width	-	-	0.04±0.005	(0.044-0.057)

Ratios

S. No.	Characters (Ratio)	Female	Male
1.	Flagellar width/ Flagellar length	-	0.17
2.	Club width/Club length	0.37	-
3.	Fore wing length/ Fore wing width	2.04	1.96
4.	Genital capsule width/ Genital capsule length	-	0.33

(Contd. Table 1)

Patiala

S. No.	Characters	Female		Male	
		Mean \pm SD (mm)	Range (mm)	Mean \pm SD (mm)	Range (mm)
1.	Body length	0.39 \pm 0.014	(0.355-0.406)	0.37 \pm 0.014	(0.361-0.393)
2.	Head length	0.16 \pm 0.015	(0.152-0.203)	0.15 \pm 0.010	(0.146-0.177)
3.	Head width	0.19 \pm 0.014	(0.184-0.228)	0.19 \pm 0.012	(0.177-0.222)
4.	Flagellar length	-	-	0.16 \pm 0.018	(0.139-0.190)
5.	Flagellar width	-	-	0.02 \pm 0.001	(0.025-0.031)
6.	Club length	0.07 \pm 0.012	(0.063-0.095)	-	-
7.	Club width	0.02 \pm 0.003	(0.019-0.031)	-	-
8.	Fore wing length	0.48 \pm 0.022	(0.444-0.507)	0.47 \pm 0.031	(0.425-0.507)
9.	Fore wing width	0.24 \pm 0.010	(0.234-0.260)	0.24 \pm 0.021	(0.215-0.266)
10.	Hind wing length	0.34 \pm 0.019	(0.317-0.368)	0.39 \pm 0.010	(0.38-0.406)
11.	Hind wing width	0.03 \pm 0.004	(0.025-0.038)	0.03 \pm 0.004	(0.025-0.038)
12.	Genital capsule length	-	-	0.12 \pm 0.008	(0.114-0.139)
13.	Genital capsule width	-	-	0.04 \pm 0.008	(0.031-0.063)

Ratios

S. No.	Characters (Ratio)	Female	Male
1.	Flagellar width/ Flagellar length	-	0.12
2.	Club width/ Club length	0.28	-
3.	Fore wing length/ Fore wing width	2.00	1.95
4.	Genital capsule width/ Genital capsule length	-	0.33

Fazilka

S. No.	Characters	Female		Male	
		Mean \pm SD (mm)	Range (mm)	Mean \pm SD (mm)	Range (mm)
1.	Body length	0.36 \pm 0.026	(0.33-0.393)	0.34 \pm 0.035	(0.285-0.380)
2.	Head length	0.14 \pm 0.017	(0.126-0.19)	0.13 \pm 0.019	(0.101-0.158)
3.	Head width	0.18 \pm 0.020	(0.158-0.222)	0.16 \pm 0.013	(0.146-0.184)
4.	Flagellar length	-	-	0.14 \pm 0.011	(0.12-0.158)
5.	Flagellar width	-	-	0.02 \pm 0.004	(0.019-0.031)
6.	Club length	0.07 \pm 0.007	(0.063-0.088)	-	-
7.	Club width	0.02 \pm 0.003	(0.025-0.031)	-	-
8.	Fore wing length	0.43 \pm 0.044	(0.374-0.495)	0.44 \pm 0.030	(0.4-0.495)
9.	Fore wing width	0.21 \pm 0.021	(0.190-0.253)	0.23 \pm 0.014	(0.209-0.253)
10.	Hind wing length	0.27 \pm 0.055	(0.241-0.380)	0.34 \pm 0.036	(0.292-0.387)
11.	Hind wing width	0.02 \pm 0.002	(0.025-0.031)	0.03 \pm 0.004	(0.025-0.038)
12.	Genital capsule length	-	-	0.12 \pm 0.004	(0.114-0.126)
13.	Genital capsule width	-	-	0.04 \pm 0.007	(0.038-0.057)

Ratios

S. No.	Characters (Ratio)	Female	Male
1.	Flagellar width/ Flagellar length	-	0.14
2.	Club width/ Club length	0.28	-
3.	Fore wing length/ Fore wing width	2.04	1.91
4.	Genital capsule width/ Genital capsule length	-	0.33

(Contd. Table 1)

Ludhiana

S. No.	Characters	Female		Male	
		Mean ± SD (mm)	Range (mm)	Mean ± SD (mm)	Range (mm)
1.	Body length	0.40±0.020	(0.38-0.440)	0.38±0.016	(0.361-0.406)
2.	Head length	0.17±0.009	(0.165-0.196)	0.17±0.008	(0.165-0.190)
3.	Head width	0.20±0.008	(0.19-0.222)	0.20±0.009	(0.19-0.215)
4.	Flagellar length	-	-	0.15±0.007	(0.146-0.171)
5.	Flagellar width	-	-	0.02±0.003	(0.019-0.031)
6.	Club length	0.07±0.005	(0.063-0.082)	-	-
7.	Club width	0.02±0.004	(0.025-0.038)	-	-
8.	Fore wing length	0.49±0.011	(0.476-0.507)	0.50±0.011	(0.476-0.526)
9.	Fore wing width	0.25±0.008	(0.234-0.260)	0.26±0.011	(0.253-0.279)
10.	Hind wing length	0.40±0.014	(0.387-0.425)	0.39±0.034	(0.361-0.431)
11.	Hind wing width	0.03±0.003	(0.031-0.038)	0.03±0.005	(0.031-0.044)
12.	Genital capsule length	-	-	0.12±0.005	(0.12-0.133)
13.	Genital capsule width	-	-	0.04±0.004	(0.044-0.057)

Ratios

S. No.	Characters (Ratio)	Female	Male
1.	Flagellar width/ Flagellar length	-	0.13
2.	Club width/ Club length	0.28	-
3.	Fore wing length/ Fore wing width	1.96	1.92
4.	Genital capsule width/ Genital capsule length	-	0.33



Fig. 1. Morphometry of *Trichogramma* spp. (a) female (b) male (c) female head (d) male head (e) female antennae (f) male antennae (g) male genitalia (h) fore wing (i) hind wing

length was nearly similar in both the sexes but width varied from 0.158-0.222 mm in females and 0.146-0.184 mm in males. Antennal club length and width in females measured 0.07 and 0.02 mm, respectively. The flagellar length and width in males measured 0.14 and 0.02 mm, respectively. In males, fore wing length and width was 0.44 mm and 0.23 mm, respectively while in females, it measured 0.43 mm (0.374 to 0.495 mm) while forewing width was 0.21 mm (0.190- 0.253 mm). The genital capsule length varied from 0.114 to 0.126 mm with the mean value being 0.12 mm and width 0.038 to 0.057 mm with an mean value of 0.04 mm. In females, the ratio of antennal club width and length was 0.28 but in males, the ratio of flagellar width and length was 0.14. The ratio of forewing length and its width was calculated as 2.04 in females and 1.91 in males. The ratio of genitalia capsule width and length in males was calculated as 0.33.

Ludhiana: The body length of females was 0.40 mm while for male was 0.38 mm. Head length and width were nearly similar in both the sexes. Antennal club length and width in females measured 0.07 and 0.02 mm, respectively. The flagellar length and width in males measured 0.15 and 0.02 mm, respectively. Fore wing length and width was relatively similar in both the sexes. The hind wing length and width measured 0.403 and 0.034 mm, respectively. In males, the hind wing length varied from 0.361 to 0.431 mm with mean value of 0.390 mm. The width ranged from 0.031 to 0.044 mm with a mean value of 0.03 mm. The genital capsule length varied from 0.12 to 0.13 mm with a mean value of 0.12 mm and width 0.044 to 0.057 mm with mean value of 0.04 mm. In females, the ratio of antennal club width and length was 0.28 but in males, the ratio of flagellar width and length was measured as 0.13. The ratio of fore wing length and its width was calculated as 1.96 in females and 1.92 in males. The ratio of genitalia capsule width and length in males was calculated as 0.33.

Thus, very small variations were observed amongst the *T. chilonis* specimens collected from locations. Like the laboratory reared strain, the species collected from all the locations were identified as *T. chilonis*. The results are supported by the findings of Yousuf et al. (2016) who studied the morphometrics of *Trichogramma* where similar characters had been analysed. Garcia-Gonzalez et al. (2009) did morphometrics of *Trichogramma* spp. mass reared in Mexico, and separated *T. pretiosum*

and *T. exiguum* from *T. fuentesi* and *T. pintoi* based on the longest funicular setae length of male antenna. Intervosellar process length of male genitalia was used to differentiate *T. pretiosum* and *T. pintoi* from *T. fuentesi* and *T. exiguum*.

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