Journal of Biological Control, 31(2): 90-94, 2017, DOI: 10.18311/jbc/2017/15947

Research Article

Morphometric analysis of *Trichogramma achaeae* Nagaraja and Nagarkatti, an important biological control agent of agriculture and forestry

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ABSTRACT: Taxonomy of *Trichogramma* species is very important for their biological control program. Various researchers have proposed the taxonomic keys based on genitalia but there are other important characters that needs consideration and these have been largely ignored by taxonomists while proposing the taxonomic keys. In the present study, the emphasis is given on morphometry of some important characters including genitalia in *Trichogramma achaeae* Nagaraja and Nagarkatti. The average body length of both male and female were measured as 0.478 mm and 0.543 mm, respectively, it showed that females are longer than males. Important characters such as head length and width, hind wing length and width, also length of its marginal fringes were measured including setae on RS1, RS2, r-m veins and statistically analyzed for significant differences. Detailed morphometry of *T. achaeae* is described and discussed.

KEY WORDS: Characters, measurements, morphometrics, *Trichogramma achaeae*

(Article chronicle: Received: 27-04-2017; Revised: 21-06-2017; Accepted: 30-06-2017

INTRODUCTION

Genus *Trichogramma* comprises of polyphagous hymenopteran parasitoids, belonging to the family Trichogrammatidae and are known for its uses in biological control throughout the world (Hoffmann *et al*., 2001). Around 239 species of *Trichogramma* are recorded from the world, which includes 37 from India (Jalali, 2013; Pinto, 2006 and Noyes, 2017). *Trichogramma* wasps parasitize the insect eggs, especially eggs of moths and butterflies including some of the most important lepidopteran pests of field crops, forests, fruit and nut trees; widely applied in the field for biological control of insect pests since three decades (Yousuf *et al.,* 2016 a &b). *Trichogramma achaeae* Nagaraja and Nagarkatti (1969) has been reported from the following hosts- *Achaea janata*, *Agrius convolvuli*, *Catopsila pyranthe*, *Clostera cupreata*, *Corcyra cephalonica*, *Earias insulana*, *Earias vitella*, *Ergolis merione*, *Helicoverpa armigera*, *Pectinophora gossypiella*, *Spodoptera litura*, *Tiracola plagiata* and *Tuta absoluta* (Yousuf *et al*., 2015).

Trichogramma achaeae is small, around 0.5 mm long and 0.15 mm wide across the head; antennae with longest hairs being 2.5 times the maximum width of the flagellum. Genitalia with chitinized DEG (Dorsal Expansion of Gonobase) being almost triangular with a blunt apex, reaching tips of the GF (Gonoforceps); CS (Chelate Structure) in the level of GF; MVP (Median Ventral Projection) minute and inconspicuous; Female ovipositor as long as hind tibia; Aedeagus about 1.5 times of apodemes.

Trichogramma species are notoriously difficult to identify because of their small size and low inter-species morphological differences. Researchers are working on male genitalia and Internal Transcribed Spacer (ITS) region of the ribosomal gene to separate and identify the different species of *Trichogramma* (Chan and Chou, 2000; Thomson *et al*., 2003).

Various researchers have tried to separate the different species of *Trichogramma* by morphometric techniques. Burks and Heraty (2002) separated *Trichogramma californicum*, *T. exiguum*, *T. minutum* and *T. platneri* through morphometric analysis. Similarly, *T. pretiosum*, *T. exiguum*, *T. fuentesi* and *T. pintoi* were morphometrically examined and separated from each other through genital studies (Garcia-Gonzalez *et al*., 2009). Querino and Zucchi (2003) worked on the morphological characterization of the ten *Trichogramma* spp. in South America. In India, morphometric studies were conducted for *T. plasseyensis* (Hassan and Yousuf, 2007) and *T. raoi* (Yousuf *et al*., 2008) and dataset for complete and accurate identification of these species were prepared. In the present study, the emphasis is given on morphometry of some important characters in-

cluding genitalia in Trichogramma achaeae Nagaraja and Nagarkatti.

MATERIAL AND METHODS

Culture of *Trichogramma achaeae* was procured from ICAR-National Bureau of Agricultural Important Resources in 2016 with accession no. NBAII-MP-TRI-06a. The culture was maintained in the laboratory at temperature 27±1^{O}C in BOD on the eggs of *Corcyra cephalonica*. Ten male and ten female specimens were dehydrated and dissected in clove oil under the stereoscopic microscope. Slides were prepared by following the methodology of Hassan and Yousuf (2007), Platner *et al*. (1999) and Yousuf *et al*. (2008). Morphological characters reported by Hassan and Yousuf (2007) were taken into consideration. A total of 33 characters (including their ratios) of males and 27 characters (including their ratios) of females of *T. achaeae* were examined for morphometric analysis.

Specimens of *T. achaeae* were identified, dissected and slides were prepared using stereoscopic zoom binocular microscope (Aark International). Similarly, morphometric measurements were taken on Leitz labor luxs (Leica, Germany).

RESULTS AND DISCUSSION

The body length of *T. achaeae* varies from 0.418 to 0.502 in males and 0.520 to 0.558 mm in females and males are smaller in size in comparison to the females. Head length, head width and eye width are nearly similar, in both the sexes. Similarly, malar space does not differ in both males and females of *T. achaeae*. Measurement range of other characters such as fore wing length and width, marginal fringe of fore wing, hind wing length and width, marginal fringe of hind wing, length of hind tibia, width of hind tibia are relatively similar for both the sexes of *T. achaeae*.

Male characters

The head length in males of *T. achaeae* was 0.9 times shorter than head width. The average eye width in the male of *T. achaeae* was recorded as 0.098 mm though it varies from 0.085 to 0.103 mm (Table. 1). The average flagellar length and width were measured as 0.183 mm and 0.037 mm, respectively. Also, the flagellar hair length was found to be 0.078 mm long on an average. The average numbers of flagellar hairs were counted as 41. The average fore wing length and width for *T. achaeae* male were measured as 0.480 mm and 0.226 mm, respectively. Similarly, the hind wing length varied from 0.333 mm to 0.407 mm with the average length of 0.371 mm, and width 0.027 mm to 0.046 mm with the average width of 0.041 mm, respectively. It was observed that the marginal fringe of fore wing (0.031±0.006) was smaller than the marginal fringe of hind wing (0.052±0.007). The hind tibia is one of the important characters to be taken into consideration for identification of the *Trichogramma* spp. and thus in the present study, the average length and width of the hind tibia were measured as 0.158 mm and 0.025 mm, respectively. The aedeagus length (average) was measured as 0.111 mm whereas, the average genitalia capsule length was 0.128 mm long. This shows that the aedeagus is almost equal to the total genital length. The setae in fore wing are again one of the important characters to be studied for correct identification of different species of *Trichogramma*. The number of setae on RS1, RS2, r-m and between RS2 and r-m were 4, 12, 18 and 43, respectively.

Table 1. Descriptive analysis of main characters for *Trichogramma Achaea*

S. No.	Male Characters	*T. achaeae* (Male)	*T. achaeae* (Female)
1	Body length	0.478±0.025	0.543±0.011
		(0.418-0.502)	(0.521-0.558)
2	Head length	0.197±0.020	0.185±0.015
		(0.154-0.228)	(0.161-0.209)
3	Head width	0.231±0.011	0.223±0.018
		(0.216-0.244)	(0.193-0.248)
4	Eye width	0.098±0.006	0.100±0.006
		(0.085-0.103)	(0.090-0.108)
5	Malar space	0.052±0.003	0.051±0.003
		(0.046-0.055)	(0.046-0.052)
6	Flagellar/Club length	0.183±0.010	0.084±0.007
		(0.172-0.209)	(0.073-0.095)
7	Flagellar/Club width	0.037±0.002	0.034±0.005
		(0.033-0.040)	(0.022-0.040)
8	Flagellar hair length	0.077±0.005	----------
		(0.070-0.084)	
9	Fore wings length	0.480±0.011	0.465±0.021
		(0.465-0.502)	(0.428-0.493)
10	Fore wings width	0.226±0.006	0.216±0.022
		(0.214-0.232)	(0.177-0.242)
11	Length of marginal fringe of Fore wings;	0.031±0.006	0.035±0.005
		(0.023-0.041)	(0.0276-0.044)
12	Hind wings length	0.371±0.023	0.361±0.023
		(0.333-0.407)	(0.327-0.398)
13	Hind wings width	0.041±0.005	0.038±0.003
		(0.028-0.046)	(0.032-0.041)
14	Length of marginal fringe of Hind wings;	0.052±0.007	0.052±0.006
		(0.032-0.057)	(0.041-0.060)
15	Length of hind tibia	0.158±0.005	0.159±0.007
		(0.150-0.165)	(0.150-0.172)
16	Width of hind tibia	0.025±0.002	0.023±0.002
		(0.022-0.029)	(0.022-0.026)
17	Ovipositor length	----------	0.164±0.014
			(0.138-0.186)
18	Fore wings setae on RS1	4-5	4-5
19	Fore wing setae on RS2	11-15	11-13
20	Fore wing setae on r-m	18-21	17-22
21	Fore wing setae b/w RS2 & r-m	34-46	33-42
22	No. of flagellar hairs	34-45	----------

Note: Means±standard deviation (top value) and range (parentheses); S. No. 1-21 are in mm and 22-26 are in numbers.

Female characters

Female *T. achaeae* were also examined for the similar characters except for genitalia and instead we studied the ovipositor for its length. The ratio of head length and head width was 0.832 which shows that head is wider than the length (Table. 1). The average eye width measured 0.1 mm which actually varies from 0.089 mm to 0.108 mm. In males, flagellum characteristics were taken for morphometric study, but in females, the antennal club is taken into account. Antennal club length and width measured 0.084 mm and 0.034 mm, respectively. Fore wing length was found to be 0.465 mm having variation from 0.428 mm to 0.493 mm. Similarly, average fore wing width was 0.216 mm with variation from 0.177 mm to 0.242 mm. The average hind wings length and width measured 0.361 mm and 0.038 mm, respectively. The marginal fringe of hind wing was found longer than the marginal fringe of fore wing which is similar to that of males. The mean length and width of the hind tibia were 0.159 mm and 0.023 mm, respectively. The ovipositor is again one of the important characters which is to be studied for the identification of the *Trichogramma* spp. if only female specimens are available. The ovipositor length was measured as 0.164±0.014 mm which ranged from 0.138 mm to 0.186 mm. The number of setae on RS1, RS2, r-m and between RS2 and r-m were 4, 12, 21 and 35, respectively.

Additional Characters

There are some additional characters (ratio of characters) which are also important along with the primary characters in species delimitation. Descriptive analysis of ratio of characters for *T. achaeae* is provided in (Table. 2). In males, the ratio of flagellar hair length and flagellar width was measured as 2.126 and similarly, the ratio of flagellar width and flagellar length was 0.201. In females, the ratio of antennal club width and length was 0.404 and the ratio of antennal club length and ovipositor length was 0.513. The ratio of fore wing length and its width was calculated as 2.124 in males and 2.170 in females. Similarly, the ratio of the marginal fringe of fore wing and fore wing width for both male and female were 0.137 and 0.165, respectively. In the case of hind wings, the ratio of the marginal fringe of hind wing and hind wing width for male and females were 1.3 and 1.4, respectively. The ratio of genitalia capsule width and length in males was calculated as 0.370 with a small variation from 0.34 to 0.40. Similarly, in females, the ratio of ovipositor length and hind tibia length was calculated as 1.04 with variation from 0.87 to 1.21.

Trichogramma achaeae was initially described by Nagaraja and Nagarkatti (1969) wherein the information for its identification up to genitalia level was provided, however other characters were not taken into consideration for taxonomic identification of the species. The characters, other than genitalia such as flagellar length, hind tibia, head length and width, malar space, eye width, marginal fringe of fore wing and hind wing are equally important. Hassan and Yousuf (2007) studied the important characters for *T. plasseyensis* and reported the morphometric analysis for correct identification of the species. In the present study, two important characters *viz*. number of flagellar hairs and marginal fringe of the hind wing have been taken into account. Yousuf *et al.* (2008) have worked on *T. raoi* for its morphometric analysis however number of flagellar hairs and hind wing marginal fringe character was not included in their study. This study complies with the work done by Yousuf *et al.* (2016a) on *T. chilonis* where similar characters have been taken for studying the morphometric of *Trichogramma*. Burks and Heraty (2002) morphometrically analysed four species of *Trichogramma* from North America. The present study is first of its kind exclusively for *T. achaeae* for its correct identification to help the future researchers on various identification issues. Description of various *Trichogramma* spp. reported from India was given by Nagarkatti and Nagaraja (1971) but identification characters on *T. achaeae* were not included. Garcia-Gonzalez *et al*. (2009) and Querino and Zucchi (2003) have studied the morphometrics of *Trichogramma* from Mexico and South America but no work was done for *T. achaeae*.

Statistical analysis

Independent sample t-test was conducted taking characters as dependent variable and gender as independent variable and it was found that characters such as head length, head width, eye width, malar space, fore wing length and width and length of its marginal fringes, hind wing length and width and length of its marginal fringe and length of hind tibia were significantly similar ($p>0.05$). But Body length in both male and female were found significantly different ($t=-7.481$, d.f.=18 and sig. (2-tailed) =0.00); since $p<0.05$ therefore, we rejected the hypothesis that the characters studied for both male and female are of similar or having no significant difference. Similarly, the width of hind tibia ($t=2.496$, d.f.=18 and sig. (2-tailed) =0.022) were found insignificant and therefore it can be concluded that the character body length and width of hind tibia are not similar in both males and females of *T. achaeae* so these characters should be taken out for identification of males and females.

Table 2. Descriptive analysis of ratio of characters for *Trichogramma achaeae*

S. No.	Male character	*T. achaeae* (Male)	*T. achaeae* (Female)
1	Flagellar hair length/Flagellar width	2.126±0.160	----------
		(1.818-2.444)	
2	Flagellar width/Flagellar length	0.201±0.017	----------
		(0.175-0.224)	
3	Flagellar length/Hind tibia length	1.158±0.058	----------
		(1.067-1.267)	
4	Antennal club width/Antennal club length	----------	0.404±0.072
			(0.286-0.500)
5	Antennal club length/Ovipositor length	----------	0.513±0.052
			(0.413-0.576)
6	Antennal club length/Hind tibia length	----------	0.529±0.041
			(0.467-0.585)
7	Fore wings length/Fore wings width	2.124±0.050	2.169±0.169
		(2.040-2.217)	(2-2.5)
8	Longest marginal fringe of fore wings/Fore wings width	0.137±0.026	0.165±0.036
		(0.099-0.185)	(0.119-0.223)
9	Longest marginal fringe of hind wings/Hind wings width	1.300±0.304	1.389±0.185
		(0.737-2.000)	(1.176-1.786)
10	Genital capsule length/ Hind tibia length	0.809±0.020	----------
		(0.753-0.903)	
11	Genital capsule width/ Genital capsule length	0.370±0.020	----------
		(0.339-0.407)	
12	Ovipositor length/Hind tibia length	----------	1.037±0.092
			(0.875-1.210)

Note: Means±standard deviation (top value) and range (parentheses).

ACKNOWLEDGEMENTS

We would like to thank Director, Forest Research Institute, Dehradun (Uttarakhand, INDIA), for providing the necessary research facilities. The authors are also grateful to Dr. Sudhir Singh, Head, Forest Entomology Division, Forest Research Institute, for providing the support and lab facility for the research work. We are also greatly indebted to the authorities of NBAIR, Bangalore, for providing the culture of *Trichogramma achaeae* for the study.

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