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On the true identity of "*Trichogramma brasiliensis* (Ashmead)" (Hymenoptera: Trichogrammatidae) being used in India

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ABSTRACT: **Investigations were carried out on the true identity of the thelytokous species purported to be *Trichogramma brasiliensis* (Ashmead) (= *T. brasiliense*), which is used in India in biological control programmes, as the holotype of Ashmead's species is placed under the genus *Trichogrammatoidea* Girault at present. Morphological and molecular studies in combination with crossing experiments conclusively proved that this species was conspecific with *T. pretiosum* Riley. Thelytoky in *T. brasiliensis* auctt. used in India is found to be *Wolbachia* - induced and reversible by administering antibiotics.**

KEY WORDS: Arrhenotoky, PCR, thelytoky, *Trichogramma brasiliensis*, *Trichogramma pretiosum*, *Trichogrammatoidea*, *Wolbachia*.

INTRODUCTION

In the late 1960s, a thelytokous culture under the name *Trichogramma brasiliensis* (Ashmead) was received in India from the USA by the Commonwealth Institute of Biological Control, Indian Station (CIBC-IS), Bangalore, under a US PL-480 project on the biosystematics of *Trichogramma* species. Its experimental hybridization with other arrhenotokous species was not possible as it was thelytokous, except for a limited unidirectional cross using the occasional rare males with the virgin females of the arrhenotokous form of *T. pretiosum* Riley, with the males of which it was identical. At that time itself, it was opined that '*T. brasiliensis*' could in fact be a thelytokous form of *T. pretiosum*. However, as it was thelytokous, it was treated as different.

This species was supplied to Indian Agricultural Research Institute, New Delhi, in the early 1970s for experimental releases against cotton bollworms. Sangwan *et al.* (1972) used it for controlling *Earias* spp. and *Pectinophora gossypiella* (Saunders) in Haryana and obtained maximum parasitization of 89% eggs of bollworms and subsequently 35% increased cotton yield. It has been used along with two other species for various studies in India such as response to sex pheromone of *Helicoverpa armigera* (Hub.) (Pawar *et al.*, 1984).

When the CIBC-IS was wound up in the late 1980's, the culture was passed on to Project Directorate of Biological Control (PDBC), Bangalore, and has been maintained there ever since. This species is listed as present in India by

Hayat and Subba Rao (1986) and Mani (1989). Singh and Jalali (1994) documented the use of "*T. brasiliensis*" in India against various pests.

Pinto (1997) found that Ashmead's (1904) holotype of *T. brasiliensis* (originally described as *Pentarthron brasiliensis*), deposited in US National Museum, belonged to the genus *Trichogrammatoidea* Girault and transferred the species to *Trichogrammatoidea*. In view of this, the present investigations were carried out to establish the true identity of the species being used in India as *T. brasiliensis* by morphological and molecular means and crossing experiments.

MATERIALS AND METHODS

Maintenance of *Trichogramma* cultures

"*Trichogramma brasiliensis*" and *T. pretiosum* were maintained on the eggs of the laboratory factitious host, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae).

Morphological studies

Specimens from the cultures of "*T. brasiliensis*" and *T. pretiosum* were studied for their taxonomic characters, particularly antenna, wing venation, and male genitalia. [Source of *T. pretiosum*- *Ex. Helicoverpa zea* (Boddie) - 1964. Rincon Insectary, USA (Nagarakatti and Nagaraja, 1971)]

Crossing experiments

(i) Without antibiotic treatment

Crossing experiments were arranged with the occasional males of '*T. brasiliensis*' with virgin females of *T. pretiosum* and the progeny sex ratio was recorded.

(ii) With antibiotic treatment

Freshly emerged adults of "*T. brasiliensis*" - thelytokous form (often mentioned as *T. brasiliense*) were fed with antibiotic (tetracycline (0.1%)) mixed with 50% diluted honey, provided as fine streaks inside the glass vials containing them. After 24h, freshly irradiated *Corcyra cephalonica* eggs were provided for parasitism. Parasitised eggs were separated with a fine brush and kept in individual vials (5 x 0.9 cm) for the emergence of F_1 adults. The progeny was observed under a microscope and the sex ratio recorded. Antibiotic treatment for 20 successive generations resulted in production of males. Such males were crossed with virgin females of *T. pretiosum* AF – arrhenotokous form and *vice versa*. Mated pairs were provided with irradiated *Corcyra* eggs for progeny production. Emerged progeny of each cross was segregated by sex and used for *Wolbachia* determination.

Molecular studies

Total DNA extraction was done from the freeze-dried adults of *Trichogramma* (Sappal *et al.*, 1995). Twenty adults of each sex of each cross and parents were collected into 1.5ml micro centrifuge tube and adults were crushed with micro pestle in 100μ l homogenisation buffer (200mMTris – HCl, pH 8.0, EDTA, 2M sodium chloride, 20mM sodium metabsulphite) till a clear homogenate was observed. N-sodium lauryl sarcosine was added to the homogenate and incubated at 60°C for 2h. The incubated mixture was centrifuged and 10M-ammonium acetate and isopanol were added to the supernatant and kept for overnight incubation at – 20°C. Centrifugation (12000g at 4°C for 15 min) allowed the complete precipitation of the extracted DNA. The precipitated DNA was washed with 70% ethanol and the pellet was dried at room temperature. DNA pellet was resuspended in 40ì l of TE buffer. The purity of DNA was checked in 0.8% agarose.

Wolbachia-specific Primer (fTsZ) PCR

PCR reaction was performed in 50ì l reaction volumes using Biorad icycler, 2 l DNA template, 5 l (10x) Taq assay buffer, 1 l dNTP's (each in 10mM concentration), 1 l forward and reverse primers (10 picomoles /μ l), 0.25 l taq polymerase (1 unit). The primers used to amplify the fTsZ region were 5' GATCCGTATGCCGATTGCAGAGCTTC 3' (forward) and 5' AATTCGCCATGAGTATTCACTTGGCT 3'

(reverse) (Minnot *et al.*, 1996). The thermal cycling condition for PCR consisted of 30 cycles (Den: 94°C for 1 min, Ann: 55°C for 1 min, Ext: 72°C for 2 min, with an initial Denaturation: 95°C for 5 min and final extension at 72°C for 10 minutes). PCR products were electrophoresed on a 1.5% agarose gel (Bioron). The gels were stained using ethidium bromide. Molecular standards were run along with the samples for reference.

RESULTS AND DISCUSSION

Morphological characters

The species used as "*T. brasiliensis*" in India belonged to *Trichogramma* by virtue of (i) its fore wing (Fig. 2) having recurrent vein tract (RS 1) containing 4-6 setae (Fig. 3) (recurrent veinlet setae absent in *Trichogrammatoidea*, Fig. 4); (ii) hind wing with incomplete anterior and posterior setal tracks above and below the complete middle track of setae (Fig.5) (only middle track of setae in *Trichogrammatoidea*, Fig. 6); (iii) male antenna with unsegmented flagellum with 26-30 long hairs, the longest about 3 times the maximum width of flagellum (Fig. 1) (5-segmented in *Trichogrammatoidea*); and (iv) scutellum with a median pair of round sensory pits below anterior pair of setae (Fig.7) (these are situated in between the posterior pair of setae in *Trichogrammatoidea* (Fig.8)).

Male genitalia (Figs 9-10): Dorsal Expansion of Gonobase (DEG) triangular without distinct lateral lobes, with notches (DEG is absent in *Trichogrammatoidea*) (Fig. 10). Aedeagus with apodemes as long as the genital capsule (this is shorter than genital capsule in *Trichogrammatoidea*) (Fig. 9). The male genitalia of "*T. brasiliensis*" and *T. pretiosum* were found to be identical.

Female genitalia (Fig. 11): The relative lengths of ovipositor, hind tibia and aedeagus in "*T. brasiliense*" were found to be identical to those of *T. pretiosum*.

Crossing experiments

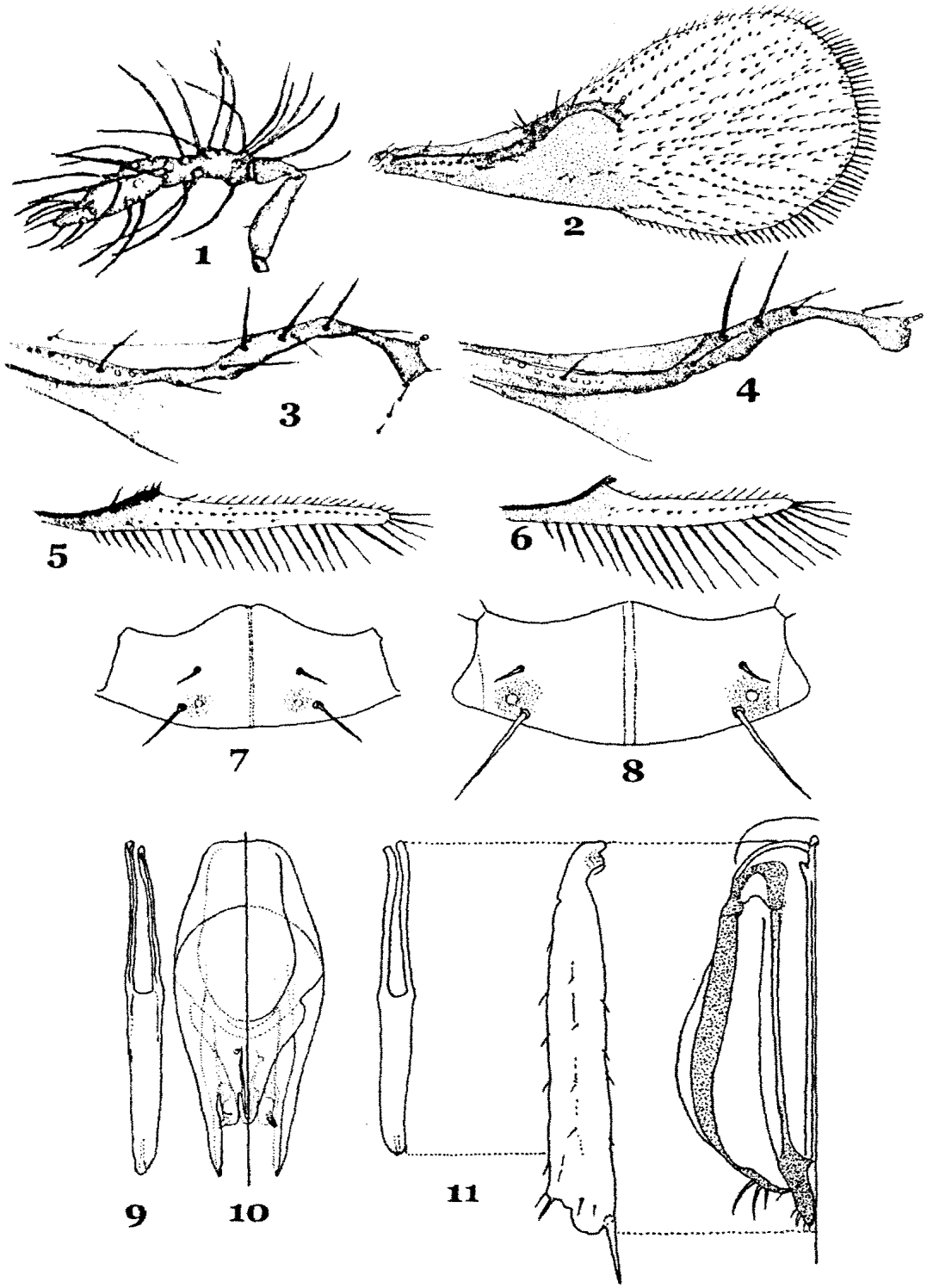
(i) Without antibiotic treatment

Table 1. Crossing experiments between males of '*T. brasiliense*' and virgin females of *T. pretiosum*.

Rep.No.	Males x Females	F_1 progeny		
		Males	Females	% Females
I	*brasiliense* 3 *pretiosum* 6	119	73	38.02

Table 2. Progeny production in crossing experiments between *Wolbachia*-cured "*T.brasiliensis*" and *T. pretiosum*

Batch No.	Species crossed		Number of pairs	F_1 progeny		% Females produced
	Males	Females		Males	Females	
I	*pretiosum*	*brasiliensis*	5	107	61	36.31
	brasiliensis	*pretiosum*	5	117	168	58.95
II	*pretiosum*	*brasiliensis*	8	125	33	20.29
	brasiliensis	*pretiosum*	1	24	23	48.94
III	*pretiosum*	*brasiliensis*	16	241	128	34.69
	brasiliensis	*pretiosum*	6	120	62	34.07
IV	*pretiosum*	*brasiliensis*	20	192	351	64.64
	brasiliensis	*pretiosum*	18	505	865	63.14



Figs. 1-11. ***Trichogramma pretiosum*****: 1. Male antenna; 2. Fore wing; 3. Basal part of fore wing showing vein track RS 1 below stigma; 4. Basal part of fore wing of** ***Trichogrammatoidea*****; 5. Hind wing of** ***T. pretiosum*****; 6. Hind wing of** ***Trichogrammatoidea*****; 7. Scutellum of** ***T. pretiosum*****; 8. Scutellum of** ***Trichogrammatoidea*****; 9-10. Male genitalia of** ***T. pretiosum*****; 11. Relative lengths of aedeagus with apodemes, hind tibia and ovipositor in** ***T. pretiosum*****.**

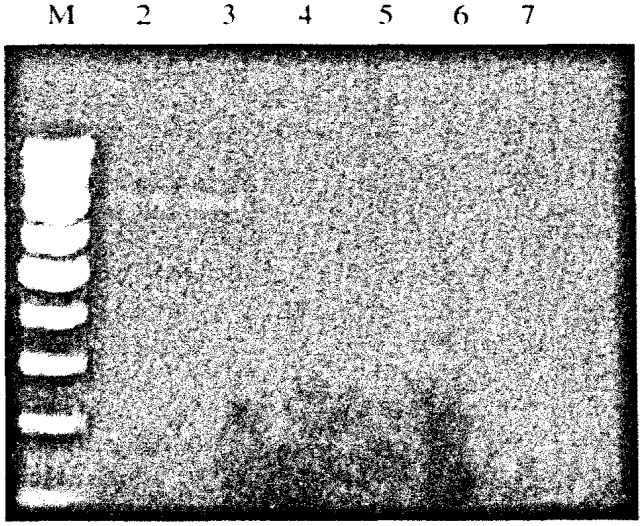


Fig 12. PCR – amplified ftsz regions in the genomic DNA of *T. brasiliense* parent, females and males of crossings and *T. pretiosum* parent. Lane 1. Ladder, Lane 2. *T. brasiliense* parent, Lane 3 and 4. Females and males of cross (*T. brasiliense* female x *T. pretiosum* male), Lane 5. *T. pretiosum* parent, Lane 6 & 7. Females and males of cross (*T. brasiliense* cured male x *T. pretiosum* female)

Female progeny production in the preliminary crossing experiment was abundant (38.02%), suggesting free gene flow in the crosses (Table 1).

(ii) With antibiotic treatment

The antibiotic treatment resulted in production of males by the thelytokous form of "*T. brasiliensis*" after 20 generations and reciprocal crossing between thelytokous and arrhenotokous forms resulted in the production of both males and females, thereby indicating both are conspecific (Table 2). The thelytoky was governed by the bacterium *Wolbachia*. *Wolbachia* was detected only in the progeny produced by thelytokous females of "*T. brasiliensis*" both in parents and in crosses where thelytokous females were used and the resultant progeny was females. *Wolbachia* was not detected when males of *T. pretiosum* (arrheotokous form) was used. The size of the WSP region band obtained by PCR was 0.7Kb (Fig. 12).

From both Tables 1 and 2, it is evident that there was free gene exchange between "*T. brasiliense*" and *T. pretiosum*, although unidirectional in the first, with female production ranging from 20.89% to 64.64% in both directions. These experiments prove reproductive compatibility between both, which should hence be considered conspecific. A breakaway population of *T. pretiosum* must have contracted *Wolbachia* earlier, which might have changed its mode of reproduction from arrhenotoky to thelytoky. Cytoplasmically inherited endosymbiotic bacterium, *Wolbachia*, is reported to be responsible for reproductive alterations in trichogrammatids (Stouthamer *et al.*, 1993). Stouthamer *et al.* (1990) cured thelytokous species of *Trichogramma* of *Wolbachia* to validate the identity of the thelytokous and arrhenotokous forms of *T. pretiosum*.

The crossing experiments clearly showed that the species hitherto called as "*T. brasiliensis*" in India is none other than a thelytokous, *Wolbachia* induced, breakaway form of *T. pretiosum*, which was corroborated by the morphological evidence. In the light of the present findings, it is evident that the most of the Indian literature pertaining to *T. brasiliensis* actually involves *T. pretiosum*.

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