

Biology of *Tetrastichus pantnagarensis* (Hymenoptera: Eulophidae) a hyperparasite of *Diaphania indica* (Lepidoptera: Pyralidae) through *Apanteles taragamae* (Hymenoptera: Braconidae)

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Abstract. *Tetrastichus pantnagarensis* Khan (Hymenoptera: Eulophidae) was recorded as a hyperparasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae) through *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). Studies were conducted on its biology. This solitary endoparasite oviposited in the host pupa within the host cocoon and the larva passes through 3 instars. Mean development time from egg to adult is 12.18 days at $26.43 \pm 2.53^\circ\text{C}$. After a preoviposition period of 1-2 days oviposition continued for 7-10 days. Mean adult longevity for males was 8.51 days and for females 11.41 days.

Keywords. *Tetrastichus pantnagarensis*; hyperparasite; biology.

1. Introduction

The pumpkin caterpillar, *Diaphania indica* (Saunders) is a major pest of several cultivated cucurbits. During the course of the studies on the bioecology of *D. indica*, the braconid, *Apanteles taragamae* was recorded as its major gregarious endoparasite. It was recorded for the first time from Sri Lanka as a parasite of *D. indica* on snake gourd (Wilkinson 1931). In 1948, Bhatnagar listed 13 hosts of *A. taragamae* from India. At Padappai, *D. indica* is a major pest on *Coccinia grandis* (L.) Voight and during certain months 80% parasitism by *A. taragamae* was recorded. However the pest was not under effective biological control. It was observed that the cocoons of *A. taragamae* were attacked by a hyperparasite which was subsequently identified as *Tetrastichus pantnagarensis* Khan. Hyperparasitism is defined as any form of parasitism other than primary (Smith 1916). There is disagreement over the importance of hyperparasitism in biological control (Narayanan 1957; Flanders 1963; Valentine 1974) but it is generally conceded that the identification of hyperparasitism is supremely important to biological control. Hyperparasitism is more common in the Tetrastichinae and is sometimes obligatory. Genera of tetrastichines that include hyperparasitic species are *Tetrastichus* Walker, *Crataepiella*, *Domenichini* and *Melittoba* Westwood (Gordh 1988). *T. pantnagarensis* was originally described as a parasite of *Apanteles* sp. on the larvae of Bihar hairy caterpillar, *Diacrisia obliqua* Walker (Khan 1983). This is the first record of *T. pantnagarensis* as a hyperparasite of *D. indica* through *A. taragamae* and there is no information on biology of *T. pantnagarensis*. Therefore in the present investigation observations on the biology of this hyperparasite were carried out.

2. Materials and methods

To study the biology of the secondary parasite it was essential to maintain a culture of the primary parasite. The following procedure was adopted to rear *A. taragamae* in the laboratory.

Twenty-five third instar larvae of *D. indica* were released on a bouquet of *Coccinia* leaves inserted into a glass vial (6 × 1.5 cm) placed inside a plastic jar (12 × 10 cm) with wire mesh fitted lid. Three mated females of *A. taragamae* were released into each jar. The larvae were exposed to the parasite for 12 h. At the end of this period the parasites were removed from the jar and the larvae allowed to feed on the leaves. The leaves were changed periodically until the parasite larvae completed their development inside the host larvae and the parasite cocoons were formed. These cocoons were collected from the rearing jars and placed in specimen tubes.

Freshly formed *A. taragamae* cocoons were exposed to *T. pantnagarensis* adults in glass specimen tubes for 1 h. After this the adults were separated from the cocoons and 12 h later the cocoons were dissected and the host pupae taken out and dissected again. This was repeated at periodic intervals of 6 h, until hatching of the hyperparasite eggs was observed. After the incubation period, the cocoons were dissected every 24 h and sketched. To establish the larval period the parasitised host cocoons were dissected at periodic intervals using a Carl Zeiss Zoom Citoval-2-Stereomicroscope. To determine the shape and size of the larval mandibles the larvae were boiled in a 10 KOH solution for 45 s for clearing the host tissues. After washing in distilled water they were mounted in Hoyer's medium on microscope slides. The parasite larvae were measured with a calibrated ocular micrometer and sketched using a camera lucida attached to a Carl Zeiss Laboval 4 compound microscope. The prepupal and pupal periods were also recorded. The rearing was done at $26.43 \pm 2.53^\circ\text{C}$ and 65% RH.

The preoviposition and oviposition periods were determined by exposing the freshly formed parasite cocoons to the hyperparasite at intervals of 24 h beginning with the day of emergence. The cocoons were dissected after each exposure and the number of parasitised hosts as well as the number of eggs laid per host were counted. The host cocoons were exposed continuously until the female died.

Adult longevity was determined by feeding honey solution to freshly emerged males and females.

The stock culture of *T. pantnagarensis* was maintained in the insectary by rearing it on *A. taragamae* cocoons. The cocoons were exposed for 6 h after which the adults were separated from the host. The exposed cocoons were placed in glass specimen tubes until the adults emerged.

3. Results and discussion

3.1 Immature stages

3.1a *Egg*: The freshly laid egg is white, slightly curved in the middle and sausage-shaped (figure 1A–D). Eggs are found mainly in the abdomen of the host. The length of 20 eggs averaged 0.31 mm and the width in the middle averaged 0.07 mm

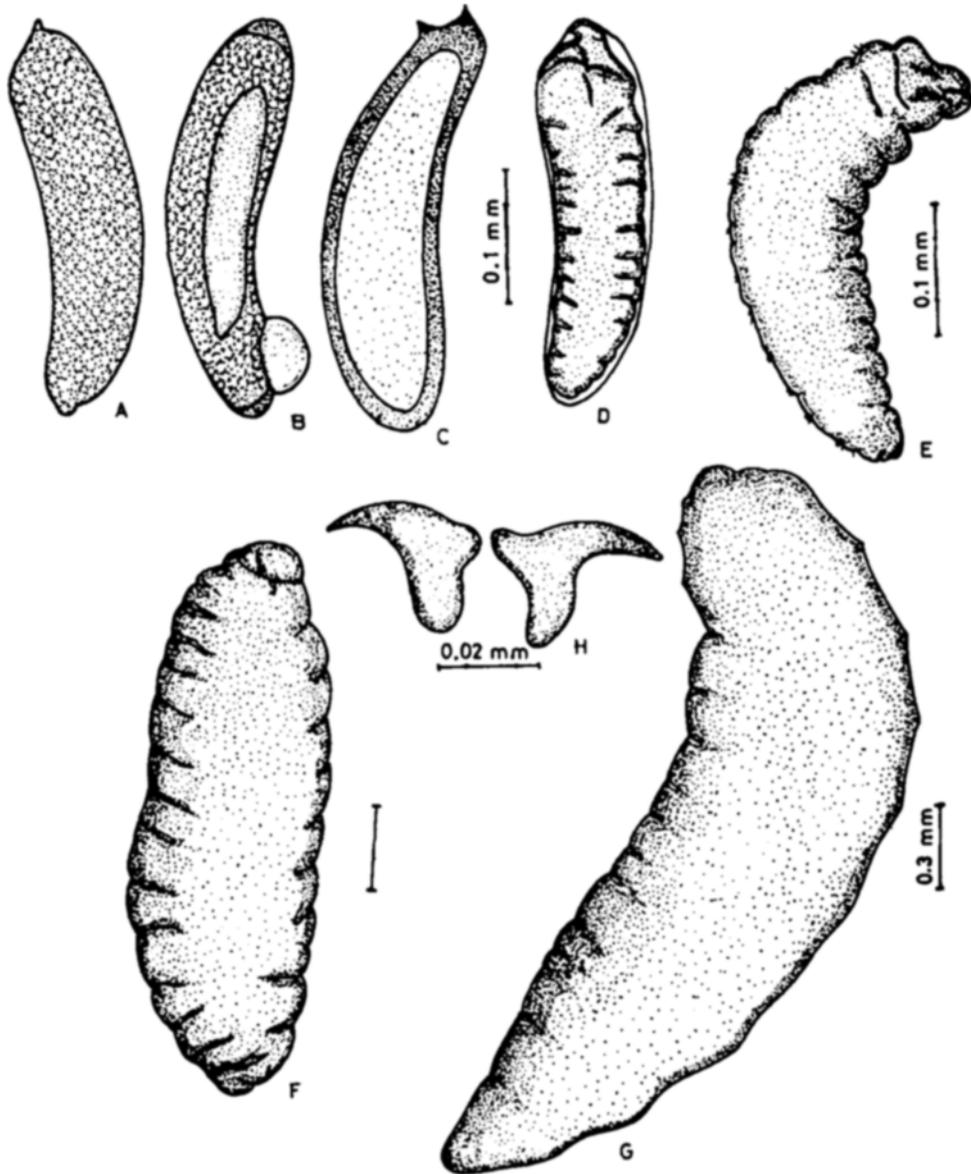


Figure 1. Developmental stages of *T. pantnagarensis*, A-D, Egg in various stages of development; E, 1st instar; F, 2nd instar; G, 3rd instar; H, Mandibles.

(table 1). Initially the chorion is smooth and shining white but with development becomes transparent and the embryo can be clearly seen.

3.1b Larva: The larva passes through 3 instars. The first instar larva is white and fusiform. The body is broad at the anterior end and tapers at the posterior end. It is broadest in the thoracic region. The body consists of 13 segments. Two rows of minute setae are visible on the dorsal side of the body (figure 1E). First instar larva averaged 1.15 mm in body length and 0.37 mm in width. The mandibles at this

Table 1. Mean size and duration of immature *T. pantnagarensis* reared at $26.43 \pm 2.53^\circ\text{C}$.

Stage	n	Length (mm)	Width (mm)	Duration (day)
		$\bar{X} \pm \text{SEX}$	$\bar{X} \pm \text{SEM}$	
Egg	20	0.31 ± 0.05	0.07 ± 0.02	40–56 h
1st instar	20	1.15 ± 0.15	0.37 ± 0.10	1–2
2nd instar	18	1.69 ± 0.12	0.61 ± 0.18	1
3rd instar	15	2.57 ± 0.31	0.77 ± 0.09	1–2
Prepupa	12	1.65 ± 0.28	0.70 ± 0.61	1
Pupa	12	2.38 ± 0.26	0.76 ± 0.14	5–6

stage are unsclerotized, simple and curved. Each measures 0.014 mm in length and 0.007 mm at the base. Spiracles are absent. Tracheal trunks are visible from the second to the tenth segment but not well defined.

The second instar larva is smooth and flabby and dorsal setae are absent. Other external structures are not visible. The head is smooth and unsclerotized (figure 1F). The larva averages 1.69 mm in length and 0.61 mm in width at the middle. This instar is less active compared to the first instar. Tracheal system similar to first instar and spiracles are absent.

The third instar larva is robust, opaque and fusiform. The head is narrow and rounded and not chitinized (figure 1G). It is smooth, dirty brown and 2.57 mm in length and 0.77 mm in width. The mandibles of the third instar larva are similar to those of the first instar but larger and measuring 0.081 mm in length and 0.048 mm in width at the base (figure 1H). The spiracles are fully developed and nine pairs are visible from the second to the tenth segment. Tracheal trunks are well formed.

3.1c Pupa: The pupa is brown in colour. The appendages adhere firmly to the body. The average length of 12 pupae was 2.38 mm and the width at the thoracic region was 0.76 mm.

3.2 Life history

T. pantnagarensis is a solitary endoparasite. The egg is laid in the abdomen of the pupa of the primary parasite. The incubation period of eggs ($n=20$) ranged from 42–56 h.

The larval duration for the 3 instars averaged 4.22 days (range 4–5 days). The size of the larva increases gradually from hatching and it entirely fills the host cocoon. When fully grown, no trace of the host pupa remains.

The pupal period recorded for 15 pupae averaged 5.82 days (range 5–6 days). The total life cycle from egg to adult emergence studied for 68 cocoon masses ranged from 11–14 days (average 12.18).

3.3 Adult

3.3a Oviposition: Mating occurs soon after emergence and lasts 20–30 s. Oviposition commences after a preoviposition period of 1–2 days. The duration of oviposition was recorded; females continue to lay eggs for about 7–10 days. Only

one egg is laid in each host even if the host cocoons were repeatedly exposed to the hyperparasite female. It is likely that a host marking mechanism by the ovipositing female prevents superparasitism. The host during the act of oviposition is paralysed and further development of the host is completely arrested.

3.3b *Longevity*: Newly emerged females when kept in tubes and fed on 20% honey solution live 11.41 days with a maximum of 16 days. The average longevity of males on the same food was 8.51 days. Without food, the adults live for 1–3 days.

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