

## Biology of *Apanteles machaeralis* Wilkinson (Hymenoptera: Braconidae) a parasite of *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae)

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**Abstract.** Studies were conducted on the endoparasite *Apanteles machaeralis* Wilkinson (Hymenoptera: Braconidae) to determine its biology when *Diaphania indica* (Lepidoptera: Pyralidae) is its host. Eggs were deposited in host larvae and increased greatly in size before hatching. The first instar larva was very active with functional mandibles, whereas the second stage larva was quiescent. The third instar larva spins its cocoon outside the body of the dead host. Mean development time from egg to adult was 12.63 days at  $29.25 \pm 1.82^\circ\text{C}$  and 59–66% RH. There is no preoviposition period. The sex ratio was 1:1.22 (males/females). Mean adult longevity was not significantly different for males (9.31 days) and females (11.68 days).

**Keywords.** *Apanteles machaeralis*; biological control; biology.

### 1. Introduction

The pumpkin caterpillar, *Diaphania indica* (Saunders) has been reported from several parts of India and other regions of the world causing damage to various cucurbitaceous plants. Patel and Kulkarny (1956) have conducted detailed studies on the biology of this insect on *Coccinia grandis* (L.) Voight in Gujarat. However literature on the natural enemies of *D. indica* is very meagre. During the course of the studies on the natural biological control of this insect pest a solitary endoparasite, *Apanteles machaeralis* Wilkinson was reared as a major parasite of *D. indica* from Padappai. Previously, Bhatnagar (1948) reported *A. machaeralis* as a parasite of *D. indica* from Bihar. The present study was carried out since there is no information available on the biology of *A. machaeralis* as a parasite of *D. indica*.

### 2. Materials and methods

#### 2.1 Rearing method and maintenance of stock culture

The adults which emerged from field collected parasite cocoons were fed with 20% honey solution. After 24 h the females were separated out. Twenty-five first instar larvae were released on *Coccinia* leaves inserted into a glass vial (6 × 1.5 cm) placed inside a plastic jar (12 × 10 cm) and 3 mated females 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

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collected from the rearing jars and placed in specimen tubes until the adults emerged. These adults were fed with 20% honey solution and used again for further rearing.

The laboratory culture of this parasite was frequently rejuvenated by mixing with field collected material.

## 2.2 *Life history and morphology of immature stages*

To study the life history of the parasite one-day old females were selected from the stock culture. They were placed in specimen tubes measuring 15 × 2 cm. A first instar larva was taken on a fine camel hair brush and placed close to the female in the tube. After oviposition by the parasite the larva was placed in a plastic jar with *Coccinia* leaves. This procedure was repeated until the female refused to show any interest in the larva offered to it. Larvae were exposed to 10 females daily and those exposed to the same female on a particular day were placed together in plastic jar and the date recorded on it.

To determine the incubation period the larvae were dissected at periodic intervals of 6 h and the development of the eggs was followed until hatching was observed. When the egg was fully developed, observation period was narrowed to 3 h. Parasite eggs were removed from the host larvae at periodic intervals and measured with a calibrated ocular micrometer using a compound microscope.

To establish the larval period the parasitized host larvae were dissected at periodic intervals of 12 h using a Carl Zeiss Zoom Citoval-2-stereo-microscope. The parasite larvae were measured with a calibrated ocular micrometer and drawn using a camera lucida attached to a Carl Zeiss Laboval 4 compound microscope. Magnification used for drawing the larval stages ranged from X32 to X640. This arrangement for measuring and drawing of the various stages was made use for all the biology studies carried out in the present investigation. The drawings formed only an outline of the various immature stages and do not represent the accurate morphological features as indicated in the text. The constant movement of the live specimens rendered the drawing process very difficult.

The larval stages were determined by studying the shape and size of mandibles at different stages. To determine the shape and size of the larval mandibles the larvae were boiled in a 10% KOH solution for 45 s for clearing but not completely removing the host tissues. After being washed in distilled water they were mounted in Hoyer's medium on microscope slides. The head, capsules and mandibles were also measured.

The cocoons were dissected using a microscissor at 12 h intervals to record the prepupal and pupal periods. Both stages were measured.

In order to determine the fecundity, 25 host larvae were exposed for 24 h to a single mated female in a plastic jar. The larvae were reared on *Coccinia* leaves. Three replications were maintained. After 24 h the host larvae were dissected and the number of eggs present in each larva was counted. This procedure was repeated until all females died. From this data the total number of eggs laid by each female was estimated.

Field collected cocoons were observed for emergence of adults which were sexed to assess the sex-ratio.

To study the host stage preference for oviposition, larvae of *D. indica* of the following age groups were selected: 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9 and 9-10-day old larvae. Twenty-five larvae belonging to each age group were released on *Coccinia* leaves and placed in a jar with wire mesh fitted lid. A single mated female was released into each jar. Three replications were maintained for each stage exposed. The number of cocoons formed for each stage was recorded at the completion of the larval development of the parasite.

The pre-oviposition and oviposition periods were determined by exposing *D. indica* larvae to the parasite females at regular intervals of 24 h beginning with the day of emergence. The larvae were dissected after each exposure and those having parasite eggs were determined. Host larvae were exposed continuously until the female died. Five replications were maintained for this study.

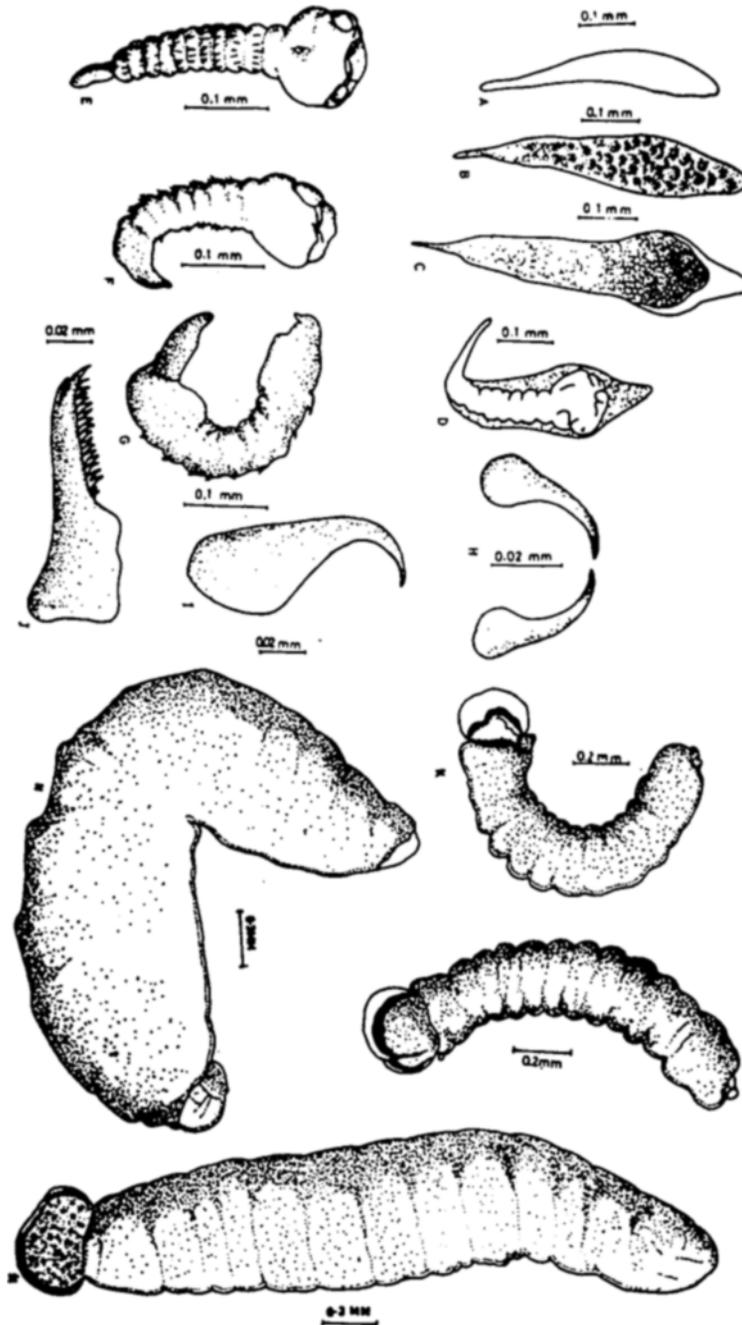
The adult specimens were initially preserved in 70% alcohol. These specimens were then mounted on slides using the following methodology. The specimen was treated in 10% KOH for 10-12 h for clearing the tissues and then transferred to glacial acetic acid to dissolve the organic matter, if any. It was passed through carboxylol. The processed specimens were dissected in clove oil and the different parts were mounted on slides in DPX mounting medium. Slide mounts of complete specimen were also prepared. The various parts of the adult were measured.

### 3. Results and discussion

#### 3.1 Immature stages

3.1a *Egg*: The egg immediately after deposition is elongate with a long thin pedicel. The chorion is thin, transparent and devoid of any sculpturing. The egg develops rapidly after deposition and changes greatly in size and shape. When freshly laid it measures 0.043 mm long from tip to the end of the pedicel and 0.056 mm wide. After 6 h the length increases to 0.48 mm and in width to 0.1 mm. At the end of 12 h after deposition, the egg measures 0.532 mm long and 0.102 mm wide at the broad end. After 20 h, the fully developed embryo is clearly seen inside the chorion. The full grown egg just before hatching measures 0.62 mm long and 0.12 mm wide at the broad end (figure 1A-D). Thus within 24 h after oviposition there is an increase of 1.45 times in size compared to the freshly laid egg. The width of the egg also increases to 2.4 times the original size. This increase is more pronounced in the transverse direction.

The general shape and size of the egg of *A. machaeralis* is similar to several other species of solitary *Apanteles* studied. The rapid increase in the size of the egg after oviposition has been reported for other species too. In *A. solitarius* Ratzeburg an increase of 1.96 times in length and 1.5 times in width was reported at the time of hatching (Parker 1935). A 4-fold increase in width and 1.25 times increase in length has been reported for *A. angaleti* Muesebeck (Narayanan *et al* 1956). Allen (1958) recorded an increase of 1.58 times in length and 2.88 times in width for *A. medicaginis* Muesebeck. Cardona and Oatman (1971) reported an increase of 1.37 times in length and 2.4 times in width for *A. dignus* Muesebeck. In certain Euphorinae and Meteorinae the increase is even greater (Balduf 1926).



**Figure 1.** Developmental stages of *A. machaeralis*. A-D, Egg; E-G, first instar larva; H, first instar mandible; I, second instar mandible; J, third instar mandible; K, second instar larva; L-N, third instar larva.

3.1b *Larva:* (i) *First instar larva:* The measurements made relating to various developmental stages of *A. machaeralis* are shown in table 1. The freshly hatched larva has a broad head about twice the width of the body. The head measures

**Table 1.** Mean size and duration of immature *A. machaeralis* reared at  $29.25 \pm 1.82^\circ\text{C}$ .

Stage	n	Length (mm)	Width (mm)	Duration (day)
		$\bar{X} \pm \text{SEM}$	$\bar{X} \pm \text{SEM}$	
Egg	25	$0.62 \pm 0.14$	$0.12 \pm 0.03$	18–24 h
1st instar	20	$0.80 \pm 0.12$	$0.08 \pm 0.01$	3–4
2nd instar	12	$1.31 \pm 0.19$	$0.29 \pm 0.08$	1–2
3rd instar	12	$3.11 \pm 1.11$	$0.82 \pm 0.10$	2–3
Prepupa	12	$2.88 \pm 1.21$	$0.78 \pm 0.14$	1–2
Pupa	20	$2.78 \pm 1.08$	$0.89 \pm 0.26$	4–7

0.12 mm in width while the width of the body is 0.06 mm. At the time of hatching it measures 0.33 mm in length and 0.06 mm wide. The larva at this stage is distinctly segmented (figure 1E–G). The body is made up of 3 thoracic and 7 abdominal segments. These segments have a single row of transverse sharp translucent dorsal spines. The caudal horn is prominent in the freshly hatched larva but it shrinks gradually as the larva develops while the anal vesicle increases in size. The caudal horn measures 0.08 mm on the day of hatching decreases to 0.04 mm on the third day and is completely absent at the end of first instar. During this period, the anal vesicle enlarges from 0.11 mm in width on the second day to 0.48 mm at the end of first instar. Even the dorsal spines which are prominent at the beginning slowly reduce in size and disappear as the larva develops.

The characteristic feature of this instar is the sickle shaped mandibles which are well fitted for tearing (figure 1H). The mean length of the mandibles measures 0.46 mm from the tip of the spine to the point of the basal process and 0.019 mm in width at the widest point. The mandibles are chitinized throughout, but more heavily so at the tips and form a good character for distinguishing this instar from the subsequent instars. The first instar larva is very active and its mandibles are constantly in motion. It is probably through the use of these mandibles that any other species or members of the same species encountered within the host are destroyed.

(ii) *Second instar larva:* The second instar larva differs considerably from the first instar larva although the change is gradual. In fact, the transition is very difficult to detect. The body tapers from the large anal vesicle toward the head (figure 1K). The head has no apparent sclerotization and the mouth parts are not visible. The mandibles, 0.09 mm in length are colourless and difficult to detect. The base of the mandible is very broad and tapers to the blade (figure 1I). The body is distinctly segmented with 10 abdominal and 3 thoracic segments. Abdominal spines are lacking. The body is opaque and creamy white. The anal vesicle, 0.259 mm in width, is a greatly enlarged and prominent structure while the caudal horn is absent.

(iii) *Third instar larva:* The change into the third instar is again difficult to differentiate from the second instar because of its similarity in appearance. The shape of the mandibles is a characteristic feature of this instar. The mandibles are strongly chitinized with series of 17 saw-like teeth on the inner edge (figure 1J). These mandibles are used by the parasite larva to cut its way out of the host.

The third instar larva is creamy white and opaque. The body tapers anteriorly and is robust. The head capsule is well sclerotized with mean width of 0.559 mm (range 0.481–0.612). The body consists of the head and 13 well developed segments.

The tracheal system is very well developed with 8 pairs of open spiracles, one pair in the second thoracic and 7 in the first 7 abdominal segments. The early third instar larva has a well developed anal vesicle which measures on average 0.581 mm in width (figure 1L). This gradually decreases in size as development proceeds and is absent by the time of emergence from the host (figure 1N). The head and the abdominal segments have a ring of minute setae. The general descriptions of the 3 larval instars of *A. machaeralis* are quite similar to those reported for other species of solitary *Apanteles*: *A. melanoscelus* (Crossman 1922), *A. solitarius* (Parker 1935), *A. angaleti* (Narayanan *et al* 1956), *A. medicaginis* (Allen 1958) and *A. dignus* (Cardona and Oatman 1971). In all cases the shape of the mandibles is the characteristic feature on which the identification of the instars was based. Further, the number and size of the saw-like teeth on the inner edge of the mandible of the third instar are characteristic for a particular species. Short (1953) found the form of the mandible was useful in classifying species of *Apanteles* into 4 main groups. Under this classification the mandible of *A. machaeralis* would be grouped under type I category as it possess a line of prominent teeth on its inner edge. Short (1953) also reported that the number of teeth on the mandible varied greatly in different species of *Apanteles*. It ranged 14 in *A. carbonarius* (Wesm.) to 25 for *A. ater* (Ratz.)

There have been considerable theories put forth to explain the function of the anal vesicle. Muesebeck (1918) and Tothill (1922) reported it to be chiefly respiratory in function and Tower (1915) was of the opinion that the anal vesicle functions as an excretory organ. According to Thorpe (1932) it is responsible for about 75% of the total oxygen uptake. Gilmore (1938) considered it to be an excretory receptacle but Narayanan *et al* (1956) indicated that it has respiratory function.

**3.1c Prepupa:** The prepupa, opaque, creamy white and found inside the cocoon can be differentiated from the third instar larva by the presence of a snout-like projection at the anterior end and by a constriction in the middle of the body.

**3.1d Pupa:** The pupa at first light cream, darkens as it matures. Before emergence it is nearly black. The pupa, of the exarate type, is slightly shorter and wider than the prepupa. The pupal appendages are loosely pressed to the body.

**3.1e Cocoon:** The cocoon, white, compact, cylindrical and rounded at both ends requires 90–120 min for construction. It is slightly flattened on its ventral surface at the point of attachment and convex laterally and dorsally. The mean length of the cocoon is 3.78 mm (range 3.66–3.89 mm) and 1.34 mm in width (range 1.28–1.42 mm) ( $n=10$ ). Gerould (1921) was of the opinion that the white colour of cocoons of *Apanteles flaviconchae* was due to the pigments available in the host larvae. Crossman (1922) reported that the colour of cocoons of *A. melanoscelus* ranges from pale yellow to light sulphur yellow. In *A. medicaginis* the colour of cocoon ranges from yellow to very pale yellow or white (Allen 1958). In most species the colour of the cocoon is white.

## 3.2 Life history

**3.2a Incubation period:** The egg is usually inserted into the anterior portion of

the body generally just behind the head capsule. Normally only one egg is laid in each host but if the same host is re-exposed, two to three eggs may be laid. The incubation period ranges from 18–24 h. The first instar larva within the egg curves its body and with the help of its mandibles bursts out of the egg.

**3.2b Larval period:** The first instar larva after hatching floats slowly to the posterior section of the body. If 3 eggs are laid, then up to one day after hatching 3 larvae are present. After that one larva survives, the other larvae are probably killed by the surviving larvae. The time required for development of the first instar ranges from 3–4 days. The second instar larva is located ventrad in the middle of the host body. This instar present on the 4th or 5th day after hatching lasts only 1–2 days. The third instar larva completes the first part of its development inside the host and after consuming the host contents emerges from the posterior, ventral position by cutting its way out with its mandible, and spins its cocoon close to the host remains. Only the head capsule and larval integument of the host remain. At the time of emergence of the full grown larva the host larva is dead. The third instar larva appears on the 6th day after oviposition and lasts for 2–3 days. The full-grown parasite larva emerges from the third instar host larva. This was determined from the width of the head capsule of the host exuviae measured after the emergence of the parasite from the host larva.

**3.2c Pupal period:** The third instar larva after spinning the cocoon discharges the meconium and enters the prepupal stage which lasts 1–2 days. Following this the pupa is formed and the pupal stage commences from the 10th day after oviposition and lasts for 4–7 days (average 4.15 days).

The duration of the life history of other species of solitary *Apanteles* varies depending on the temperature. In *A. melanoscelus* the incubation period ranges from 48–72 h and the larval stage 2–3 for the first and second instars and 1–3 days for third instars. The pupal period ranges from 5–9 days and the total life cycle from egg to adult 12–21 days (Crossman 1922). Parker (1935) reported the incubation period of egg as 69 h. Allen (1958) studied the biology of *A. medicaginis* and reported that at 26.7°C the incubation period took 24–30 h, the first instar 2.5–4.5 days, second instar 4.0–4.5 days and the pupal stage 4.5 days. For *A. angaleti* the period for egg, first and second instars at 30°C averaged 26.3 h, 15.8–20 days and 8.5–13.2 days, respectively (Narayanan *et al* 1956). The duration recorded for *A. angaleti* was unusually longer than for the other species of *Apanteles*. The probable reason is that *A. angaleti* was reared on an alternative host (*Corcyra cephalonica*) and not its natural host. Under such circumstances, there is a possibility that the life cycle of the parasite was influenced by the host life cycle which required about 40 days for larval development. Probably in this host the larval development of the parasite was also extended. In *A. dignus* the life cycle studied at 26.6° ± 1°C ranged from 16–19 days with the average being 18 days. The duration of the developmental stages was egg 1 day, 1st instar 4 days, 2nd instar 1 day, 3rd instar 1–2 days, prepupa 1 day and pupa 8–10 days (Cardona and Oatman 1971). In the present investigation, the shortest life cycle from egg to adult was 12 days and the longest 19 days. However, the mean duration of life cycle studied for 73 individuals during May–July 1986 in the insectary at 29.25°C and 59–66% RH, averaged 12.63 days from oviposition to adult emergence.

### 3.3 Adult

3.3a *Description*: Female: 3.03 mm long. Body black in colour. Head 0.55 mm long and 0.61 mm wide; antennae 2.99 mm long, 19 segmented; eyes black, 0.27 mm long and 0.13 mm wide. Thorax 1.03 mm long and 0.80 mm wide. Wings hyaline and transparent, veins pale in colour, fore wings 2.35 mm long and 0.55 mm wide; hind wing 1.18 mm long and 0.23 mm wide. Legs black to yellowish brown; foreleg femur black at the base, the rest of the femur, tibia and tarsomeres yellowish brown; middle leg femur more or less completely black, tibia and tarsomeres yellowish brown as in foreleg; hind leg femur completely black, tibia light yellowish brown to black; tarsomeres yellowish brown. Abdomen black, 1.37 mm long; ovipositor 1.32 mm long and black in colour.

Male: Identical to female except slightly smaller in size, body length 2.49 mm. Antennae 3.03 mm long and longer than that of female.

3.3b *Mating*: Mating occurs soon after emergence. The males actively follow the females with vibrating wings. If the female is receptive it stops and spreads its wings and the male approaches the female from behind. The male, after mounting, curves its abdomen down and inserts its aedeagus into the genital aperture of the female. Copulation lasts 10–20 s. After mating, the female move away and begins to groom itself while the male moves about wildly in search of other females. The position taken during mating is common to other species of *Apanteles* and the act is completed in less than a minute.

3.3c *Preoviposition period*: The experiment conducted to determine the preoviposition period of *A. machaeralis* females revealed that egg laying commenced on the day of emergence irrespective of mating. The adults of *A. melanoscelus* (Crossman 1922), *A. solitarius* (Parker 1935) and *A. dignus* (Cardona and Oatman 1971) were reported to be ready for oviposition within a few hours after they issue from the cocoons.

3.3d *Oviposition behaviour*: Oviposition takes about a second and the female inserts its egg into the thorax close to the head. The host larva thrashes out violently when the female thrusts its ovipositor into the body. If the same larva is exposed again to the female another egg is laid and in some cases up to 3 eggs were deposited in one larva but only one survives after hatching. Oviposition is similar to other species of *Apanteles* and is completed very rapidly. It was reported to take one second for *A. melanoscelus* (Crossman 1922), 3–12 s in *A. angaleti* (Narayanan *et al* 1956), 1 s in *A. medicaginis* (Allen 1958) and 2–3 s in *A. dignus* (Cardona and Oatman 1971). The site of oviposition varied in some species of *Apanteles*. While *A. melanoscelus* favours the posterior half of the larva (Crossman 1922) the female of *A. solitarius* oviposits in any part of the host larva (Parker 1935). In the solitary species of *Apanteles* only one egg is generally laid with each thrust. In *A. medicaginis* the number of eggs ranges from 1–6 per host (Allen 1958) but only one larva completes development in the solitary forms of *Apanteles* sp.

3.3e *Oviposition period*: The average oviposition period of *A. machaeralis* was

8-60 days (range 3-11 days). In most cases oviposition continued as long as the female was mobile. However, the rate of egg laying gradually reduced with age. In *A. dignus* the average oviposition period was reported to be 6.7 days which was almost the same as average female longevity (Cardona and Oatman 1971).

3.3f *Host stage preference for oviposition:* Maximum parasitism occurs in 1-2-day old larvae (72%) and 2-3-day old larvae (76%). Larvae up to 5 days old were also attacked but to a lesser extent (<10%). It was determined from the present study, that first instar larvae of *D. indica* are preferred for oviposition by *A. machaeralis*. Certain variations in the host age preference for oviposition by other solitary forms of *Apanteles* spp. have been recorded in literature. Crossman (1922) reported that third instar larvae are preferred by *A. melanoscelus*. In *A. medicaginis* the first instar larva is generally selected for oviposition (Allen 1958) and in *A. dignus* 2-3-day old larvae are the most suitable ages for parasitization (Cardona and Oatman 1971).

3.3g *Sex ratio:* Parasite cocoons obtained from field collected larvae were held until adults emerged. The sex ratio of adults from 140 cocoons was 1.22:1 in favour of females. *A. machaeralis* is an arrhenotokous species. Unfertilised females deposit eggs that develop into males and the progeny of mated females contains both sexes. This type of reproduction which is common in most *Apanteles* is described in detail by Allen and Smith (1958). In *A. angaleti* the male: female sex ratio was reported to be 1:2 (Narayanan *et al* 1956), while for *A. dignus* the sex-ratio was recorded to be 1.47:1 in favour of males (Cardona and Oatman 1971).

3.3h *Fecundity:* The fecundity of mated females of *A. machaeralis* ranges from 80-126 eggs per female with a mean of 118.56 eggs under insectary conditions. In the field the oviposition ratio may be higher. There was considerable variation in the fecundity of other solitary *Apanteles* spp. Crossman (1922) reported that females of *A. melanoscelus* were capable of laying about 1000 eggs under natural conditions. However in the laboratory the fecundity was estimated to be 535 eggs per female. The reproductive capacity of *A. solitarius* ranged from 311-516 with an average of 402 (Parker 1935). In *A. angaleti* the average fecundity was reported to range from 61.83-86.33 per female (Narayanan *et al* 1956). Cardona and Oatman (1971) reported that in *A. dignus* the total production of progeny by mated females ranged from 126-182.

3.3i *Longevity:* In the absence of food the mean longevity of females was 1.63 days and of males 1.48 days. Both sexes lived longer when provided with 20% honey solution with the mean longevity being 11.68 days for females and 8.31 days for males. *A. melanoscelus* was reported to live for 30-32 days (Crossman 1922) with no difference in the longevity of the two sexes. Parker (1933) reported that *A. solitarius* lived for an average of 3-4 weeks and the females lived longer than males. *A. angaleti* was reported to live for 2-8 days (Narayanan *et al* 1956). Cardona and Oatman (1971) observed that in *A. dignus*, males lived longer (13.6 days) than females (10.2 days) and without food or water the mean longevity was 1.2 days for males and 1.1 days for females.

### 3.4 Hyperparasite of *A. machaeralis*

During this study only one hyperparasite emerged from the cocoon of *A. machaeralis*; it was subsequently identified as *Elasmus hyblaeae* Ferriere. This is the first record of *E. hyblaeae* as a hyperparasite of *D. indica* through *A. machaeralis*.

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