

## Morphological Studies of the Predatory Ladybird Beetle *Stethorus vagans* (Blackburn) (Coleoptera: Coccinellidae)

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**Abstract** Morphological features of the ladybird beetle *Stethorus vagans* (Blackburn) were studied at  $25 \pm 2^\circ\text{C}$  with a photoperiod of 16L: 8D. All stages of *S. vagans* were examined under a stereo-zoom microscope. Newly laid eggs were translucent white, turning pale yellow after 4-5 hours. The mean egg dimension was  $0.36 \times 0.19$  mm. Eggs laid by unmated females did not hatch or show any signs of development. Newly emerged larvae were white in color, but soon became pale creamy-white. There were four larval instars, which were differentiated from each other by the presence of exuviae and differences in head capsule size. The pre-pupa, not a distinct stage in the life cycle but a quiescent period at the end of the 4th larval instar, lasted for several hours. Pupae were oval, flattened and black-brown with fine hair like setae on their dorsal sides with a mean length and width of  $1.06 \times 0.74$  mm. The adults were oval, convex and black with small yellow setae on their dorsal side.

**Key words** morphology, *Stethorus vagans*, measurements

### Introduction

A less familiar group of the ladybird is the tiny black beetle that belongs to the genus *Stethorus*. The importance of *Stethorus* species as a biological control agent of spider mites is recognized worldwide (Colburn and Asquith, 1970; Gordon and Anderson, 1979; Parrella *et al.*, 1980; Dhoori 1981; Rustamova 1981; Gikorashvili 1983; Nageshchandra and ChannaBasavanna, 1983; Chazeau, 1985; Houck, 1991; Congdon *et al.*, 1993; and Majerus, 1994). Successful manipulation (predator and/or prey) of any organism depends on adequate

morphological, biological and ecological studies of the organism(s). In this regard much documentation has been done on the morphology, biology and ecology of *Stethorus* species, but very little study has been done on the morphological features of *Stethorus vagans*, i.e., adult and last larval instar (Britton and Lee, 1972; Houston, 1980). Two-spotted mite, *Tetranychus urticae* Koch, which is spread world wide and is a major pest of wide range of crops such as, fruits, vegetables, flowers and ornamental plants (Rabbinge, 1985; Hills, 1987). A number of predators are reared commercially for its control. *S. vagans* is also one of the best predators of *T. urticae*. From the viewpoint of its commercial production this study was, therefore, initiated to understand the morphological features of this potential predator, use their features to differentiate it from their close relatives.

### Materials and Methods

#### Morphological Studies

Morphological studies were carried out on the first generation ( $F_1$ ) of *S. vagans* adults caught from the wild, which were randomly selected and paired over 2.5cm diameter leaf disc infested with all stages of *T. urticae*. These leaf discs were maintained on water-saturated foam in 5 cm modified sealable petri dishes. These dishes were allocated to  $25 \pm 2^\circ\text{C}$  constant temperature at 16L: 8D hours photoperiod in the laboratory. Eggs laid by pairs were randomly selected 12 hourly and individually examined under a stereo-zoom microscope ( $\times 400$ )\* to note length and width as well as other morphological characteristics. These eggs were then exposed singly on a 4.7 cm diameter dry filter paper in similar petri dish at the same temperature. The first as well as the subsequent instar larvae were also observed under the

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\* Stereo-zoom microscope fitted with an ocular micrometer (compound microscope, BM series Olympus, Olympus Optical Co. Ltd., Japan).

microscope to record their morphological characteristics. The presence of exuviae as well as changes in head capsule size was used to differentiate between larval instars. These instars were fed daily with all stages of *T. urticae* and were retained in the same petri dish until adult emergence. The detail description of pre-pupae, pupae and adult were also noted at 12-hour intervals. Measurements of the head capsule as well as dimensions of all life stages of *S. vagans* were made by the aforementioned microscope.

### Statistical analysis

The mean length and width of all stages of *S. vagans* were calculated by using Excel 5.0 (Microsoft Office, SYBEX Inc., 2021 Challenger Drive, Alarmed, CA 94501, USA). The head capsule measurements of all larval instars were analyzed by using analysis of variance (ANOVA) of the data using the statistical software package CoStat (CoHort Software P.O.Box 19272, Minneapolis, MN 55419, USA).

## Results

### Description of *S. vagans* stages

**Egg** Newly laid eggs were translucent white, turning pale yellow after 4-5 hours. They were smooth, elongate and rounded at both ends (Fig. 1). The mean size of the eggs was 0.36 x 0.19 mm (Table 1). They were laid horizontally on the lower surface of the leaves along the midrib and lateral veins in an exposed position. They were commonly deposited singly, but occasionally in pairs among large mite colonies.

Fertilized eggs appeared granular in the first two days, while two red eyespots developed one-day before hatching. The eggs became transparent on the day of hatching and the developing embryo could clearly be seen through the chorion of the egg. Unfertilized eggs were slightly smaller and did not show any color changes and they gradually shriveled, and died.

**Larva** Larvae molted three times with instars closely resembling each other. The presence of exuviae and head capsule size was used to differentiate between instars (Table 1). Prior to molting, larvae stopped moving and fixed themselves by the 10th abdominal segment to a surface and remained inactive for 3-5 hours. Ecdysis began at the head and continued along the back throughout the abdomen. The shed exuviae remained fixed with the substrate and the larva emerged from the exoskeleton of the former instar. Four larval instars were recorded (Figs. 2~5). The newly emerged larvae were white, but soon become pale creamy-white. The pink colored contents of the alimentary canal (turning dark after feeding) were visible through the larval body. All instars possessed numerous dark brown setae over the tergites and pleurites with dark brown pigmentation at the bases of the dorsal setae. The measurements of body length, width and head capsule are presented in Table 1. The analysis of variance showed that the mean body length, width and head width of each instar are different significantly ( $p \leq 0.05$ ).

**Pre-pupa** When the final instar stopped feeding, it attached itself by the anal cremaster to the substrate. The larva shrank and gradually hunched up its back. This marked the prepupal stage, which was cream in color. This stage was not a distinct stage in the life cycle, but a quiescent period at the end of 4th instar, lasting for only a few hours (Fig. 6).

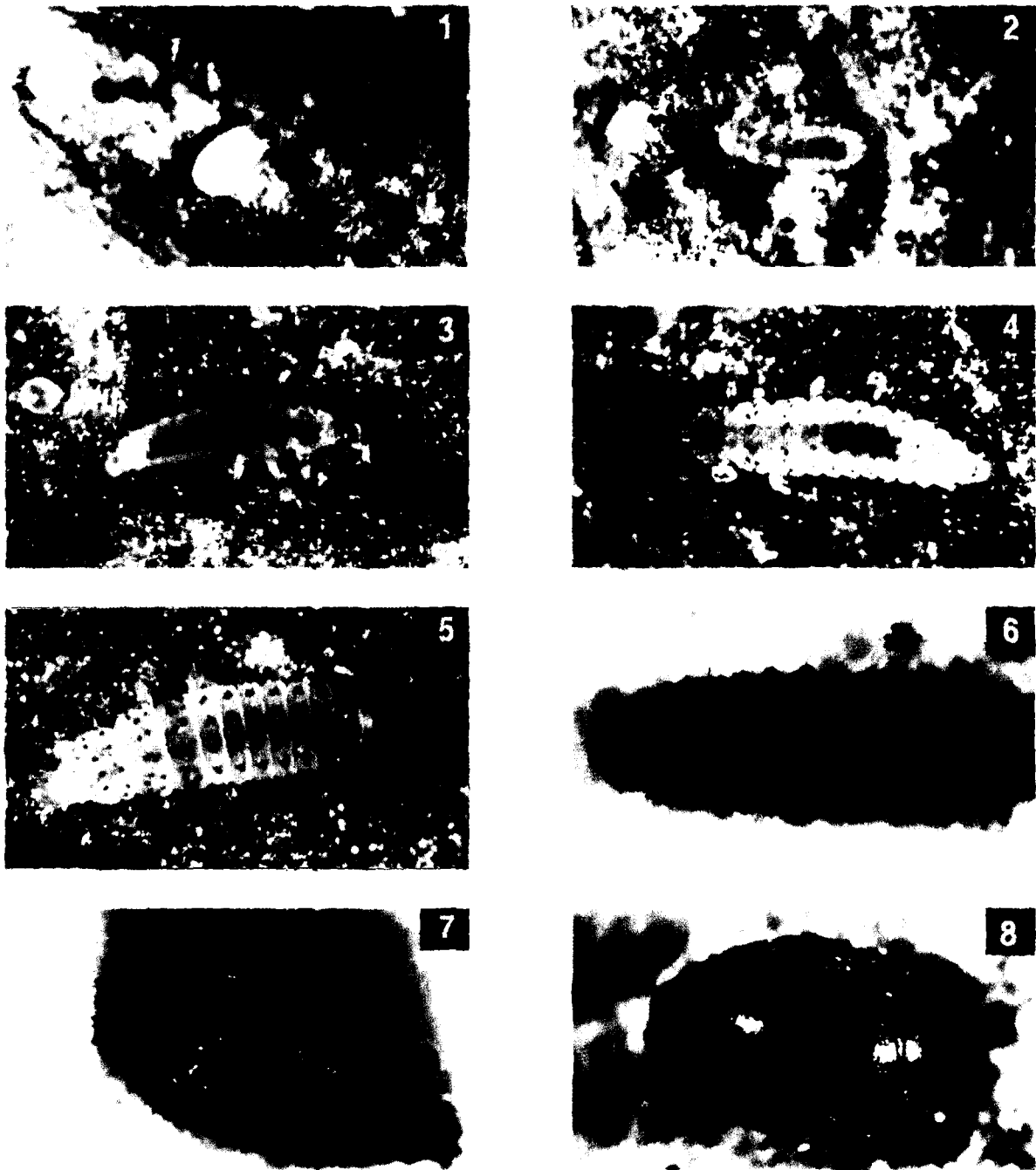
**Pupa** The pupa was oval, flattened, and subtruncate anteriorly and tapered posteriorly. It was creamy in

**Table 1.** Measurements (mm) of all stages of *S. vagans*<sup>1</sup>

Stages	No. of individual	Length		Width		Head capsule		
		Range	Mean $\pm$ S.E	Range	Mean $\pm$ S.E	Range	Mean	
Egg	20	0.33-0.40	0.36 $\pm$ 0.01	0.18-0.20	0.19 $\pm$ 0.002	-	-	
1st instar	20	0.50-0.75	0.64 $\pm$ 0.02	0.10-0.13	0.12 $\pm$ 0.002	0.10-0.11	0.106a	
2nd instar	20	0.85-0.98	0.91 $\pm$ 0.01	0.15-0.18	0.16 $\pm$ 0.002	0.13-0.14	0.131b	
3rd instar	20	1.08-1.25	1.14 $\pm$ 0.04	0.20-0.28	0.22 $\pm$ 0.005	0.15-0.16	0.156c	
4th instar	20	1.5-2.08	1.84 $\pm$ 0.02	0.38-0.45	0.41 $\pm$ 0.005	0.18-0.19	0.181d	
Pupa	20	1.0-1.25	1.06 $\pm$ 0.01	0.63-0.80	0.74 $\pm$ 0.001	-	-	
Adult	Male	20	0.98-1.08	1.04 $\pm$ 0.01	0.70-0.80	0.75 $\pm$ 0.007	-	-
	Female	20	1.10-1.20	1.15 $\pm$ 0.01	0.78-0.80	0.79 $\pm$ 0.002	-	-

Means in same column followed by same letters are not significantly different at  $p \leq 0.05$ .

<sup>1</sup>Figures represent range and mean of length and width of all stages, and head capsule of larval stages.



Figs. 1-8. Ladybird beetle, *Stethorus vagans*: 1, Egg; 2, First instar larva; 3, Second instar larva; 4, Third instar larva; 5, Fourth instar larva; 6, Pre-pupa; 7, Pupa; 8, Adult. (All photographs are enlarged x 400).

color for the first few hours then became uniform black-brown (Fig. 7). The body was covered with fine hair-like setae and the abdominal segments, wing pads and legs of the adult were very prominent. In the field, pupae were found in different locations but most frequently on the underside of the leaves. In the laboratory they were found on leaf discs as well as on the inner surface of petri dishes. The mean length and width at

widest point of the pupa was 1.06 x 0.74 mm, respectively (Table 1).

**Adult** Adults emerged from the pupal skin, by splitting it transversely and longitudinally on the ecdysial sutures in the thoracic region. The beetles took several minutes to emerge completely. Once free they remained for approximately an hour beside their pupal cases. The newly emerged beetles were light yellow in color for

the first few minutes then slowly changed through orange to black (Fig. 8). The pronotum, however, was completely black at eclosion. Adults were convex and oval, being wide in the middle and narrow at both ends. Males were smaller than females and were differentiated from the females by a cleavage on their 10th abdominal sternite. The mean length and width of males and females were 1.04 x 0.75 mm and 1.15 x 0.79 mm, respectively (Table 1).

## Discussion

### Description of *S. vagans* stages

**Egg** *S. vagans* appears to have a similar egg morphology and development as that reported for a number of other *Stethorus* species, such as *S. punctillum* (Putman, 1955), *S. gilvifrons* (Mathur, 1969; Ahmed and Ahmed, 1989) *S. keralicus* (Daniel, 1976) *S. pauperculus* (Puttaswamy and ChannaBasavanna, 1977) and *S. nigripes* (Richardson, 1977). However the mean egg size of *S. vagans* is relatively small (0.36 x 0.19 mm), compared with *S. punctum* (0.43 x 0.33 mm) (Colburn and Asquith, 1971), *S. keralicus* (0.37 x 0.32 mm) (Daniel, 1976) and *S. pauperculus* (0.38 x 0.20 mm) (Puttaswamy and ChannaBasavanna, 1977). No measurements have been reported for *S. nigripes* (Richardson, 1977).

**Larva** We recorded four larval instars for *S. vagans* the same as that recorded for most other ladybirds including *Stethorus* spp., although five instars have been recorded for the *Chilocorus bipustulatus* (Yinon, 1969). The larvae were similar to that described by Britton and Lee (1972) and Houston (1980). The larval instars were differentiated by body length and width as well as head capsule width (Table 1). *S. vagans* (i.e. 1st, 2nd, 3rd and 4th instar larvae were 0.64 x 0.12, 0.91 x 0.16, 1.14 x 0.22 and 1.84 x 0.41 mm, respectively) is relatively smaller than *S. punctum*, i.e. 1st larval instar (1.03 x 1.57 mm) and 4th larval instar (2.5 x 2.2 mm) (Colburn and Asquith, 1971), but similar to *S. pauperculus*, i.e. 0.61 x 0.20, 1.0 x 0.32, 1.2 x 0.46 and 1.8 x 0.68 mm, for 1st, 2nd, 3rd, and 4th larval instars, respectively (Puttaswamy and ChannaBasavanna, 1977).

We differentiated larval instars from one another by the presence of shed exoskeletons as well as by head capsule size. Head capsule measurements are the most reliable method of differentiating between larval instars (Dyar, 1890). However, it appears that apart from Daniel (1976) who reported head capsule measurements for 1st instar of *S. keralicus*, no such measurement for other *Stethorus* species has been under

taken.

**Pre-pupa** The duration of the prepupal stage for *S. vagans* we recorded at 25°C varied from 8 to 13 hours, which is similar to that reported for most other species of *Stethorus*. For example, Daniel (1976) recorded 10-15 h intervals for *S. keralicus* at 26-34°C, while Puttaswamy and ChannaBasavanna (1977) reported 8 hours for *S. pauperculus* at 24-26°C. However it is much shorter than that reported for *S. nigripes* (24 hours) at 25-35°C (Richardson, 1977).

**Pupa** The pupal appearance was similar to that reported by Britton and Lee (1972) and Houston (1980). Its duration was shorter than that of *S. keralicus* (3.5~4.0 days at 26~30°C) (Daniel, 1976), *S. pauperculus* (3.8~4.0 days at 25.4~26°C) (Puttaswamy and ChannaBasavanna, 1977), but longer than *S. nigripes* (3.16 days at 25°C) (Richardson, 1977) and *S. gilvifrons* (2-5.6.0 days at 20-35°C) (Ahmed and Ahmed, 1989) and *S. loi* (3.3 days at 23.8°C) (Shih et al., 1991). An extended prepupal stage was recorded for *S. nigripes* (Richardson, 1977).

The length and width of pupae of *S. vagans* was 1.06 and 0.74 mm, which are larger than *S. keralicus* (1.03 and 0.7 mm, respectively) (Daniel, 1977), but smaller than *S. punctum* (1.36 and 0.97 mm) (Colburn and Asquith 1971) and *S. pauperculus* (1.8 and 1.1 mm).

**Adult** The color of newly emerged adult of *S. vagans* was light yellow, which turned to a uniform black-brown. Similar results were obtained by Majerus (1994) for a number of ladybirds other than *Stethorus*, for *S. pauperculus* by Puttaswamy and ChannaBasavanna (1977) and for *S. nigripes* by Richardson (1977). The males and females were easily distinguished on the basis of characteristics described by Britton and Lee (1972) and Houston (1980). Britton and Lee (1972) also reported a mean length only for "adult" *S. vagans*, of 1.12mm, while our dimensions were 1.04 and 0.75mm for males and 1.15 and 0.79 mm for females, respectively. Like the immature stages, the adults of *S. vagans* are smaller than most other *Stethorus* spp., such as *S. pauperculus* (1.47mm length and 1.0 mm width) (Puttaswamy and ChannaBasavanna, 1977) and *S. punctillum* (1.5 and 1.01 mm, respectively) (Gordon and Chapin, 1983).

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