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SEM studies on immature stages of *Aphaenogaster beesoni* Donisthorpe, 1933 (Hymenoptera: Formicidae)

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Abstract

Larval instars of *Aphaenogaster beesoni* Donisthorpe, 1933 are herein described in detail by scanning electron microscopy. Based on the body profile, body size, types of body hairs and head capsule size a total of five larval stages are recognised. All five instars show clear variations in their body shape and mouth parts. Body of the first instar is Aphaenogastroid, while that of the second, third and fourth is Pogonomyrmecoid, and in fifth it again becomes Aphaenogastroid type.

Keywords: SEM, immature stages, *Aphaenogaster,* India.

Introduction

The ant genus *Aphaenogaster* Mayr includes 180 extant species, 45 subspecies and 19 fossil species; these ants are widely distributed in all geographical regions of the world except Ethiopian region (Bolton *et al.,* 2007; Bolton, 2013). The genus includes one of the most primitive and generalized ant species with fossil records in the Baltic amber. Currently, it is represented by 176 extant species from the World of which 14 belong to Indian region (Bharti, 2011).

The significance of larval descriptions to ant systematics has been discussed in a number of sources (Wheeler and Wheeler 1976, 1986, 1988; Fox *et al.,* 2007, 2010, 2012; Solis *et al.,* 2007, 2010a, b, 2011; Jesus *et al.,* 2010; Nondillo *et al.,* 2010; Bharti and Gill, 2011 and Bharti and Kaur, 2011). As rightly put by Wheeler and Wheeler (1976), "Modern taxonomy is complete only when variety of data is pushed as far as possible towards the limit of practicability. The object of classification should be holomorph i.e. studying all the characteristics of an individual throughout its life". Apart from their importance in the study of general biology (Peeters and Holldobler, 1992), the larval characters of ants have also been used for phylogenetic and behavioural studies (Petralia and Vinson, 1979a, b; Schultz and Meier, 1995; Masuko 2003, 2008; Pitts *et al.,* 2005 and Signorotti *et al.,* 2013).

Aphaenogaster beesoni Donisthorpe, 1933, a high altitude species is widely distributed in Northwest Himalaya. It nests under stones and is found mainly in forested areas with scarce undergrowth and fairly moist surfaces. Intensive ecological and taxonomic studies have been made on *Aphaenogaster beesoni* but there is a void in our knowledge, as far as its larval morphology is concerned.

Here we provide reliable distinguishing larval characters, to fill this gap in our understanding of *Aphaenogaster* immatures. The present study marks the first ever comprehensive study on immatures of *Aphaenogaster beesoni.* Based on ultrastructural images, minute

morphological details of different larval forms have been studied, to generate baseline data and to aid in future interspecific diagnosis.

Materials and Methods

To study the immature stages of *Aphaenogaster beesoni,* larval forms were collected from 5 different colonies located at Solang valley (2560m), Himachal Pradesh, India. The larvae were fixed in the Dietrich's solution for 24-48 hrs, and then preserved in 80% alcohol. The larvae were separated into five instars on the basis of body profile, body size, types of body hairs and head capsule size.

After separation of larval forms, all instars (n=10) were prepared for scanning electron microscopy using following steps: a) instars preserved in 80% alcohol were post fixed in 1% Osmium tetraoxide and then placed in refrigerator for 2 hours; b) after post fixing, the specimens were dehydrated in a graded acetone series; c) specimens were critical point dried in desiccators; d) dried specimens were then attached to the aluminium stubs with double faced conductive adhesive tape; e) specimens were then placed in the sputter for coating with the palladium and f) specimens were studied under a Zeiss EVOMA10 Scanning Electron Microscope at 20 KV/EHT.

The terminology given by Wheeler and Wheeler (1976) and Fox *et al.* (2007) has been used to describe the larvae of *Aphaenogaster beesoni.* As body profile of *Aphaenogaster beesoni* is of curved type, it is therefore measured as cursor width and cursor height. Body hairs are measured at full length. Head capsule width, mouthparts and other morphological characters are measured for at least twenty individuals per larval instars.

Results

The detailed description of important morphological larval characters of different instars of *Aphaenogaster beesoni* is as follows:

First larval instar

Body: Whitish, Aphaenogastroid in shape head bent ventrally, anus slightly subterminal transverse slit with 10 body spiracles (Figs. 1a, 1b). Head and body covered with abundant hairs. Mainly two types of hairs present on body surface: smooth, unbranched flexuous hairs and anchor-tipped hairs (Fig. 1c). Body length 865.3µm, width 564.1µm; body diameter at thorax region 246.6µm, at abdomen region 336.4µm (Fig. 1a).

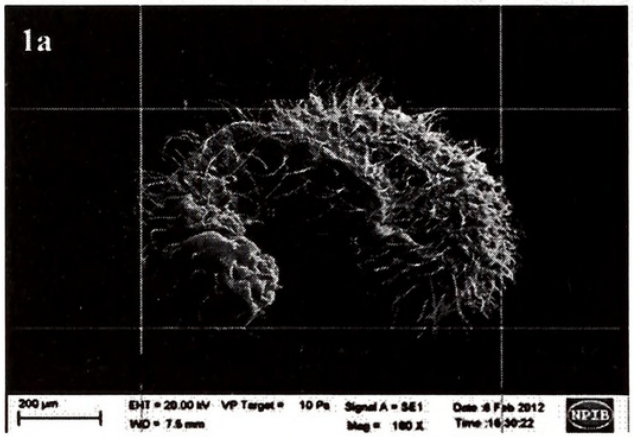
Head capsule: Cranium 224.9µm high and 278.8µm wide; roughly subcircular in shape (Fig.1d). Head surface smooth with bilaterally symmetrical tip-bifid hairs.

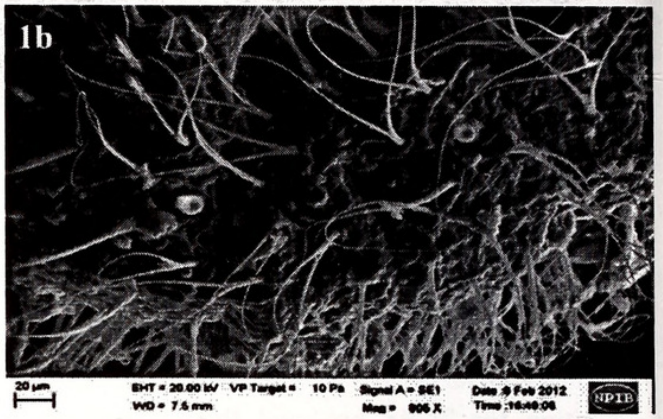
Mouthparts: Clypeus not clearly delimited from the cranium, upper surface of clypeus smooth, without sensilla, with two types of hairs: simple and tip-bifid; labrum bilobed (Fig. 1e). Mandibles simple, sharp pointed; Pogonomyrmecoid in shape, 92.38µm long and 32.55µm wide from the base with three medial teeth lying approximately in same plane (Fig. 1f).

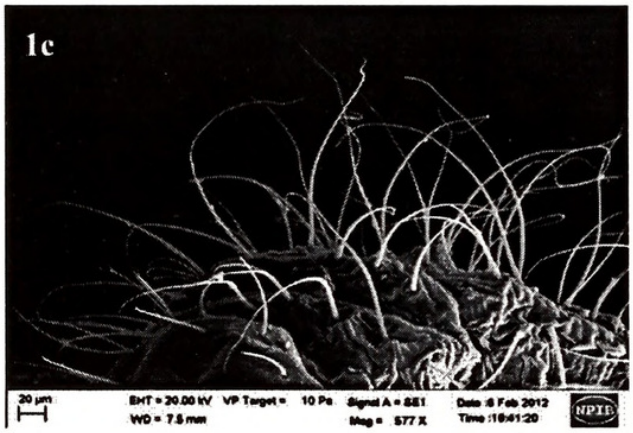
Second larval instar

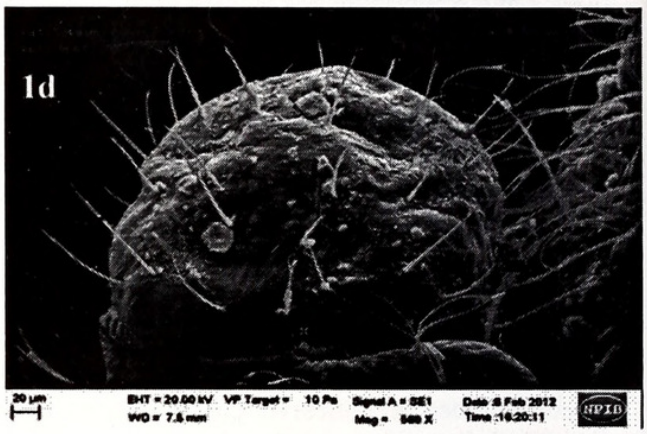
Body: Body shape changed from Aphaenogastroid in first instar to Pogonomyrmecoid in second larval instar. In this case the diameter is greatest near middle of abdomen, decreasing gradually toward head and more rapidly toward rounded posterior end; thorax more slender than abdomen, forming a ventrally curved neck (Fig. 2a). Body hairs are less as compared with the first instar larva and are of two types: deeply bifid measuring about 146.5µm and flexuous i.e. whiplike or flagelliform, measuring about 154.6µm (Fig. 2b); anus subterminal in position (Figs. 2c, 2d). Body length 1.403mm; width 949.7µm; body diameter at thorax region 300.7µm, at abdomen region 629.0µm (Fig. 2a).

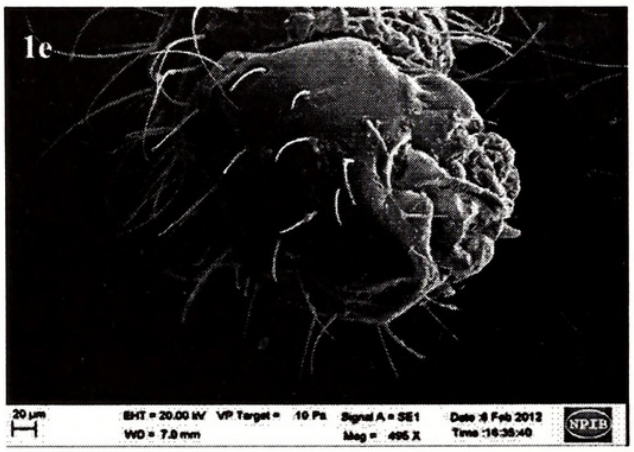
Head capsule: Cranium 230.7µm high and 290.5µm wide; antennae distinct in diameter.

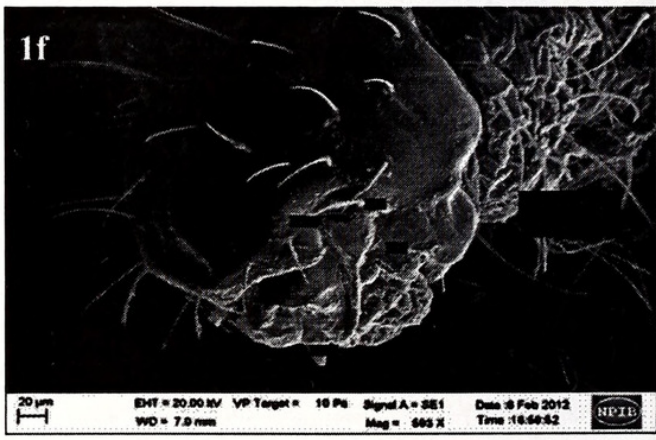




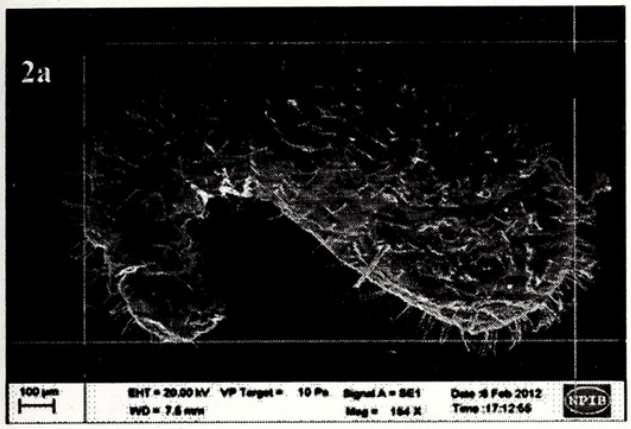


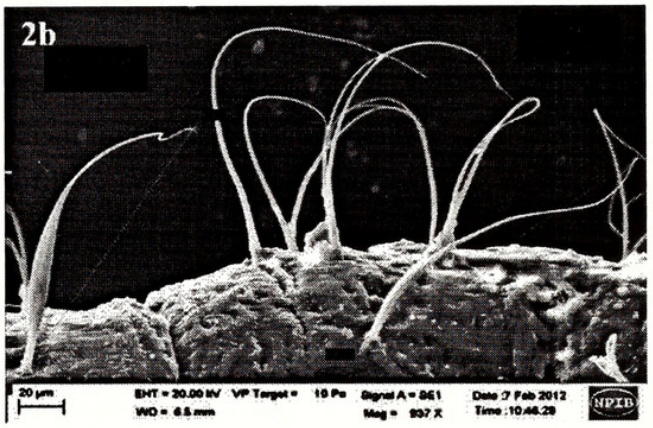


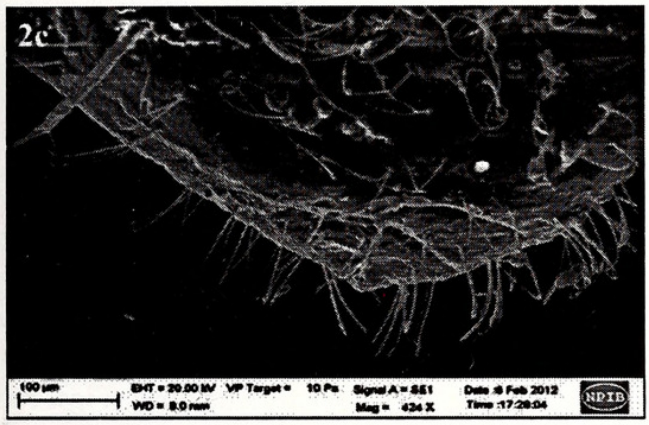


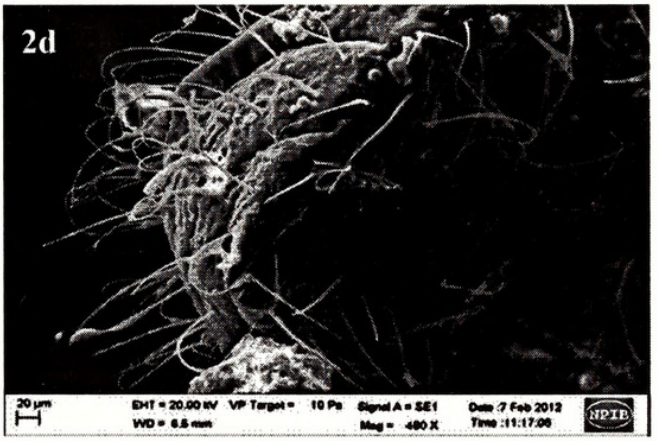


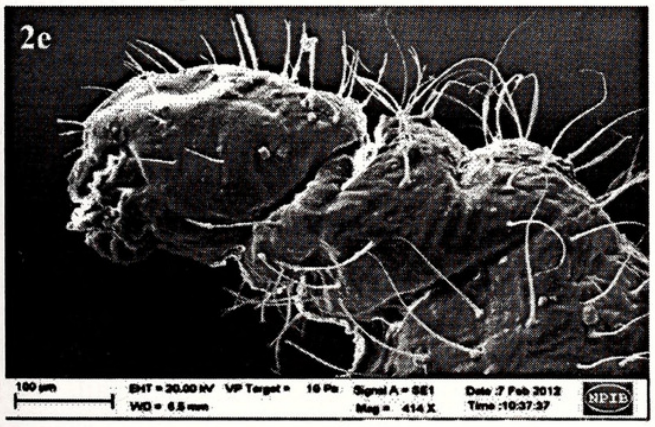
Figures 1a-f: First larval instar; *Aphaenogaster beesoni* 1a. Body profile; 1b. Abdominal spiracles; 1c. Body hairs; 1d. Head cranium; 1e, 1f. Head and mouth parts.

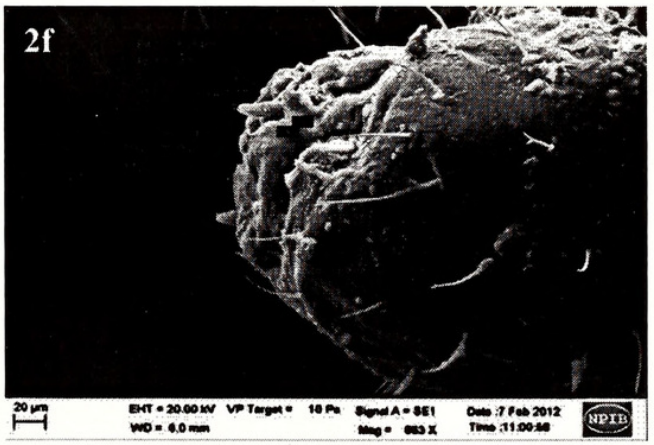












Figures 2a-f: Second larval instar; *Aphaenogaster beesoni* 2a. Body profile; 2b. Body hairs; 2c. Anal region; 2d. Body hair and anal region; 2e. Cranium and thorax; 2f. Mouth parts.

Head hairs which include simple, straight and tip-bifid hairs are present on the occipital border, on the vertex, on the genal region and on the gula (Fig. 2e).

Mouthparts: Labrum bilobed; mandibles Pogonomyrmecoid i.e. subtriangular in shape, with three conspicuous medial teeth lying in the same plane, mandibular surface spinulose. Maxillae paraboloidal, long, wide with widely spaced setaceous sensilla. Galeae simple 28.25µm long × 10.35µm wide. Labium stout and hemispherical with some scattered setaceous sensilla over the surface. Clypeus clearly delimited from the cranium (Fig. 2f).

Third larval instar

Body: Body profile Pogonomyrmecoid (Fig. 3a), anus subterminal in position. Body hairs of three types: straight, deeply bifid and dichotomously branching (Fig. 3b), more dense on the posterior surface of body. Spiracles about 1.10µm in diameter, unadorned peritreme (Figs. 3c, 3d). Total body length 677.2 µm, from thorax to anus 782.2 µm; body diameter at thorax 444.9µm, at abdomen 625.6µm (Fig. 3a).

Head capsule: Cranium 250.6µm high and 298.5µm wide. Hairs mainly two type: simple, slightly curved, long and tip-bifid. Antennae distinct, shallow pits on upper half of cranium with three sensilla (Fig. 3e).

Mouthparts: Clypeus not delimited from the cranium, surface smooth, sensilla absent. Labrum bilobed, with setaceous sensilla over the anterior surface. Mandibles wide, subtriangular, 102.9µm long × 28.61µm (Fig. 3f).

Fourth larval instar

Body: Body profile Pogonomyrmecoid (Fig. 4a), anus subterminal in position (Fig. 4b); body hairs of three types: smooth flexuous; tip bifid and deeply bifid; hairs less dense as compared to the other instars. Body length 1.980 mm, width 932.6µm (Fig. 4a).

Head capsule: Cranium 244.0µm high × 255.2µm wide (Figs. 4c, 4d). Hairs on the head are of two types: simple, slightly curved and tip bifid.

Mouthparts: Clypeus delimited from the cranium, two rows of simple slightly curved and tip bifid hairs present on the head; labrum bilobed with setaceous sensilla over the anterior surface. Mandibles long, subtriangular. Maxillae paraboloidal in shape. Galeae 25.94 µm long × 12.05 µm wide (Fig. 4e).

Fifth larval instar

Body: Body Aphaenogastroid i.e. diameter increasing gradually towards the middle of thorax and abdomen; thorax arched ventrally but not forming a distinct neck; posterior end broadly rounded (Fig. 5a). Body hairs of two types: simple 103.4µm long and slightly curved at the tip 123.5µm long (Fig. 5b); anus subterminal 415.4µm in diameter (Fig. 5c); diameter at waist 654.2µm, at thoracic region 959.7µm and at abdominal region 1084.0µm (Fig. 5a).

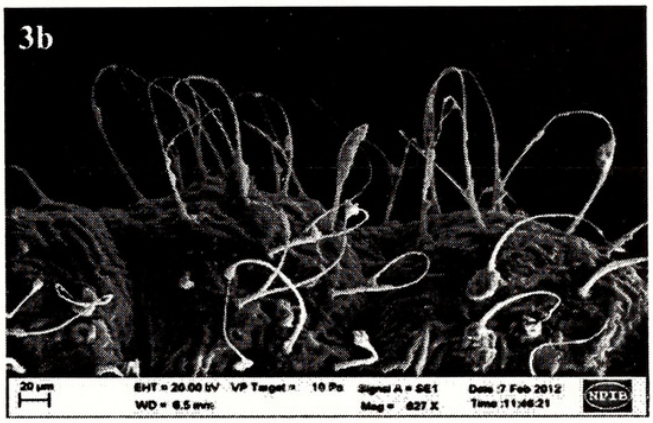
Head capsule: Cranium 763.2µm high × 985.9µm wide from upper view and 221.0µm high × 317.8µm wide from side view (Figs. 5d, 5e). Antennae present on the upper cranium measuring 44.13µm long (Fig. 5e). Hairs slightly curved and less in number (Fig. 5e).

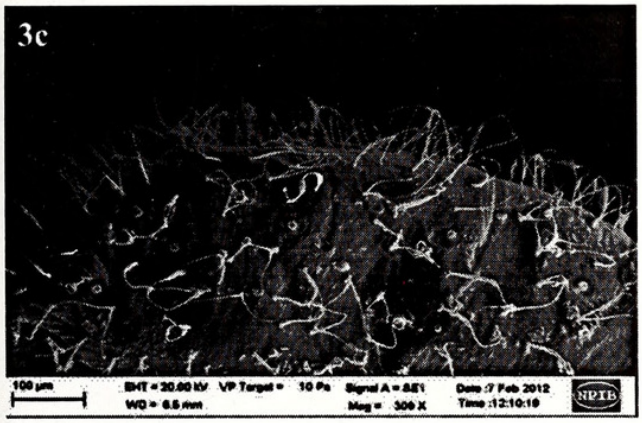
Mouthparts: Clypeus delimited from the cranium. Hairs almost absent from mouth region (Fig. 5f).

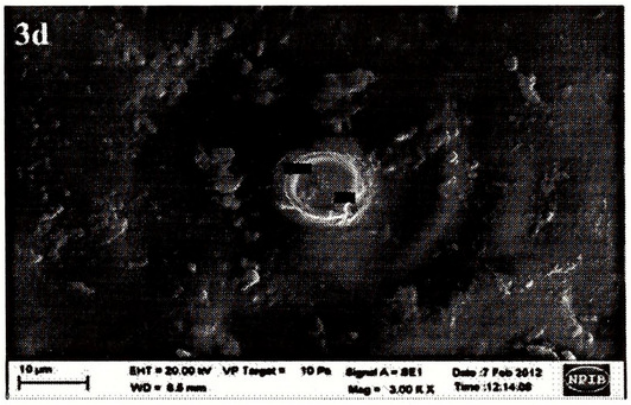
Discussion

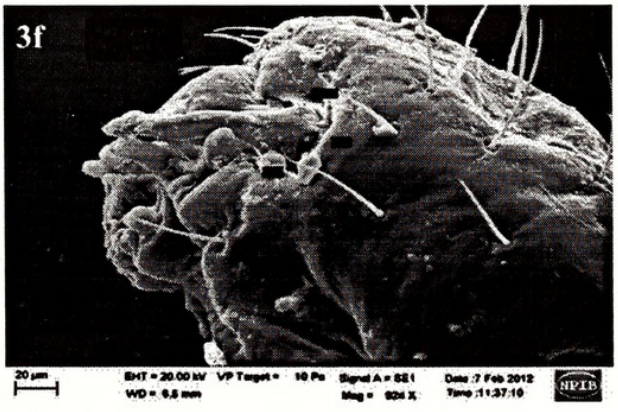
The present study marks first ever comprehensive study on immatures of *Aphaenogaster beesoni*. Using scanning electron microscopy, the larvae of species were separated into five instars; their minute details were observed and later described in detail. This division of larvae into 5 instars is carried for the very first time (Table 1 and 2).

The body profile of 1st and 5th larval instar is Aphaenogasteroid and that of 2nd, 3rd and 4th larval instar is Pogonomyrmecoid type (Figs. 1a,

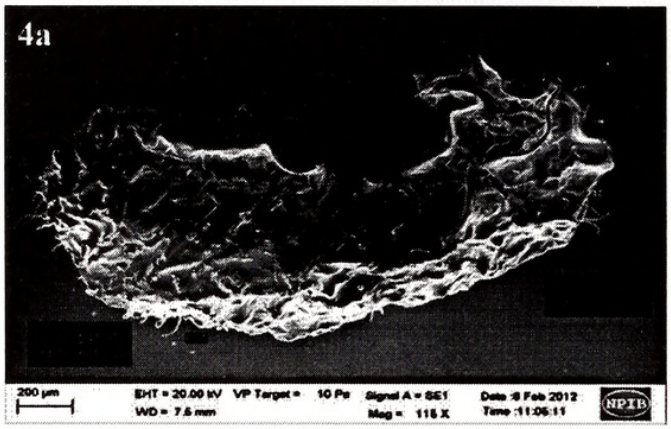


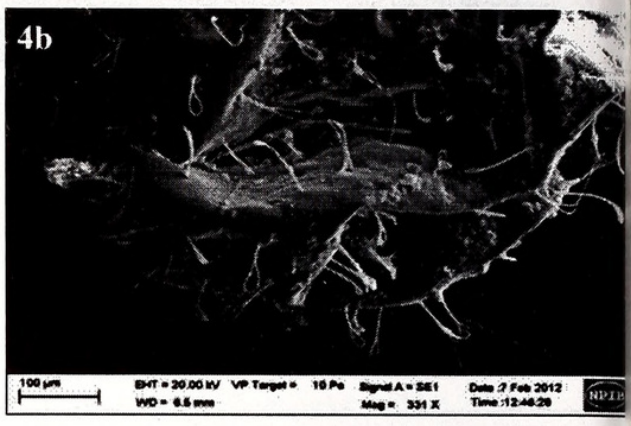


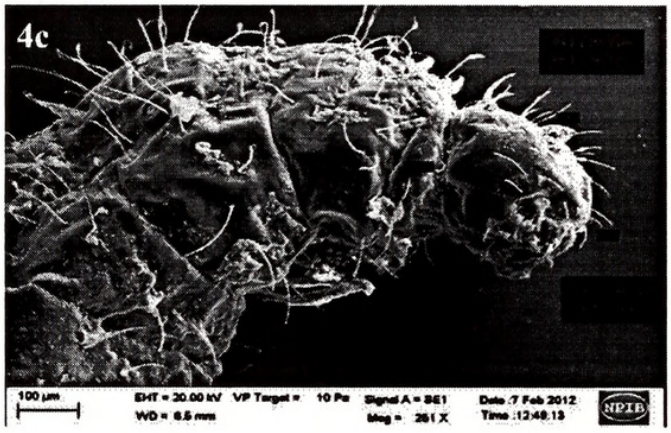


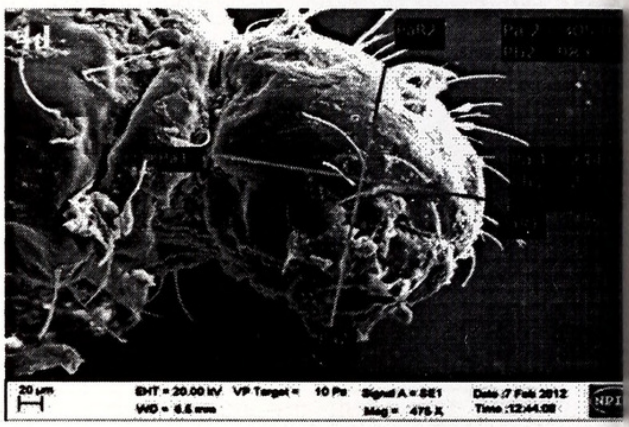


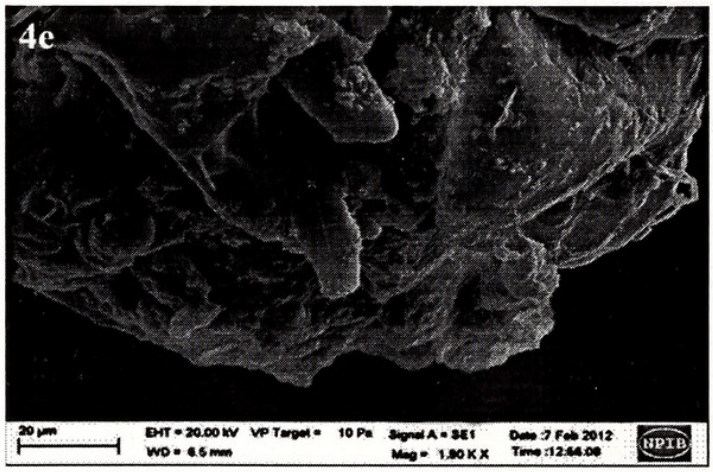
Figures 3a-f: Third larval instar; *Aphaenogaster beesoni* 3a. Body profile; 3b. Body hairs; 3c. Body spiracles; 3d. Spiracle with measurement; 3e. Cranium and sensilla; 3f. Mouth parts.



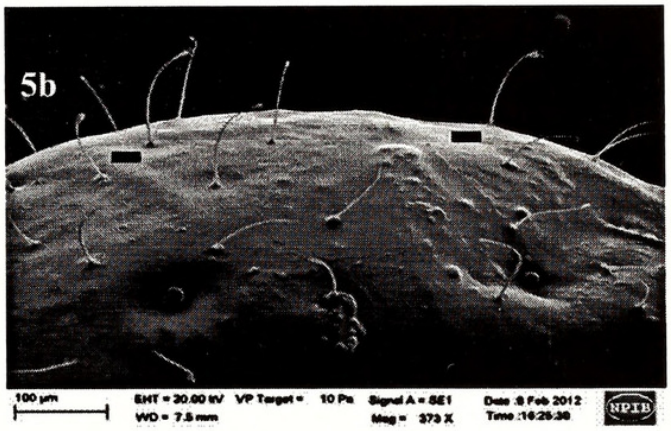


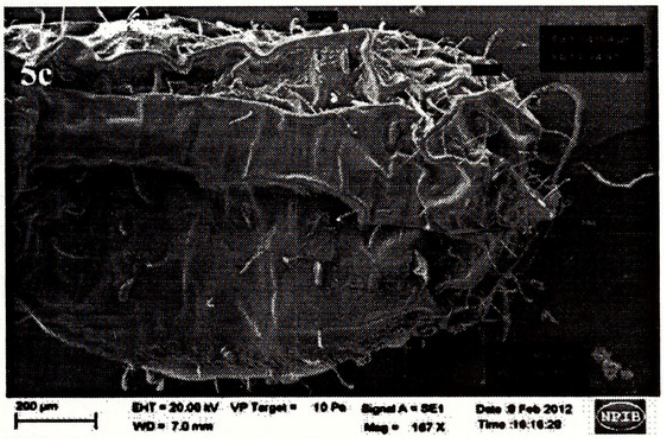


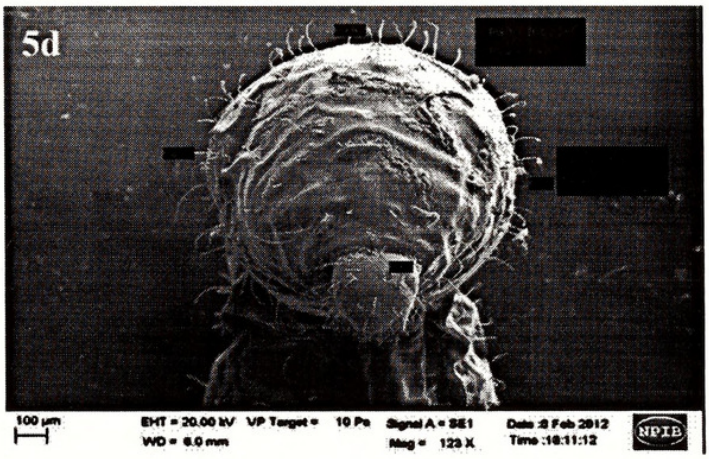


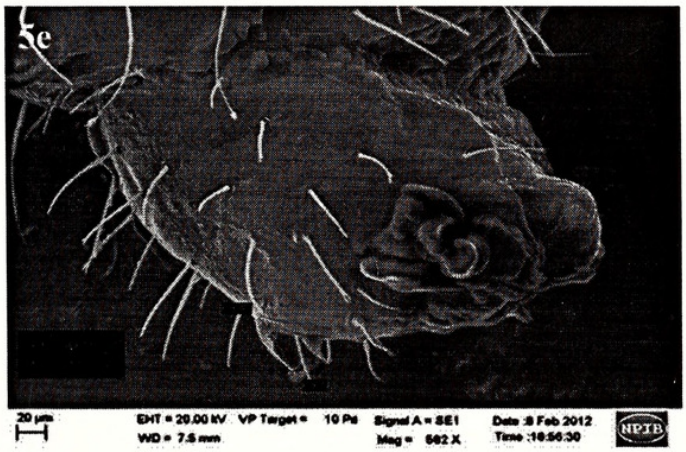


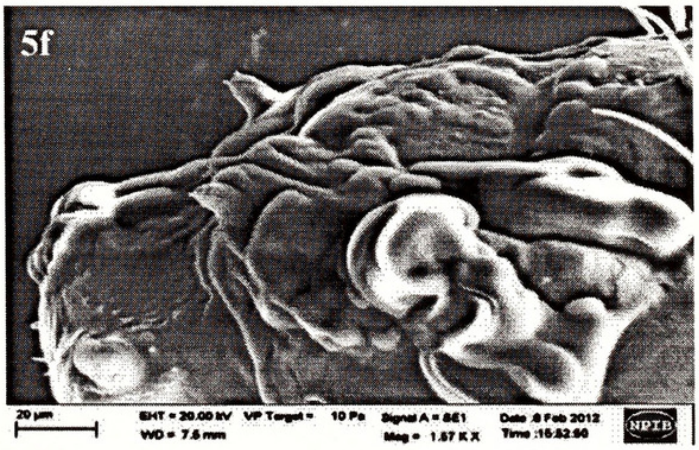
Figures 4a-e. Fourth larval instar; *Aphaenogaster beesoni* 4a. Body profile; 4b. Anal region; 4c. Thorax and cranium; 4d. Head capsule; 4e. Galeae with measurement.











Figures 5a-f: Fifth larval instar; *Aphaenogaster beesoni* 5a. Body profile; 5b. Body hairs; 5c. Anal region; 5d. Head capsule; 5e. Antennae with measurement; 5f. Mouth region.

2a, 3a, 4a, 5a respectively). The body size of all larval instars increases as they grow. The diameter from thorax and abdomen region in all instars measured corresponds to 246.6μm : 336.4μm in 1st larval instar, 360.7μm : 629.0μm in 2nd larval instar, 444.9μm : 635.6μm in 3rd larval instar, 775.6μm : 932.6μm in 4th larval instar and 959.7μm : 1084.0μm in 5th larval instar (Figs. 1a, 2a, 3a, 4a, 5a). Anus shifts from slightly ventral in position in first instars to subterminal in rest of the larval instars (Figs. 2c, 2d, 4b, 5c).

Hair type is one of the characters considered in calculating the "specialization indices" proposed by Wheeler and Wheeler (1986). The presence of bifurcations in the head hairs is being recently proposed as a character of considerable importance for separating species in the genus *Solenopsis* Westwood (Pitts, 2005). Fox *et al.* (2007) pointed out that intraspecific variation in types of head hairs might occur in other ant species as well; the present study is in accordance with the above study and suggests the revision of all the previously described ant larvae. Two types of hairs observed on the body and head surface of immature larval instars include:

Simple Hairs: Present on body and head surfaces of almost all larval instars and are further of two subtypes:

(i) Slightly curved or straight, present on head and body of all instars.

(ii) Flexuous, present on the body of 1st and 2nd larvae and absent on head region.

Bifid hairs: These also have two subtypes:

(i) Tip bifid, observed on head surfaces of almost all instars but were only present on the body surfaces of 1st and 3rd larval instars.

(ii) Deeply bifid, branched long and flexuous hairs, observed on body surfaces of all instars (Figs. 1c, 2e, 2f, 3c, 3e, 4d, 5b).

Size of cranium of larval instars increases as they grow in size; measurements of cranium of all instars corresponds to 224.9μm high × 278.8μm wide in 1st larval instar, 234.6μm high × 289.6μm wide in 2nd larval instar, 239.0μm high × 294.4μm wide in 3rd larval instar, 244.0μm high × 305.2μm wide in 4th larval instar and 251.0μm high × 317.8μm wide in 5th larval instar (Fig. 1d, 2e, 3e, 4c, 5d).

Clypeus is not clearly delimited from the cranium in all larval instars. Labrum is bilobed in all larval instars. Mandibles are simple, sharp, pointed and Pogonomyrmecoid in shape with three medial teeth approximately in same plane and increase in size from 1st larval instar to the 5th larval instar. Mandible measured in the first larval instars is 92.38μm long × 32.55μm wide from base. In third larval instar it is measured as 102.9 μm long × 38.91μm wide from base (Figs. 2f, 3f).

Maxillae and maxillary palps of all the five instars are found to be very distinct. As the growth proceeds in larvae the mouth parts also increase in size. Galeae measurements of 2nd and 4th larvae are 28.25μm long × 10.35μm wide and about 35.94μm long × 12.05μm wide (Fig. 2f, 4e).

The details added to the larval description of *Aphaenogaster beesoni* will provide an insight into minute details of immature stages and aid in future interspecific diagnosis.

Table 1: Hair types observed in larvae of *Aphaenogaster beesoni*.

Hair type	Subtypes	Description	Location on instars (L)	
			Body	Head
Simple (S)	S1	Slightly curved/straight	All instars	All instars
	S1	Flexuous	L1*, L2*	Absent
Bifid (B)	B1	Tip bifid	L1*, L3*	All instars
	B2	Deeply bifid branches long and flexuous	All instars	Absent

*** = (L1) First instar, (L2) Second instar and (L3) Third instar**

Table 2: Body measures of different instars of *Aphaenogaster beesoni.*

Observed Structures		Larval instars				
		1st	2nd	3rd	4th	5th
Body Types		Aphaenogasteroid	Pogonomyremocoid	Pogonomyremocoid	Pogonomyremooid	Aphaenogasteroid
Body Width	Thorax	246.6µm	360.7µm	444.9µm	775.6µm	965.4µm
	Abdomen	336.4µm	629.0µm	635.0µm	932.6µm	1084.0µm

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