

Description of the Ant Larvae of Two Species of the Genus *Myrmica* Latreille, 1804 (Hymenoptera: Formicidae) from Indian Himalayas

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ABSTRACT. We provide detailed description of three larval instars of two ant species namely; *Myrmica hecate* Weber 1947 and *Myrmica rupestris* Forel 1902. *Myrmica rupestris* larvae exhibit the “pogonomyrmecoid” body shape which is considered ancestral in the genus *Myrmica*, whereas *M. hecate* larvae exhibit the “pheidoloid” shape.

Keywords larval external morphology, Myrmicinae, *Myrmica hecate*, *Myrmica rupestris*.

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INTRODUCTION

The species of genus *Myrmica* (Hymenoptera: Formicidae), are mainly concentrated in Pa-laeartic and South-east Asian tropical and sub-tropical regions. The genus includes 164 species from the Old World of which 33 occur in Indian Himalayas (Radchenko and Elmes 2010; Bharti and Sharma 2011a, b, c; Bharti 2012a, b; Bharti and Sharma 2013; Bharti *et al.* 2016a, b; Bharti 2019). Most of these species are generally found in meadows, forests, under stones and grasslands. The larvae of *Myrmica* like other boreal myrmecines develop slowly; and in Himalayas the development from an egg to a fertile queen is interrupted by six months long harsh winter season (Kipyatkov 1993).

Although the adult morphology of the genus *Myrmica* have been extensively studied (Radchenko and Elmes 2010; Bharti 2012a, b; Bharti 2013; Bharti and Sharma 2011a, b, c; Bharti and Sharma 2013; Bharti *et al.* 2016a, b), the larval morphology has so far been poorly studied.

Wheeler and Wheeler (1976) initiated the mammoth task of describing the larval forms of nearly 800 species belonging to the subfamilies Myrmicinae, Ponerinae, Formicinae, Cerapachyinae, Dolichoderinae and Dorylinae. However, these descriptions were mainly based on few specimens and lacked the information regarding the number of instars (Solis *et al.* 2010). So far, complete larval descriptions of merely 0.5% of extant ant species are known. Given the importance of larval forms in taxonomic stud-

ies, Fox *et al.* (2007; *Paratrechina longicornis*), Vieira *et al.* (2009; *Ectatomma vizottoi*), Jesus *et al.* (2010; *Tapinoma melanocephalum*), Solis *et al.* (2010; *Monomorium floricola*) and Fox *et al.* (2017a, b; *Odontomachus sp.*, *Camponotus senex* and *Camponotus textor*) investigated the fine details of immature stages, number of larval instars as well as growth rate between each instar with the help of scanning electron microscope and light microscope. From India, larval forms of two species of the subfamily Myrmicinae, i.e., *Pheidole indica* Mayr 1879 and *Aphaenogaster beesoni* Donisthorpe 1933, were described in detail using scanning electron microscope (Bharti and Gill 2011, Bharti *et al.* 2013).

Based on morphology of adults, the 33 Indian Himalayan *Myrmica* species have been clustered into six species groups (Radchenko and Elmes, 2010, Bharti *et al.* 2016 b). These species groups have been defined by a combination of external morphological characters of the adults. But in contrast, the species are distinguished from the related ones by at least one morphological autapomorphy (Radchenko and Elmes, 2010). Given this backdrop the study aims to test whether the species delimitation based on adult morphology holds true for larval stages as well. In the present study, the treated species viz., *M. hecate* and *M.*

rupestris belong to the *M. rugosa* species group. The morphometric measurements of larval cephalic capsule width suggests the presence of three instars in these species.

MATERIALS AND METHODS

Sampling

Immatures of *M. hecate* and *M. rupestris* from seven colonies of each species were collected from Solang valley (2560 m), Kothi (2600 m) and Kelong (3080 m) located in the state of Himachal Pradesh.

Identification and depository of voucher specimens

Larvae were identified based on the external morphology of their adult nestmates by referring to Radchenko & Elmes, 2010. The voucher specimens used for identification and examination of larval morphology will be deposited in the collection of PUAC (Punjabi University Ant Collection).

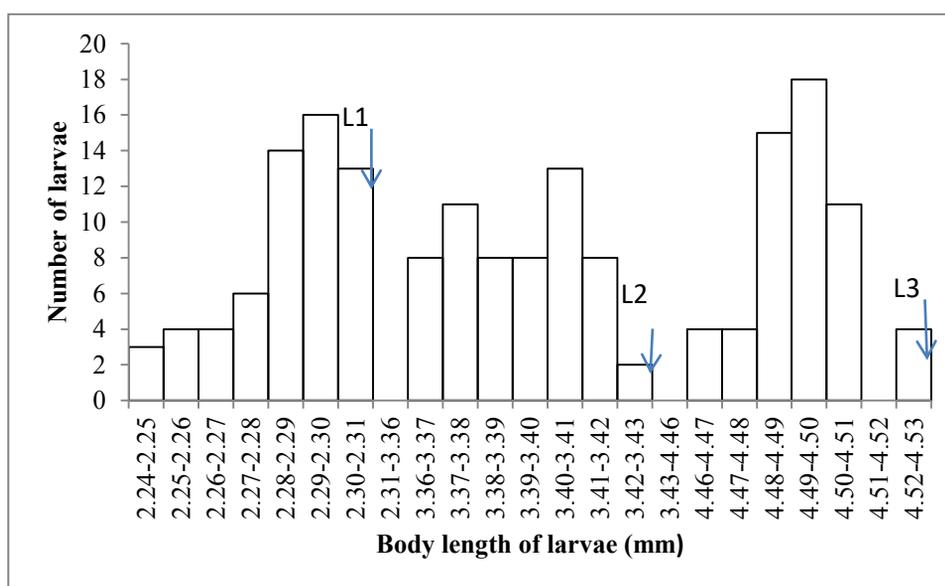


Fig. 1. Frequency distribution of the body length of *Myrmica hecate* larvae.

Specimen preparation and imaging

The larval stages were fixed in the dietrich's solution for 24-48 hours period and there after preserved in 80% alcohol. The larvae were separated into three instars/stages based on head capsule width. After separation of larval forms, all instars ($n = 5$) were prepared for scanning electron microscopy. For this purpose, the instars preserved in 80% ethanol were post fixed in 1% osmium tetroxide and then placed in refrigerator for 2 hours. After post fixing, the specimens were dehydrated in a graded acetone/ethanol series and

then critical point dried in a desiccator. The dried specimens were attached to the aluminium stubs with the help of double faced conductive adhesive tape. The specimens were then placed in the sputter for coating with the palladium. Specimens were studied under a Zeiss EVOMA10 scanning electron microscope at 20 KV/EHT. Additional larvae of each instar ($N = 15-20$) were warmed for 15 min in an aqueous solution of KOH (10%) and placed in a small drop of glycerin on a microscope slide for observations under a radical compound microscope.

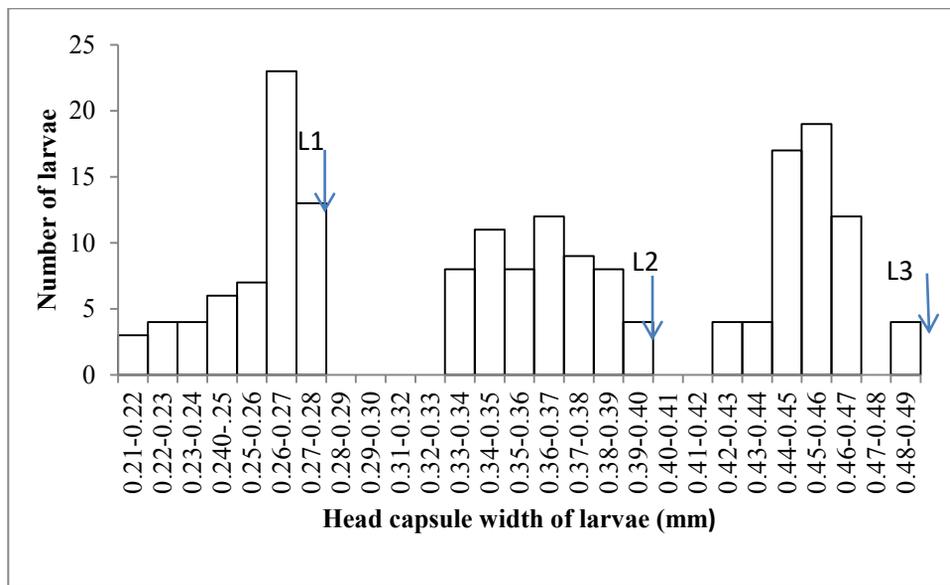


Fig. 2. Frequency distribution of the maximum widths of head capsules of *Myrmica hecate* worker larvae: (L1) first larval instar, (L2) second larval instar, and (L3) third larval instar.

Determination of larval Instars

As we are unable to directly observe moults, the number of larval instars was determined using the method described in Parra and Haddad (1989). The maximum head widths of the larvae were measured and plotted in a frequency distribution graph, where every distinct peak was considered to correspond to a different larval instar; the obtained number of larval instars was then tested against Dyar's rule (Figs. 1, 2, 3, 4).

Terminology

The terminology given by Wheeler and Wheeler (1976) and Fox *et al.* (2007) has been used to describe the larval stages of *M. rubestris* and *M. hecate*. Herein, all body measurements are presented as approximate measures, size intervals or mean \pm standard error followed by number of individuals analysed.

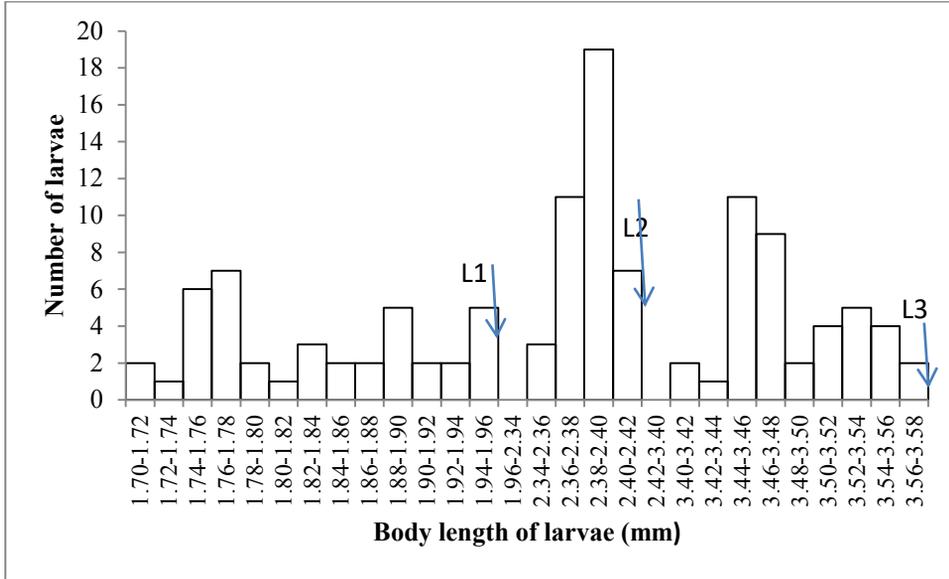


Fig. 3. Frequency distribution of the body length of *Myrmica rupestris* larvae.

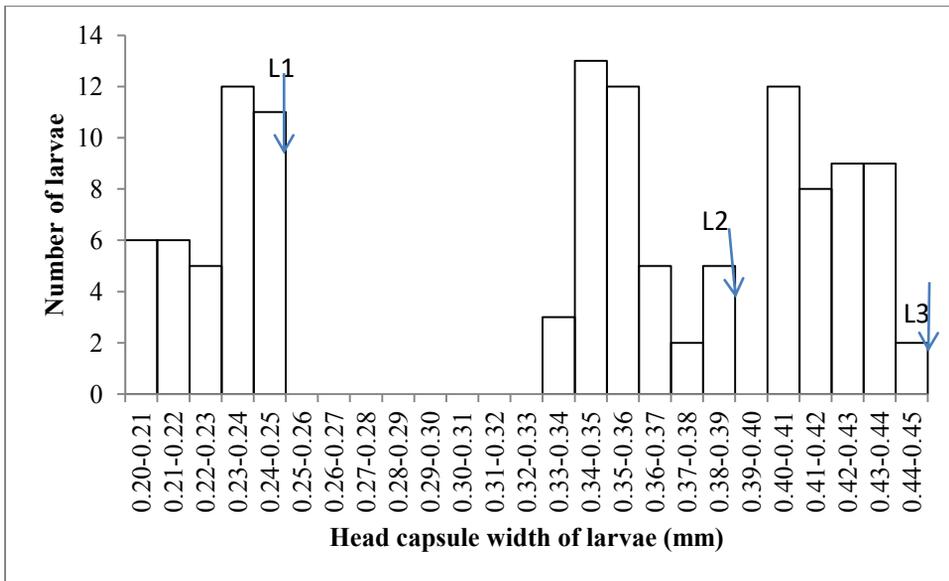
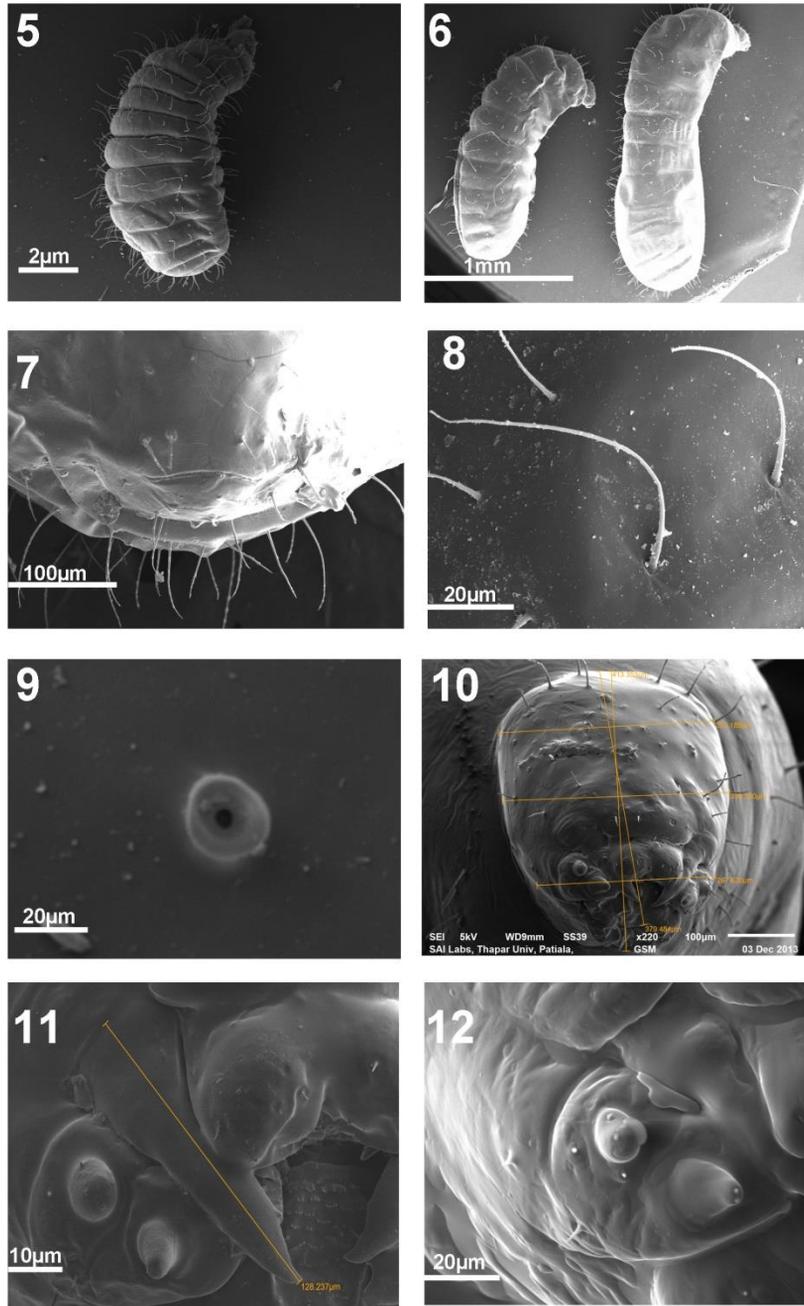
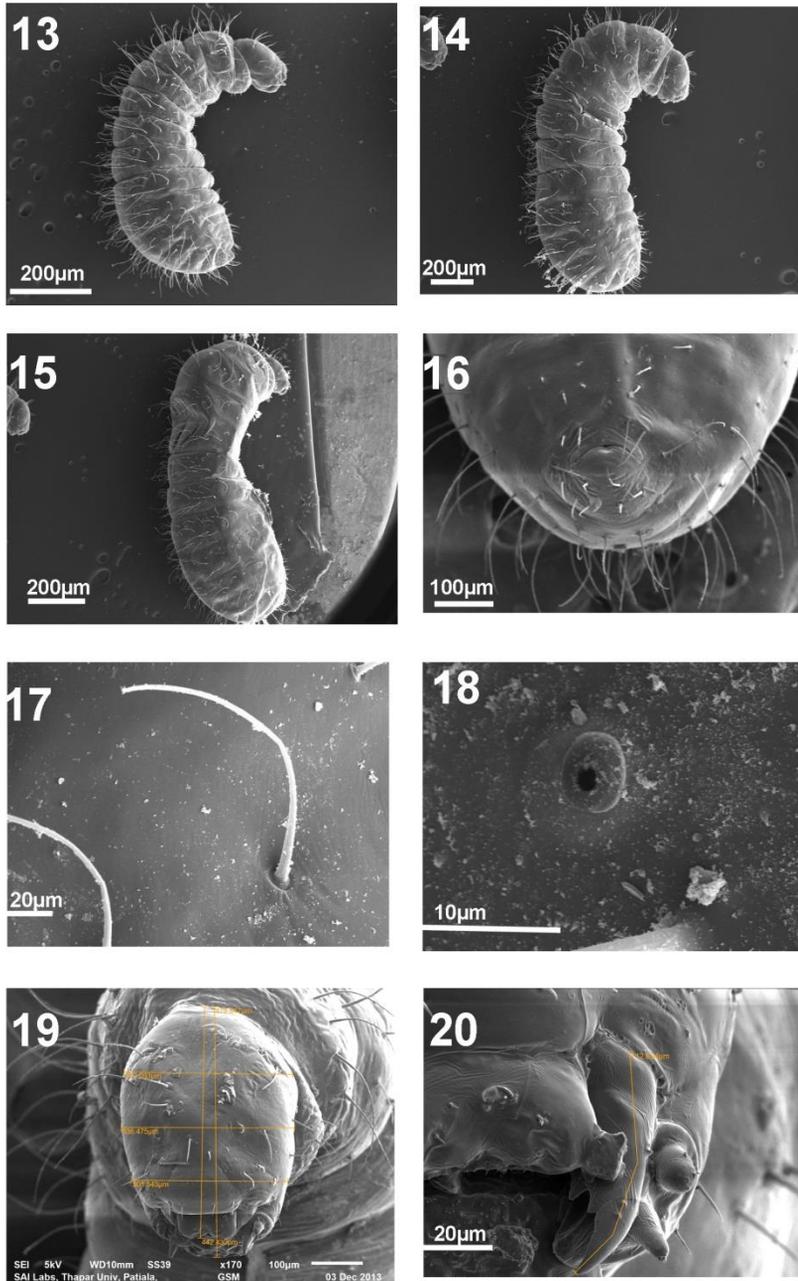


Fig. 4. Frequency distribution of the maximum widths of head capsules of *Myrmica rupestris* worker larvae: (L1) first larval instar, (L2) second larval instar, and (L3) third larval instar.



Myrmica hecate Figure (5) first instar larva (6) side view of 2nd and 3rd instar larvae (7) position of anus of 3rd instar larva (8) unbranched denticulate hair (9) unornamented spiracle (10) subquadrate shaped head (11) ectatomoid mandible (12) mouthparts of 3rd instar larva.



Myrmica rupestris Figure (13) first instar larva (14) second instar larva (15) third instar larva (16) position of anus (17) unbranched denticulate hair (18) unornamented spiracle (19) subpyriform shaped head (20) mandible.

RESULTS

Myrmica hecate Weber 1947

First instar

Body: Whitish, slender in profile, rather pheidoloid in shape (Fig. 5); thorax more slender than abdomen and forming a neck, which is curved ventrally; head bent ventrally & slightly curved; anus a slightly subterminal transverse slit with the lower lip bigger than the upper lip. Body about 2.24–2.30 mm long, 1.20–1.24 mm wide (n = 15). Length through spiracles 1.8 mm (n = 1). Width of anterior, median and posterior end of body 540.3 μm , 810.2 μm & 680.9 μm (n = 1), respectively. Body hair unbranched denticulate, 124.6–166.9 μm long (n = 12), distance between 2 adjacent hairs 89.9–112.7 μm (n = 10). Body with 10 spiracles; distance between two spiracles 192.6–230.1 μm (n = 10). Spiracle opening unornamented; diameter of spiracle 17.1–18.1 μm (n = 3).

Head capsule: Cranium subquadrate shaped, 218.7–221.6 μm in height, 324.7–326.8 μm in width; cranium index (height/width x 100) = 67.35–67.80 (n = 4); head surface smooth with unbranched denticulate hairs; length of hair on the occipital border 62.2–64.6 μm (n = 3). Antennae positioned on the upper half of cranium, with three sensilla. Tentorial pits well-demarked.

Mouthparts: Clypeus not clearly delimited from the cranium; upper surface of clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border. Labrum 90.9–94.7 μm in width (n = 3), with the anterior margin weakly bilobed, with about four sensilla on the anterior surface. Mandibles simple, sharp-pointed, with one medial tooth, roughly ectatommoid in shape, 96.6–98.7 μm long (n = 3). Maxilla conical in shape. Galea present as a small hump culminating with two sensilla, 15.8 μm in length (n = 1); maxillary palpus paxilliform. Labium strongly hemispherical, 98.8–100.8 μm wide (n = 3). The medial opening of sericteries positioned at the base of hypopharynx. The hypopharynx covered with dense spinules.

Second instar

Body: Whitish, body profile pheidoloid shaped as in the first instar (Fig. 6); head bent ventrally and slightly curved; anus a slightly ventral transverse slit. Body about 3.36–3.42 mm long, 1.84–2.05 mm wide (n = 15). Length through spiracles measures about 3.0 mm (n = 1). Width of anterior, median and posterior end of body was found to be 874.4 μm , 1.2 mm & 1.2 mm (n = 1) respectively. Body hair unbranched denticulate and ranges about 181.7–200.6 μm in length (n = 12), distance between 2 adjacent hairs 155.7–189.3 μm (n = 10). Body with 10 spiracles. Each spiracle possess unornamented opening; maximal diameter including peritreme 21.7 μm (n = 10); maximal diameter of the opening 4.1 μm (n = 2); distance between two spiracle 272.4 μm (n = 3).

Head capsule: Cranium subquadrate shaped, 327.5–329.4 μm in height, 329.9–331.8 μm in width (n = 5); cranium index = 99.27–99.54; head surface smooth with unbranched denticulate hairs; four hairs present on the genal region; two hairs present on the vertex; six hairs circling the occipital area; length of hairs on the occipital border 78.41–79.27 μm (n = 3).

Mouthparts: Clypeus delimited from the cranium by a suture; upper surface of clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border. Labrum 113.9–116.2 μm in width (n = 3), with the anterior margin markedly bilobed, with about six sensilla on the anterior surface. Mandibles simple, sharp-pointed, with one medial tooth, roughly ectatommoid in shape, 126.4–130.4 μm long (n = 3). Maxilla conical in shape. Galea, simple, skewed peg-formed, 20 μm in length (n = 1), with two sensilla at the top. Maxillary palpus paxilliform. Labium stout and hemispherical, 136.9–139.5 μm in width (n = 3). The medial opening of sericteries positioned at the base of hypopharynx. The hypopharynx covered with dense spinules.

Third instar

Body: Whitish, pheidoloid in profile, less curved; anus slightly ventral in position (Figs. 6 & 7). Body 4.46–4.52 mm long, 2.21–2.30 mm wide (n = 15). Length through spiracles 4.0 mm (n = 1). Width of anterior, median and posterior end of body 945.2 μ m, 1.2 mm & 1.4 mm, respectively (n = 1). Body hair unbranched denticulate in shape (Fig. 8), about 212 μ m long; distance between 2 adjacent hairs 279.5–321.6 μ m (n = 10). Body with 10 spiracles. Each spiracle with unornamented openings (Fig. 9); maximal diameter including peritreme 22.1–22.7 μ m (n = 2); maximal diameter of the opening 4.1–4.3 μ m (n = 2). Distance between two spiracles 446.8–486.5 μ m (n = 10).

Head capsule: Cranium subquadrate shaped, 378.7–380.8 μ m in height, 338.6–340.1 μ m in width (n = 4); cranium index = 111.84–111.96 (Fig. 10); head surface smooth with unbranched denticulate hairs; four hairs present on the genal region, two hairs present on the vertex; seven hairs circling the occipital area (n = 3); hair on the occipital border 80.2–82.6 μ m long. Tentorial pits well-demarcated.

Mouthparts: Clypeus clearly delimited from the cranium by a suture; a distinct row of four simple hairs present along the distal clypeal border. Labrum 117.8–119.4 μ m wide (n = 3), with the anterior margin markedly bilobed, with about six sensilla on the anterior surface. Mandibles simple, sharp-pointed, roughly ectatommoid in shape, without medial tooth, 180.9–182.2 μ m long (n = 3). Maxilla conical in shape. Galea present as a small hump culminating with two sensilla, 32.3 μ m long (n = 1). Maxillary palpus paxilliform (Fig. 11 & 12). Labium stout and strongly hemispherical, 140.5–142.6 μ m wide (n = 3). The medial opening of sericteries positioned at the base of hypopharynx.

Pupa

Pupae exarate, with no coccon, 6.0 mm long, milky-white when young; eyes becoming black and body gradually darkened to a deep brown as they develop into imago.

Myrmica rupestris Forel 1902

First instar

Body: Whitish, slender in profile, rather pognomyrmecoid in shape (Fig. 13); thorax more slender than abdomen and forming a neck, which is curved ventrally; head bent ventrally & more curved; anus slightly subterminal transverse slit with the lower lip bigger than the upper lip. Integument covered with minute spinules. Body about 1.74–1.95 mm long x 1.05–1.12 mm wide (n = 20). Length through spiracles 1.6 mm (n = 1). Width of anterior, median and posterior end of body 492.8 μ m, 524.8 μ m & 615.0 μ m (n = 1) respectively. Body hair unbranched denticulate, 127.2–195.7 μ m long (n = 10), distance between 2 adjacent hairs 84.5–124.1 μ m (n = 10). Body with 10 spiracles, distance between two spiracle 92.7–171.9 μ m (n = 10). Spiracle opening unornamented; diameter of spiracle 10.6–12.5 μ m (n = 3), diameter of opening of spiracle 3.2–3.6 μ m (n = 3).

Head capsule: Cranium subpyriform shaped, 379.7–397.6 μ m in height, 311.7–318.8 μ m in width (n = 5); cranium index = 121.81–124.71 μ m; head surface smooth with unbranched denticulate hairs; length of hair on the occipital border 76.9–77.5 μ m, a total of 12 hairs were observed on the head which include four hairs on the genal region, four on the vertex, and eight circling the occipital area (n = 3). Tentorial pits well-demarcated.

Mouthparts: Clypeus clearly delimited from the cranium; upper surface of clypeus smooth without sensilla; a distinct row of four simple hairs present along the distal clypeal border. Labrum weakly bilobed, with about six sensilla on the anterior surface. Mandibles simple, sharp-pointed with one medial tooth, roughly ectatommoid in shape, 88.2–88.6 μ m long (n = 2). Maxilla conical in shape. Galea present as a small hump culminating with two sensilla, galea 29.9 μ m (n = 1) in length; maxillary palpus paxilliform. Labium pronounced and strongly hemispherical, 140.8 μ m wide. The medial opening of sericteries positioned at the base of hypopharynx. The hypopharynx covered with dense spinules.

Second instar

Body: Whitish, slender in profile, rather pogonomymecoid in shape (Fig. 14); thorax more slender than abdomen and forming a neck, which is curved ventrally; head bent ventrally and slightly curved; anus a slightly ventral transverse slit. Body about 2.37–2.41 mm long, 1.29–1.33 mm wide (n = 20). Length through spiracles measures 1.7 mm (n = 1). Width of anterior, median and posterior end of body was found to be 505.4 μm , 721.5 μm & 777.2 μm (n = 1) respectively. Body hair unbranched denticulate and ranges about 197.6–227.3 μm in length (n = 10), distance between 2 adjacent hairs 137.5–167.6 μm (n = 10). Body with 10 spiracles and distance between two spiracle 190.6–245.9 μm (n = 10). Each Spiracle possess unornamented opening; maximal diameter including peritreme 13.3–13.9 μm (n = 3); maximal diameter of the opening of spiracle 3.9–4.1 μm (n = 6).

Head capsule: Cranium subpyriform shaped, 437.5–442.7 μm in height, 331.3–340.2 μm in width (n = 5); cranium index = 130.12–132.05 μm ; head surface smooth with unbranched denticulate hairs; ;four hairs present on the genal region; four hairs present on the vertex, eight hairs circling the occipital area; Length of hairs on the occipital border 90.6–91.9 μm (n = 3).

Mouthparts: Clypeus delimited from the cranium by a suture; upper surface of clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border. Labrum markedly bilobed, with about six sensilla over the anterior surface. Mandibles simple, sharp-pointed, with one medial tooth roughly ectatommoid in shape, 102.1–106.5 μm long (n = 2). Maxilla conical in shape; Galea present as a small hump culminating with two sensilla and 40.7 μm long (n = 1); maxillary palpus paxilliform. Labium stout and strongly hemispherical, 131.4 μm wide.

Third instar

Body: Whitish, slender in profile, rather pogonomymecoid in shape (Fig. 15); head bent ventral-

ly & less curved as compared to first instar of *M. rupestris*; anus slightly ventral in position (Fig. 16). Integument covered with minute spinules. Body 3.40–3.58 mm long, 1.56–1.62 mm wide (n = 20). Length through spiracles 3.0 mm (n = 1). Width of anterior, median and posterior end of body 877.4 μm , 890.3 μm & 1.2 mm respectively. Body hair unbranched denticulate (Fig. 17), about 260.9–283.7 μm long (n = 10); distance between 2 adjacent hairs 147.6–202.5 μm (n = 10). Body with 10 spiracles. Each spiracle with unornamented opening; the opening of spiracle about 6.0 μm and distance between two spiracles 355.2 μm (Fig. 18).

Head capsule: Cranium subpyriform shaped, 445.4–450.2 μm in height, x 352.7–357.0 μm in width (n = 5); cranium index = 136.10–136.46 μm , (Fig. 19); antennae present on the upper half of cranium; head surface smooth with unbranched denticulate hairs; four hairs present on the genal region, four hairs present on the vertex ;eight circling the occipital area (n = 1); hairs on the occipital border 96.5–98.7 μm long (n = 3), Tentorial pits well-demarcated.

Mouthparts: Clypeus clearly delimited from the cranium by a suture; upper surface of clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border. Labrum markedly bilobed, with about six sensilla over the anterior surface. Mandibles simple, sharp-pointed, roughly ectatommoid in shape, with one medial tooth, 124.7–127.3 μm long (n = 3) . Maxilla conical in shape. Galea present as a small hump culminating with two sensilla, 29.6 μm long (n = 1); maxillary palpus paxilliform. Labium stout and strongly hemispherical, 132.7 μm wide (n = 1) (Fig. 20). The opening of sericteries positioned at the base of hypopharynx.

Pupa

Pupae exarate, with no cocoon, 5.7 mm long, milky-white when young; eyes becoming black and body gradually darkened to a deep brown as they develop into imago.

Table 1. Comparison of larval morphology between *Myrmica hecate* Weber, 1947 and *Myrmica rupestris* Forel, 1902

S.No.	Morphological Characters	<i>Myrmica hecate</i> Weber, 1947	<i>Myrmica rupestris</i> Forel, 1902
1.	Color	Brownish	Dull white
2.	Profile	Pheidoloid	Pogonomyrmecoid
3.	Head	Ventral in position and slightly curved	Ventral in position and comparatively more curved
4.	Shape of head	Subquadrate	Subpyriform
5.	Shape of antennae	Depressed in 1 st instar & Elevated in 2 nd , 3 rd instar	Elevated
6.	No. of hairs in occipital region	7-8	8
7.	No. of hairs in vertex region	2	4
8.	No. of hairs in genal region	4	4

DISCUSSION

Wheeler & Wheeler (1976) designated pogonomyrmecoid as the body shape for the third instar in genus *Myrmica*, and Mizutani and Yamane (1978) also observed it in *Myrmica ruginodis* Nylander, 1846 (s. lat.). These observations were conducted under dissecting binocular microscope thus limiting the critical examination of some characters and obliterating few as well. During present investigations the body profile of the two species i.e. *Myrmica hecate* and *Myrmica rupestris* were found to be different. *Myrmica hecate* possesses a pheidoloid body type whereas *Myrmica rupestris* retained the primitive pogonomyrmecoid shape (Table 1). The morphological description of the larval forms of the above stated species has been attempted for the first time thus paving the way for future biologists.

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