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Scanning Electron Microscopy on the Ant Larvae of Three Species of *Myrmica* Latreille, 1804 (Hymenoptera: Formicidae) from the Indian Himalayas

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Abstract

The genus *Myrmica* is represented by 32 species categorized into five species groups from India: *ritae* group, *inezae* group, *pachei* group, *smythiesii* group, and *rugosa* group. The *rugosa* group is further subdivided into two species complexes, the *rugosa*-complex and the *cachmiriensis*-complex. Herein, we present Scanning Electron Microscopy of larval instars of two species falling in *cachmiriensis*-complex (*Myrmica cachmiriensis* Forel, 1904, and *Myrmica wardi* Radchenko & Elmes, 1999) and *Myrmica nitida* Radchenko & Elmes, 1999, which has not been assigned to any group because of its unique combination of morphological characters. Based on the frequency distribution of larval head widths, three larval instars were observed in the studied species of the genus *Myrmica*. There were discrete differences in the body constitution between larval instars. The body profile is different in all three species; *M. cachmiriensis* is of Pheidoloid type, *M. nitida* Aphaenogastroid type, and *M. wardi* Myrmecoid type. Prominent interspecific differences were observed in body hair and mandibles, as well as in morphometric analysis.

Introduction

The taxonomy of the ant subfamily Myrmicinae is notoriously complex owing to its immense species diversity and morphological variability across castes and regions. Frequent occurrence of cryptic species, morphological convergence, and poorly resolved species complexes further obscure generic and species boundaries. Although socially parasitic taxa represent only a small proportion of myrmicines, their morphological reduction and host-specificity pose additional challenges for accurate classification (Ward et al., 2015). The group is a hyperdiverse assemblage of 155 genera, and over 6700 described species, constituting about half of all named ants on Earth (Ward et al., 2015; antCat.org). The phylogenetic analysis of the subfamily Myrmicinae (Branstetter et al., 2017) divides the subfamily into six clades, of which the Myrmicini clade points towards the sister group relationship between *Myrmica* and *Manica*. Within this diverse assemblage, *Myrmica* and *Manica* are temperate myrmicines that share broadly similar ecological habits, such as nesting in soil or decaying wood and foraging close to the ground (Radchenko & Elmes, 2010). However, research on their biology is uneven: *Myrmica* has been the focus of extensive ecological, behavioural, and parasitological studies (Elmes et al., 1998; Czechowski et al., 2012), whereas *Manica*, a much smaller genus, remains comparatively poorly understood (Ward et al., 2015).

The recognition of immature stages in investigating an organism's natural history is widely accepted. The life history patterns of an organism provide invaluable insights into the evolutionary trajectory and developmental processes of a species (Bharti et al., 2023c). Ants of the genus *Myrmica* Latreille, 1804 (Hymenoptera: Formicidae: Myrmicinae) are well distributed in the Holarctic realm and high mountains

in Southeast Asia. Ants of the genus Myrmica Latreille, 1804 (Hymenoptera: Formicidae: Myrmicinae) are widely distributed throughout the Holarctic region and extend into the high mountains of Southeast Asia. In the Indian Himalayas, 32 *Myrmica* species are known, of which 19 are endemic to the region (Bharti & Sharma, 2011a,b,c, 2013; Bharti, 2012a,b, 2013; Bharti et al., 2016a,b,c). Despite this richness, the immature stages of most *Myrmica* species remain poorly documented. Previous studies have described the larvae of only a few species using scanning electron microscopy (Bharti et al., 2019). The present work expands this knowledge by providing detailed morphological analyses, through scanning and light microscopy, of the larval instars of three Himalayan Myrmica species – *M. cachmiriensis* Forel, 1904*, M. wardi* Radchenko & Elmes, 1999, and *M. nitida* Radchenko & Elmes, 1999 – which are restricted to the northwestern and central Himalayan regions (Bharti et al., 2016c).

Materials and Methods

Depository and specimens

PUAC = "Punjabi University Patiala Ant Collection" at Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India

Colonies of *M. cachmiriensis* and *M. wardi* were collected from Manali, located in the state of Himachal Pradesh, and *M. nitida* from Sonmarg, Kashmir. All immatures were preserved in 80% alcohol. The larvae were separated into three instars/stages based on the width of the head capsule. After separating larval forms, all instars (n = 5 for each stage) were prepared for scanning electron microscopy. For SEM analysis, the specimens were dehydrated in a graded ethanol series. Then, they were dried in a desiccator at a critical point to remove water and air from the samples and maintain a dry environment. The dried specimens were attached to the aluminum stubs using double-faced conductive adhesive tape. The specimens were then placed in the sputter for coating with palladium in a Quorum Q150 ES Plus coater (Quorum Technologies, Laughton, East Sussex, UK). Specimens were examined under a Zeiss Gemini 1 Sigma 500 scanning electron microscope (Carl Zeiss, Jena, Germany) at 20 KV/EHT. Additional larvae of each instar were warmed for 15-20 min in an aqueous solution of KOH (10%) and placed in a small drop of glycerin on a microscope slide for observation under a Radical compound microscope.

The number of larval stages was determined using the method described by Parra and Haddad (1989). The maximum head widths of the larvae were measured and plotted in a frequency distribution graph, and the recognized numbers of larval instars were then tested against Dyar's rule (Dyar, 1890) (Figs 1, 2, 3). For comparative morphometric analysis between the *cachmiriensis* and *rugosa* species groups, larval data from Bharti et al. (2019) and Bharti et al. (2023b) were incorporated and are summarized in Table 4.

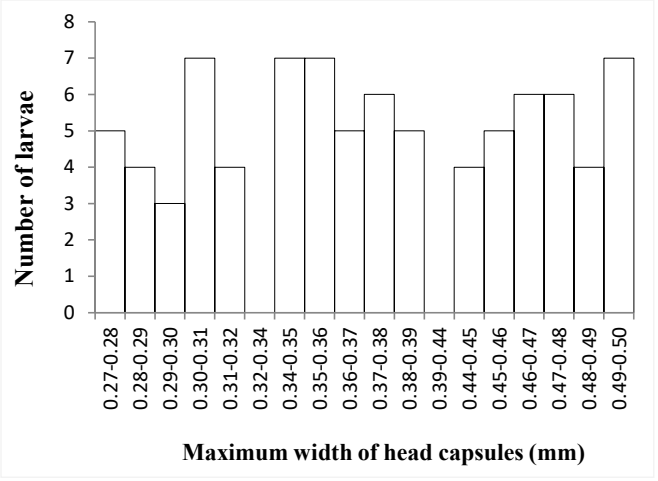


Fig 1. Frequency distribution of the maximum widths of head capsules of larvae of *Myrmica cachmiriensis*: (L1) first instar, (L2) second instar, and (L3) third instar.

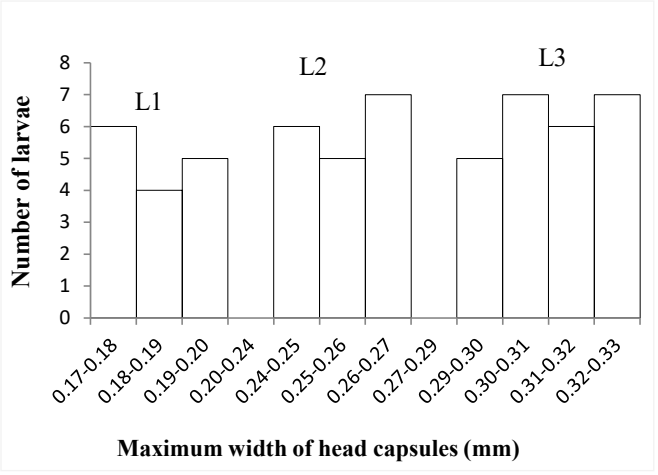


Fig 2. Frequency distribution of the maximum widths of head capsules of larvae of *Myrmica nitida*: (L1) first instar, (L2) second instar, and (L3) third instar.

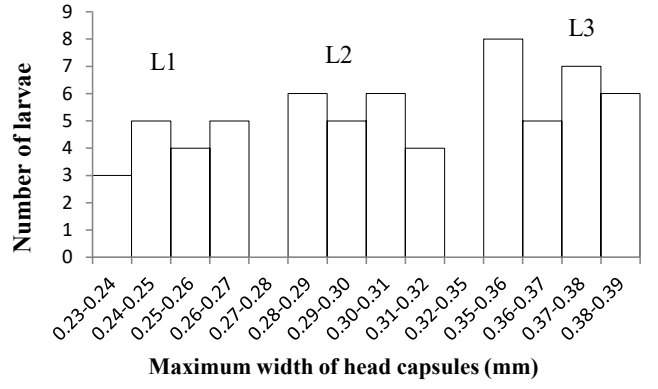


Fig 3. Frequency distribution of the maximum widths of head capsules of larvae of *Myrmica wardi*: (L1) first instar, (L2) second instar, and (L3) third instar.

Results

*Morphological description of the larval stages of **Myrmica cachmiriensis** Forel, 1904*

General aspect of larvae: The three instars share many characteristics; thus, a general description is given below, and the differences among the instars are given in Table 1.

Body: The body whitish in color, and in the first instar, the body profile is "pogonomyrmecoid" (i.e., "diameter greatest near middle of the abdomen, decreasing gradually toward the head and more rapidly toward posterior end, which is rounded; thorax slenderer than the abdomen and forming a neck, which is curved ventrally") but in the second and third instar, the body profile changes from "pogonomyrmecoid" to "pheidoloid," (i.e., "abdomen short, stout and straight; head ventral near anterior end, mounted on the short, stout neck, which is prothorax; ends rounded, one end more so than other; anus a ventral transverse slit" as in Wheeler & Wheeler, 1976) (Fig 4, 6, 10, 16). The body is covered with numerous unbranched hairs in three instars, denticulate on the distal half only (Fig 7, 12, 18). Ten pairs of unornamented spiracles, two thoracic and eight abdominal (Fig 13, 19). Anus ventral in position (Fig 5, 11, 17).

Head capsule: Head subpyriform in shape in all the instars; antennae positioned on the upper half of the cranium, with three sensilla (Fig 8, 14, 20). Head surface smooth with denticulate on the distal half only; two hairs on the genal region; three hairs on the vertex; two hairs on the frons region; four hairs on the clypeal region, and six on the occipital region. Clypeus clearly delimited from the cranium; upper surface of the clypeus smooth, without sensilla. A distinct row of four simple hairs is present along the distal clypeal border.

Mouthparts: Labrum bilobed, with six sensilla on the anterior surface (Fig 9, 21). Mandibles sharp-pointed, with one medial tooth, roughly ectatommoid in shape (Fig 9, 21). Maxilla paraboloidal in shape (Fig 9, 15, 21). Galea present as a small hump with three sensilla. Labium strongly hemispherical. The medial opening of the sericteries is positioned at the base of the hypopharynx. The hypopharynx is covered with dense spinules.

Table 1. Comparative morphological traits of larval stages of *Myrmica cachmiriensis* Forel, 1904.

Measured characters	First Larval Instar	Second Larval Instar	Third Larval Instar
Body length	1.44-1.53 mm (n = 23)	1.71-1.77 mm (n = 30)	2.79-2.88 mm (n = 32)
Body length through the spiracle	1.39 mm	1.46 mm	2.58 mm
Body width	1.05 mm	1.15 mm	1.39 mm
Length of body hairs			
Denticulate on the distal half only	53.37-74.91 µm (n = 10)	89.73-104.19 µm (n = 7)	151.37-176.53 µm (n = 7)
Diameter of mesothoracic spiracle	10.98-11.92 µm (n = 3)	12.63-12.98 µm (n = 3)	14.15-15.86 µm (n = 4)
Head length	0.37-0.39 mm (n = 4)	0.40-0.43 mm (n = 6)	0.48-0.50 mm (n = 6)
Head width	0.27-0.32 mm (n = 23)	0.34-0.35 mm (n = 30)	0.44-0.49 mm (n = 32)
Length of head hair			
Denticulate on the distal half only	101.87 µm	105.69 µm	107.24 µm
Width of labrum	102.48-103.29 µm (n = 3)	107.22-112.69 µm (n = 3)	128.69-135.43 µm (n = 3)
Mandible length	130.57-132.39 µm (n = 3)	135.72-138.65 µm (n = 4)	145.17-148.63 µm (n = 4)
Width of labium	125.14-130.90 µm (n = 3)	135.85-137.24 µm (n = 2)	144.71-148.39 µm (n = 3)
Length of galea	21.65 µm (n = 1)	23.63 µm (n = 1)	27.30 µm (n = 1)

*Morphological description of the larval stages of **Myrmica nitida** Radchenko and Elmes, 1999*

General aspect of larvae: The three instars share many characteristics; thus, a general description is given below, and the differences among the instars are given in Table 2.

Body: Body whitish in color, and in the first and second instar, the body profile is "pogonomyrmecoid" (i.e., "diameter greatest near middle of the abdomen, decreasing gradually toward the head and more rapidly toward posterior end, which is rounded; thorax slenderer than the abdomen and forming a neck, which is curved ventrally") but in the third instar, the body profile changes from "pogonomyrmecoid" to "aphaenogastroid," (i.e., "slightly constricted at first abdominal somite, diameter increasing gradually toward the middle of the thorax and the abdomen; thorax arched ventrally but not forming a distinct neck; posterior end broadly rounded" as in Wheeler & Wheeler, 1976) (Fig 22, 28, 40). The body is covered with numerous unbranched and branched hairs in the three instars; denticulate on the distal half only, deeply bifid branches long and flexuous, lanceolate, deeply bifid, and tip bifid (Fig 23, 24, 29, 30, 41, 42). Ten pairs of unornamented spiracles, two thoracic and eight abdominal (Fig 25, 31, 43). Anus ventral in position.

Head capsule: Head subpyriform in shape in all the instars; antennae positioned on the upper half of the cranium, with three

sensilla (Fig 32, 44). Head surface smooth with denticulate on the distal half only and Tip bifid hairs (Fig 33, 34); two hairs present on the genal region (Fig 26, 35); three hairs on the vertex; two hairs on the frons region; four hairs on the clypeal region and six on the occipital region. Clypeus clearly delimited from the cranium; upper surface of the clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border.

Mouthparts: Labrum bilobed, with six sensilla on the anterior surface (Fig 36). Mandibles sharp-pointed, with two medial teeth, roughly ectatommoid in shape (Fig 27, 37). Maxilla paraboloidal in shape. Galea present as a small hump with three sensilla (Fig 39). Labium is strongly hemispherical (Fig 36). The medial openings of the sericteries are positioned at the base of the hypopharynx. The hypopharynx is covered with dense spinules (Fig 38, 45).

Table 2. Comparative morphological traits of larval stages of *Myrmica nitida* Radchenko and Elmes, 1999.

Measured characters	First Larval Instar	Second Larval Instar	Third Larval Instar
Body length	1.46-1.50 mm (n = 15)	1.66-1.70 mm (n = 18)	1.92-1.96 mm (n = 25)
Body length through the spiracle	1.21 mm	1.32 mm	1.70 mm
Body width	0.93 mm	1.00 mm	1.16 mm
Length of body hairs			
a. Denticulate on the distal half only	135.86-140.62 µm (n = 3)	151.38-156.82 µm (n = 3)	191.95-200.49 µm (n = 3)
b. Deeply bifid branches long and flexuous	131.84-142.02 µm (n = 4)	151.46-160.74 µm (n = 4)	200.59-215.32 µm (n = 4)
c. Lanceolate	120.86-124.98 µm (n = 3)	-	-
d. Deeply bifid	-	-	160.23-168.49 µm (n = 3)
e. Tip bifid	-	107.92 µm	162.84 µm
Diameter of mesothoracic spiracle	5.16-5.35 µm (n = 3)	7.32-7.72 µm (n = 3)	8.08-8.98 µm (n = 4)
Head length	0.26-0.27 mm (n = 5)	0.30-0.31 mm (n = 5)	0.32-0.33 mm (n = 5)
Head width	0.17-0.19 mm (n = 15)	0.24-0.26 mm (n = 18)	0.30-0.34 mm (n = 25)
Length of head hair			
a. Denticulate on the distal half only	124.05 µm	160.83 µm	168.53 µm
b. Tip bifid	-	67 µm	78.46 µm
Width of labrum	114.36-117.29 µm (n = 3)	120.71-123.68 µm (n = 3)	124.58-127.20 µm (n = 3)
Mandible length	120.72-122.56 µm (n = 2)	130.26-134.02 µm (n = 3)	135.62-137.96 µm (n = 2)
Width of labium	101.54-103.96 µm (n = 3)	115.16-117.43 µm (n = 2)	182.37-186.52 µm (n = 3)
Length of galea	16.20 µm (n = 1)	21.34 µm (n = 1)	21.73 µm (n = 1)

Morphological description of the larval stages of ***Myrmica wardi*** *Radchenko and Elmes, 1999*

General aspect of larvae: The three instars share many characteristics; thus, a general description is given below, and the differences among the instars are given in Table 3.

Body: Body whitish in color, and in the first instar, the body profile is "pogonomyrmecoid" (i.e., "diameter greatest near middle of the abdomen, decreasing gradually toward the head and more rapidly toward posterior end, which is rounded; thorax slenderer than the abdomen and forming a neck, which is curved ventrally") but in the second and third instar, the body profile changes from "pogonomyrmecoid" to "myrmecoid," (i.e., "elongate and rather slender; curved ventrally; without differentiated neck; diameter diminishing only slightly from fifth abdominal somite to the anterior end; anus a subterminal transverse slit" as in Wheeler & Wheeler, 1976) (Fig 46, 47, 52, 53, 58). Body covered with numerous unbranched denticulate on the distal half, with hairs only in three instars (Fig 59). Ten pairs of unornamented spiracles, two thoracic and eight abdominal (Fig 48, 54, 60). Anus ventral in position.

Head capsule: Head subpyriform in shape in all the instars; antennae positioned on the upper half of the cranium, with three sensilla (Fig 49, 55, 61). Head surface smooth, with denticulate on the distal half only head hair (Fig 56, 62); two hairs present on the genal region; three hairs on the vertex; two hairs on the frons region; four hairs on the clypeal region, and six on the occipital region. Clypeus clearly delimited from the cranium; upper surface of the clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border.

Mouthparts: Labrum bilobed, with six sensilla on the anterior surface (Fig 50, 57, 63). Mandibles sharp-pointed, with two medial teeth, roughly ectatommoid in shape (Fig 50, 57, 63). Maxilla paraboloidal in shape (Fig 51). Galea present as a small hump with three sensilla. Labium strongly hemispherical. The medial opening of the sericteries is positioned at the base of the hypopharynx. The hypopharynx is covered with dense spinules.

Table 3. Comparative morphological traits of larval stages of *Myrmica wardi* Radchenko and Elmes, 1999.

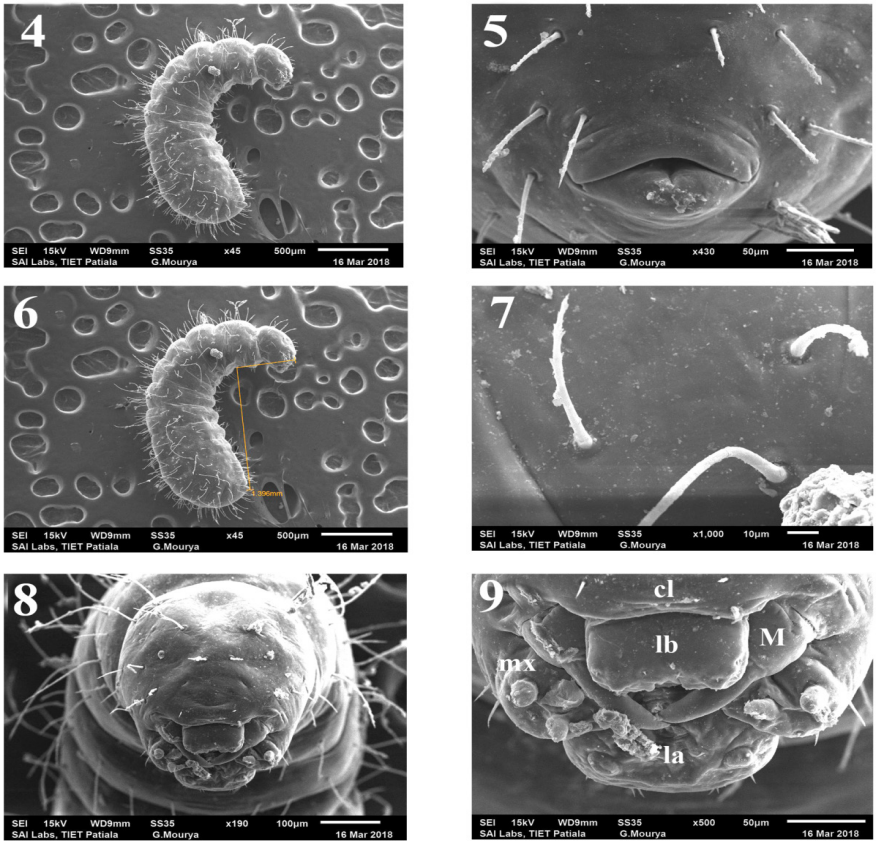
Measured characters	First Larval Instar	Second Larval Instar	Third Larval Instar
Body length	2.1-2.16 mm (n = 17)	2.49-2.58 mm (n = 21)	2.7-2.79 mm (n = 26)
Body length through the spiracle	1.32 mm	1.80 mm	2.74 mm
Body width	1.46 mm	1.34 mm	1.39 mm
Length of body hairs			
a. Denticulate on the distal half only	64.51-119.85 µm (n = 10)	154.11-179.98 µm (n = 8)	192.68-238.47 µm (n = 10)
Diameter of mesothoracic spiracle	14.35-15.95 µm (n = 3)	16.2-16.89 µm (n = 3)	17.54-19.14 µm (n = 4)
Head length	0.34-0.35 mm (n = 4)	0.36-0.37 mm (n = 5)	0.37-0.38 mm (n = 5)
Head width	0.23-0.27 mm (n = 10)	0.28-0.32 mm (n = 15)	0.35-0.39 (n = 26)
Length of head hair			
Denticulate on the distal half only	21.39 µm	32.10 µm	38.52 µm
Width of labrum	95.40-99.36 µm (n = 3)	103.81-108.36 µm (n = 3)	106.58-108.63 µm (n = 3)
Mandible length	98.43-101.60 µm (n = 3)	106.17-107.54 µm (n = 3)	112.31-114.38 µm (n = 3)
Width of labium	136.74-137.19 µm (n = 2)	139.26-140.87 µm (n = 2)	142.05-143.86 µm (n = 3)
Length of galea	11.74 µm (n = 1)	13.51 µm (n = 1)	20.64 µm (n = 1)

Discussion

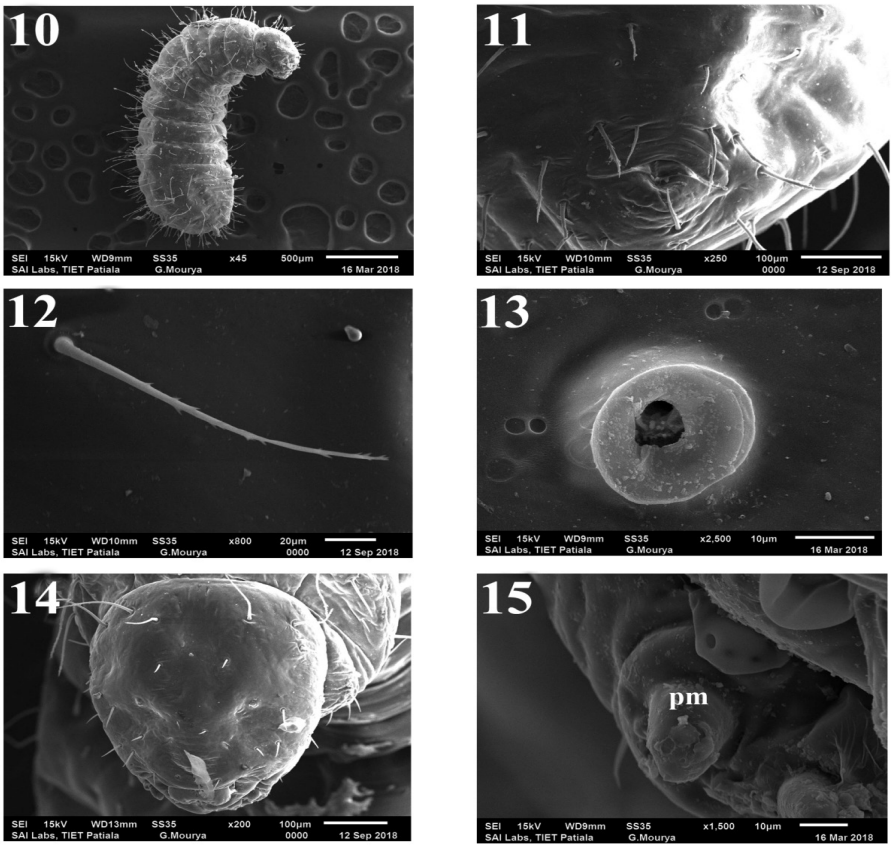
Wheeler and Wheeler (1976) proposed that the larvae of the genus *Myrmica* possess a relatively generalized body profile, described as Pogonomyrmecoid, and share several features, such as the position of the anus, segmentation, integumentary spinules, mandibular shape, and hypopharyngeal spinules with ancestral forms of Myrmicinae. In contrast, observations from the present study reveal marked variation in larval body profiles among the examined species: *M. cachmiriensis* shows a Pheidoloid form, *M. nitida* an Aphaenogastroid form, and *M. wardi* a Myrmecoid form. Hair morphology also differs considerably among these species (Table 4). The number of mandibular teeth varies as well, with *M. cachmiriensis* possessing a single medial tooth, whereas *M. nitida* and *M. wardi* exhibit two. Scanning electron microscopy further demonstrates notable interspecific diversity among *Myrmica* larvae, particularly in mandibular structure and pilosity patterns (Bharti et al., 2023b). These findings indicate that larval morphology within *Myrmica* is more heterogeneous than previously recognized, reflecting multiple adaptive modifications rather than a single "primitive" type. The expanded morphological dataset presented here provides valuable characters for comparative taxonomy. It may also serve as a foundation for future studies on the developmental biology and social behaviour of these ants.

Table 4. Comparison of 3rd larval instar of species categorized in the *cachmiriensis* and *rugosa* species group.

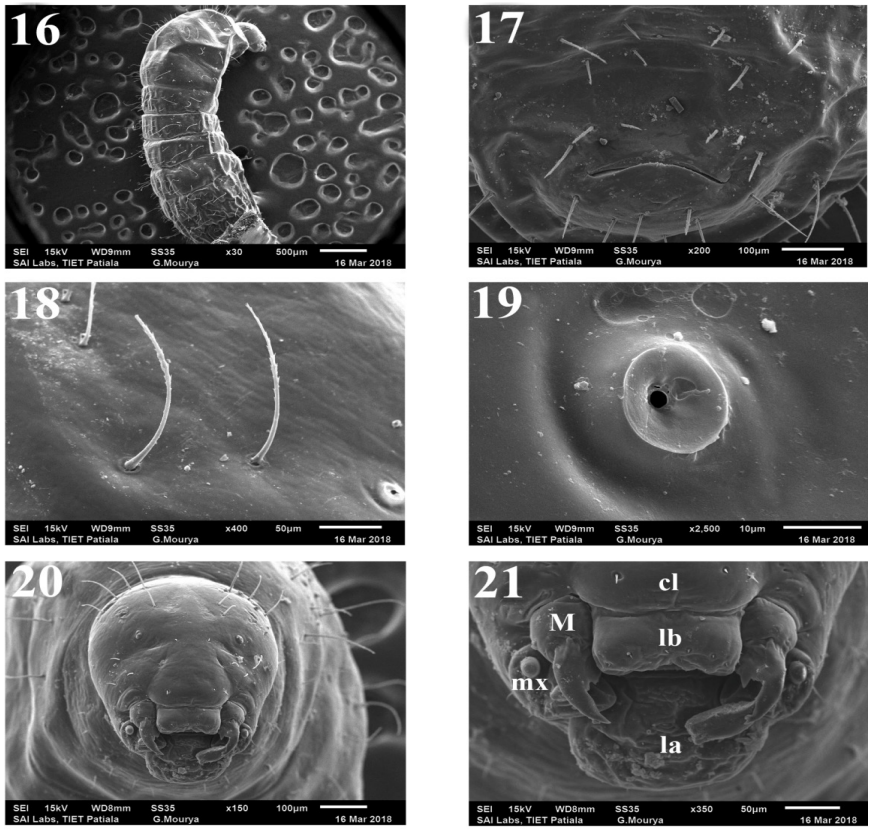
Morphological Characters		*cachmiriensis*	*nitida*	*wardi*	*hecate*	*rupestris*	*inezae*
Color		Whitish	Dull Whitish	Whitish	Brownish	Dull Whitish	Dull white
Profile		Pheidoloid	Aphaenogastroid	Myrmecoid	Pheidoloid	Pogonomyrmecoid	Pheidoloid
Body hair	**Shape of body hair**	Denticulate on the distal half	a. Denticulate b. Deeply bifid branches, long and flexuous c. Deeply bifid d. Tip bifid	Denticulate on the distal half	Denticulate on the distal half	Denticulate on the distal half	Denticulate on the distal half
Head	**Shape of head**	Subpyriform	Subpyriform	Subpyriform	Subquadrate	Subpyriform	Subpyriform
Type of Head hair	**Shape of head hair**	Denticulate on the distal half	Denticulate Tip bifid	Denticulate on the distal half	Denticulate on the distal half	Denticulate on the distal half	Denticulate on the distal half
Occipital border	**No. of hairs**	6	6	6	7-8	8	6
Vertex region	**No. of hairs**	3	3	3	2-3	4	3
Genal region	**No. of hairs**	4	4	4	4	4	2
Labrum	**Shape of labrum**	Bilobed	Bilobed	Bilobed	Bilobed	Bilobed	Bilobed
Mandibles	**Shape of mandibles**	Ectatommoid	Ectatommoid	Ectatommoid	Ectatommoid	Ectatommoid	Ectatommoid
	Medial teeth	1	2	2	1	1	1
Maxilla	**Shape**	Paraboloidal	Paraboloidal	Paraboloidal	Paraboloidal	Paraboloidal	Conoidal



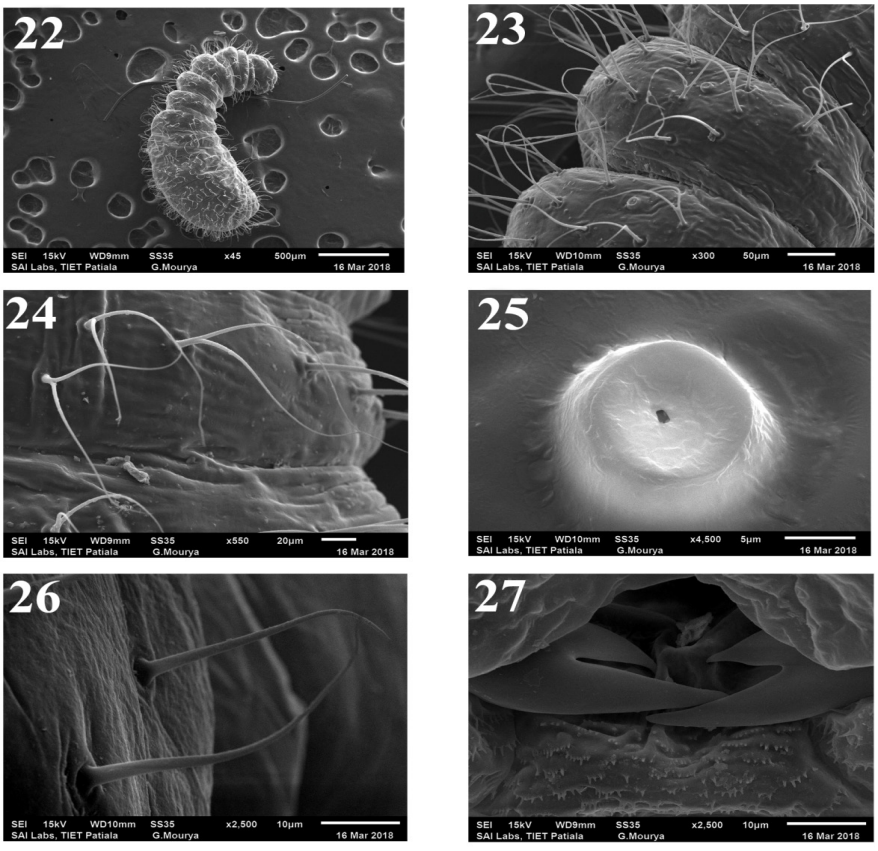
Figs 4-9. 4: Body profile of 1st larval instar of *M. cachmiriensis*; 5: detail of the terminal portion and transverse anus; 6: length through the spiracle; 7: unbranched denticulate hair; 8: subpyriform shaped head; 9: detail on the mouthparts: labrum (la), mandibles (M), labium (lb), clypeus (cl), maxilla (mx).



Figs 10-15. 10: Body profile of 2nd larval instar of *M. cachmiriensis*; 11: detail of the terminal portion and transverse anus; 12: unbranched denticulate hair; 13: unornamented mesothoracic spiracle; 14: subelliptical shaped head; 15: maxillary palp (pm).



Figs 16-21. 16: body profile of 3rd larval instar of *M. cachmiriensis*; 17: detail of the terminal portion and transverse anus; 18: unbranched denticulate hair; 19: unornamented mesothoracic spiracle; 20: subpyriform shaped head; 21: detail on the mouthparts: labrum (la), mandibles (M), Labium (lb), clypeus (cl), maxilla (mx).



Figs 22-27. 22: body profile of 1st larval instar of *M. nitida*; 23: denticulate on distal half only and lanceolate hair; 24: deeply bifid branches long and flexuous branched hair; 25: unornamented mesothoracic spiracle; 26: unbranched smooth slightly curved genal hairs; 27: mandible with one medial tooth.

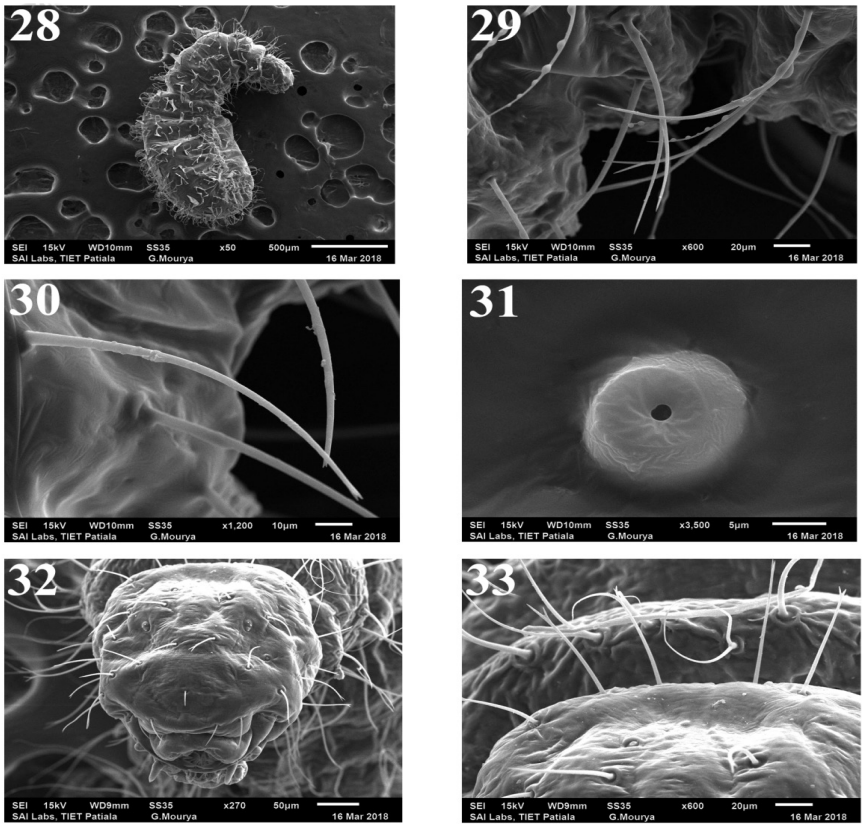
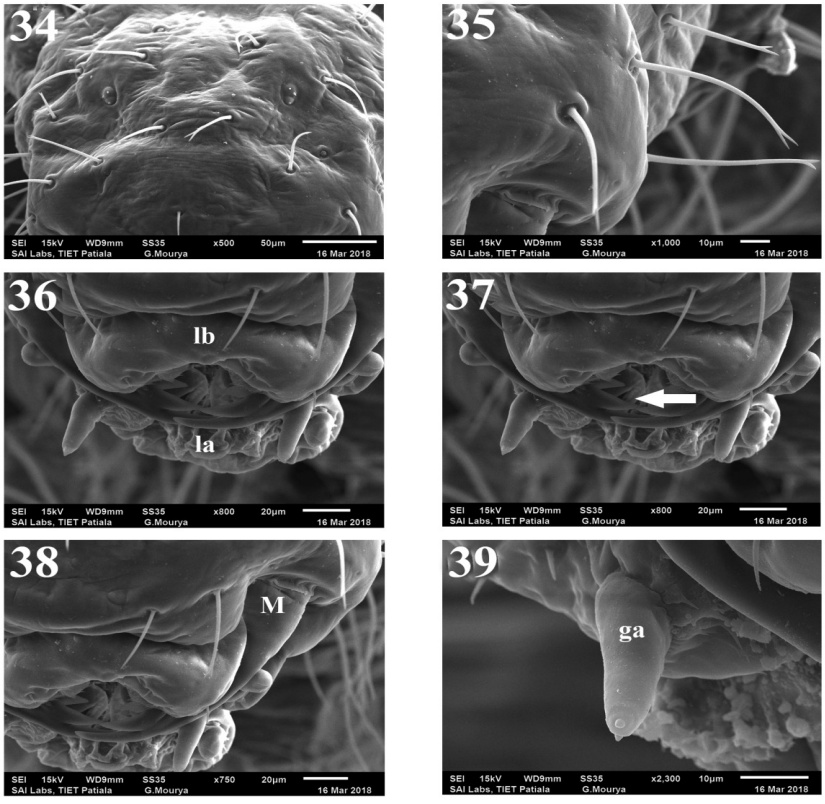
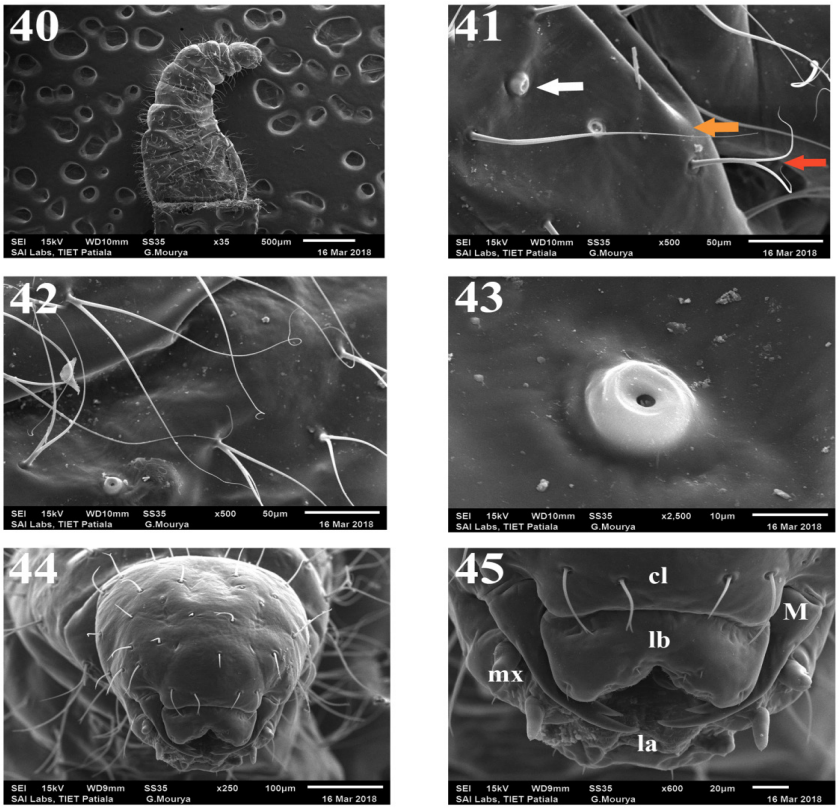


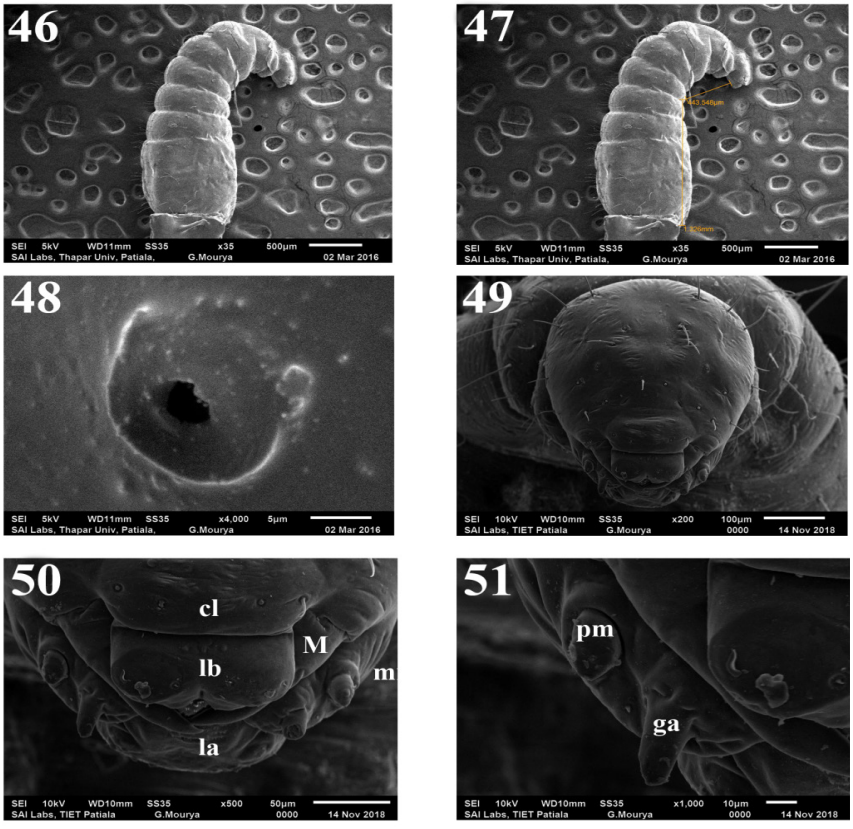
Fig. 28-33. 28: body profile of 2nd larval instar of *M. nitida*; 29: deeply bifid and Tip bifid hair; 30: unbranched denticulate hair; 31: unornamented mesothoracic spiracle; 32: subpyriform shaped head; 33: unbranched denticulate on distal half only and Tip bifid head hair



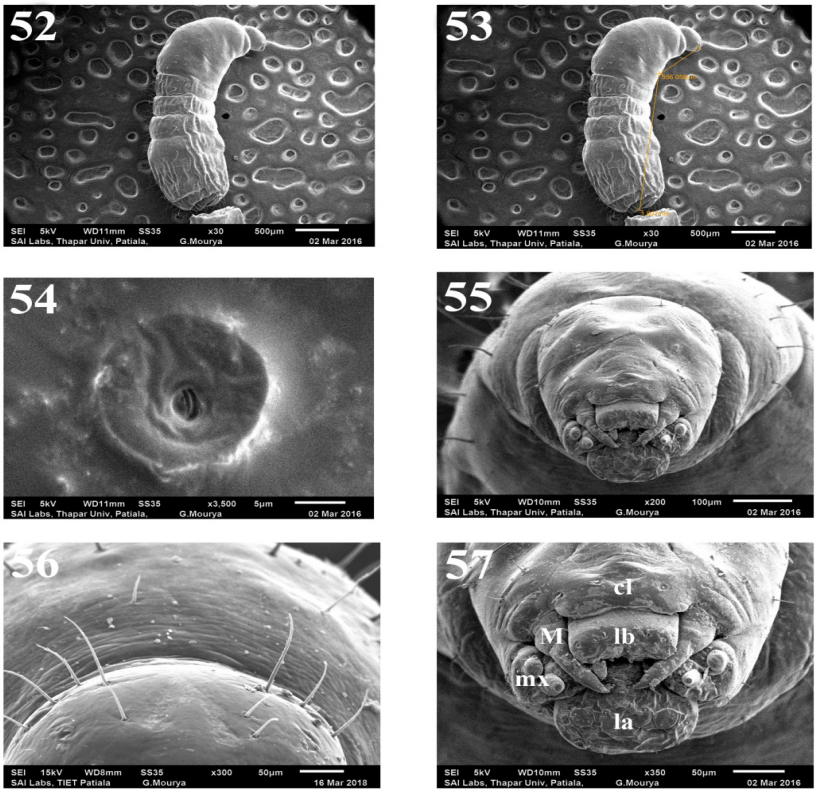
Figs 34-39. 34: unbranched denticulate on distal half only and tip bifid head hair; 35: unbranched smooth slightly curved genal hairs; 36: detail on the mouthparts: labrum (la) and Labium (lb); 37: mandible with two medial teeth; 38: detail on the mouthparts: labrum (la), mandibles (M), Labium (lb), clypeus (cl); 39: galea (ga).



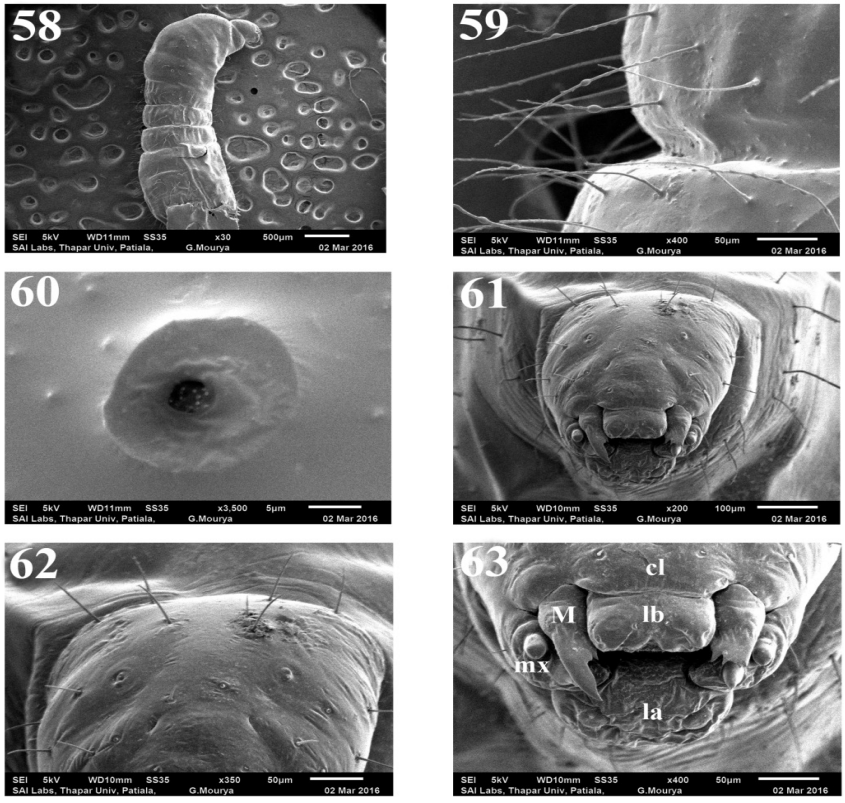
Figs 40-45. 40: body profile of 3rd larval instar of *M. nitida*; 41: Denticulate on distal half only (orange arrow), deeply bifid branched hair (red arrow) and abdominal spiracle (white arrow); 42: deeply bifid branches long and flexuous branched hair; 43: unornamented mesothoracic spiracle; 44: subpyriform shaped head; 45: detail on the mouthparts: labrum (la), mandibles (M), Labium (lb), clypeus (cl), maxilla (mx).



Figs 46-51. 46: body profile of 1st larval instar of *M. wardi*; 47: length through spiracle; 48: unornamented mesothoracic spiracle; 49: subpyriform shaped head; 50: detail on the mouthparts: labrum (la), mandibles (M), labium (lb), clypeus (cl), maxilla (mx); 51: detail on right maxilla, showing Galea (ga) and maxillary palpus (pm).



Figs 52-57. 52: body profile of 2nd larval instar of *M. wardi*; 53: length through spiracle; 54: unornamented mesothoracic spiracle; 55: subpyriform shaped head; 56: unbranched denticulate on distal half head hair; 57: detail on right maxilla, showing Galea (ga) and maxillary palpus (pm).



Figs 58-62. 58: body profile of 3rd larval instar of *M. wardi*; 59: unbranched denticulate hair; 60: unornamented mesothoracic spiracle; 61: subpyriform shaped head; 62: unbranched denticulate on distal half head hair; 63: detail on the mouthparts: labrum (la), mandibles (M), Labium (lb), clypeus (cl), maxilla (mx).

Authors' Contribution

HB: Conceptualized and supervised the study.

MB: Data compilation and preparation of manuscript.

PK: Conducted scanning electron microscopy and light microscopy analyses, performed morphometric measurements, and prepared figures and tables.

JK: Data compilation, and literature review.

All authors contributed to data interpretation, reviewed, and approved the final manuscript.

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