



RESEARCH ARTICLE - ANTS

On the Description of the Larval Stages of *Myrmica inezae* Forel, 1902 (Hymenoptera: Formicidae: Myrmicinae), an Endemic Himalayan Species, with Notes on its Natural History

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Abstract

Of the known species groups in *Myrmica*, the *M. inezae* belong to the *inezae*-group, which is restricted between an altitude range of 1900-3000m in the himalayas. The species in question is probably relictual as it looks like more basal species of the *ritae*-group. Herein, a detailed description of larval instars of *Myrmica inezae* Forel, 1902 is provided. Three larval instars are reported in this species. The larvae exhibit a pheidoloid body profile, which otherwise is pogonomyrmecoid in the larval stages of genus *Myrmica* Latreille, 1804 reported hitherto. *M. inezae* differs from the earlier described larval stages of *M. rugosa* and *M. rupestris* (*rugosa*-group) based on number of occipital hairs (six in *M. inezae* while these are eight in *M. hecate* and *M. rupestris*); mandibular teeth (two in *M. inezae* as compared to one in *M. hecate* and *M. rupestris*) and shape of maxilla (conoidal in *M. inezae* whereas paraboloidal in *M. hecate* and *M. rupestris*).

Introduction

The genus *Myrmica* Latreille, 1804 encompasses 164 valid species from the Old World, out of which 43 known species are distributed in the Central Asian Mountains (Radchenko & Elmes, 2010; Bharti, & Sharma 2011a, b, c; Bharti 2012a, b; Bharti & Sharma, 2013; Bharti et al., 2016a, b). The *Myrmica* species generally occur in grasslands, under stones, in logs, in meadows, and forests. As an adaptation to high altitude, the larvae of *Myrmica* develop slowly; the estimated development period from an egg to a fertile queen is almost 1.5 to 2 years. The hostile winter season interrupts the development process (Bharti et al., 2016b; Bharti et al., 2023).

Myrmica inezae Forel, 1902 is distributed at an altitudinal range of 1,900-3,000m in the Himalaya. The workers are characterized by a specific morphology, long scape, petiole, and propodeal spines, which place them close to members

of the *ritae* species group (Radchenko & Elmes, 2010). The species is endemic to Indian Himalaya; the colonies are polygynous and nest under stones and rotten logs in shady places. This ant dwells well at a temperature range of 21-30 °C. Each colony consists of 100-150 workers. Alates emerge in June and July and immediately take up a nuptial flight. The mated queen overwinters till March of next year. The mated queens are mostly adopted by the established colonies, probably a strategy to overcome harsh environmental conditions, as solitary hibernation could be lethal (Bharti et al., 2016b).

The immature stages of an organism provide a window into its developmental patterns. The significance of this was realized much early in the 18th century by Ernst Haeckel. Unfortunately, very few studies have been conducted to describe the immature stages of insects. Out of almost 15,000 described species of ants, larval forms of nearly 800 species (a mere 0.5% of the extant species) have been described



to date (Wheeler & Wheeler 1953, 1986, 1990). Most of these descriptions are based on examining a few specimens and do not provide exact information about the number of larval instars (Solis et al., 2010; Bharti et al., 2019). Probably biologists are yet to realize the significance of larval forms in providing a holistic view of an organism's natural history. Undoubtedly, some recent studies have addressed this issue, but it still requires greater attention (Fox et al., 2017 a, b). Nevertheless, some of the prominent works have provided complete larval descriptions of ant species (Masuko, 1990; Alvares et al., 1993; Solis et al., 2010; Fox et al., 2017 a, b). From India, ultra-structure studies on the immature stages have been carried out on *Pheidole indica* (Bharti & Gill, 2011) and *Aphaenogaster bessonii* (Bharti et al., 2013). More recently, Bharti et al., 2019 described ant larvae of two species of genus *Myrmica* that is *Myrmica hecate* Weber, 1947 and *Myrmica rupestris* Forel, 1902. Herein, we provide detailed description of three larval instars of Himalayan species of *Myrmica inezae* Forel, 1902, which will provide an insight in better understanding of immature stages in ants.

Materials and Methods

Immatures of *M. inezae* were collected from Kothi (2,600 m.a.s.l.) located in the state of Himachal Pradesh. All immatures were 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 for each stage) were prepared for scanning electron microscopy. For SEM analysis, the specimens were dehydrated in a graded ethanol series and then critical point dried in a desiccator so as to remove water and air from samples and to maintain a dry environment. The dried specimens were attached to the aluminum 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 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 observations under a Radical compound microscope.

The terminology given by Wheeler and Wheeler (1976) and Fox et al. (2007) has been used to describe the larval stages of *M. inezae*. The voucher specimens of *M. inezae* have been deposited at PUAC (Punjabi University Patiala Ant Collection) hosted at Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India. The following abbreviations are employed in Table 2 with adaptations as in Solis et al. (2012): (BL) body length; (BLS) body length through spiracles; (BW) medial width of body; (DS) diameter of spiracles; (HW) head capsule width; (LLA) length of labium; (LBH) length of body hairs; (ML) length of mandible; (LM) length of maxilla; (WL) width of labrum; (n) number of observations; (NHH-B) number of hairs on occipital border; (NHH-C) number of hairs on clypeus; (NHH-F) number of hairs on frons; (NHH-G) number of hairs on gena; (NHH-V) number of hairs on vertex; (NSA) number of sensilla on antennae; (NSLP) number of sensilla on labial palp; (NSMP) number of sensilla on maxillary palp.

Results

Larval Instar Determination: According to the Dyar's rule, the head capsules of insect larvae grows in geometrical progression, increasing at a constant rate that varies between 1.1 to 1.9, usually around 1.4 (Parra & Haddad, 1989). The mean head growth rate between successive instars in *M. inezae* was calculated as 1.29 (Table 1).

Table 1. Mean maximum head capsule widths and growth ratio of the three larval instars of *Myrmica inezae*.

Instar	Range (mm)	Mean (mm)	Growth rate between instars	Mean growth rate
L1 (n = 23)	0.24-0.28	0.25		
L2 (n = 26)	0.34-0.36	0.34	1.36	1.29
L3 (n = 29)	0.40-0.44	0.42	1.23	

First larval instar

Body: Whitish, slender in profile, aphaenogastroid in shape (Fig 2), slightly constricted at first abdominal somite, diameter increasing gradually toward middle of thorax and of abdomen; thorax arched ventrally but not forming a distinct neck; posterior end broadly rounded. Body 1.41-1.47 mm long (n=23) x 1.00 mm wide (n=1). Width of anterior, median and posterior end of body 377.09 μ m, 490.76 μ m & 597.08 μ m (n=1) respectively (Fig 3). Length through spiracles 1.30 mm (n=1) (Fig 4). Unbranched denticulate (distal half) hair measures about 60.39-86.50 μ m (n=10) in

length, distance between two adjacent hairs 75.35-98.66 μ m (n=8). Body spiracle ten in number, diameter of mesothoracic spiracle 12.85-13.69 μ m (n=3) (Fig 5), spiracle opening unornamented, diameter of spiracle opening 2.47 μ m (n=1). Distance between mesothoracic to metathoracic spiracle 190.63-199.73 μ m (n=6).

Head capsule: Head 0.29-0.30 mm long (n=5) x 0.24-0.28 mm wide (n=23) and subpyriform in shape (Fig 6). Height of cranium 211.64 μ m (n=1). Elevated antennae positioned on the upper half of cranium with 3 sensilla and distance between antennae measures about 146.0 μ m (n=1). Head surface smooth with unbranched denticulate (distal half) hair (Fig 7).

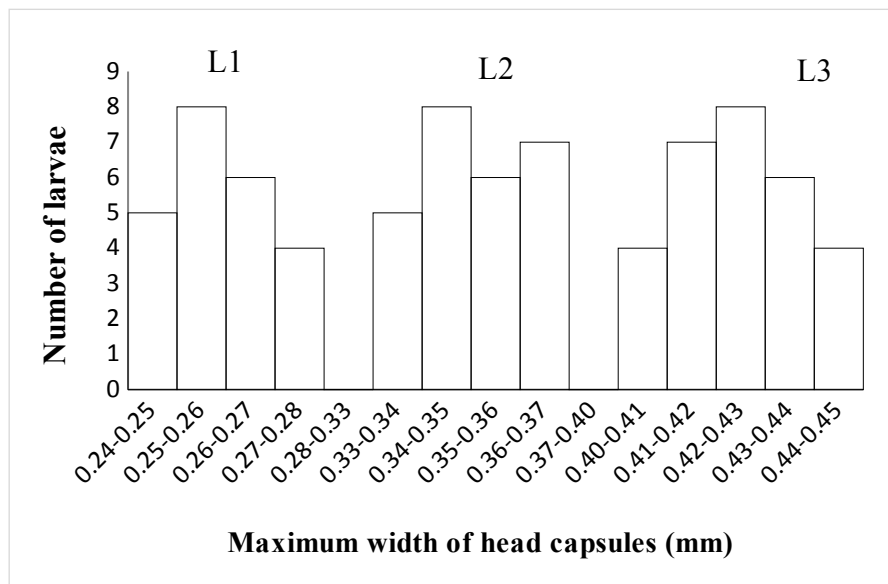
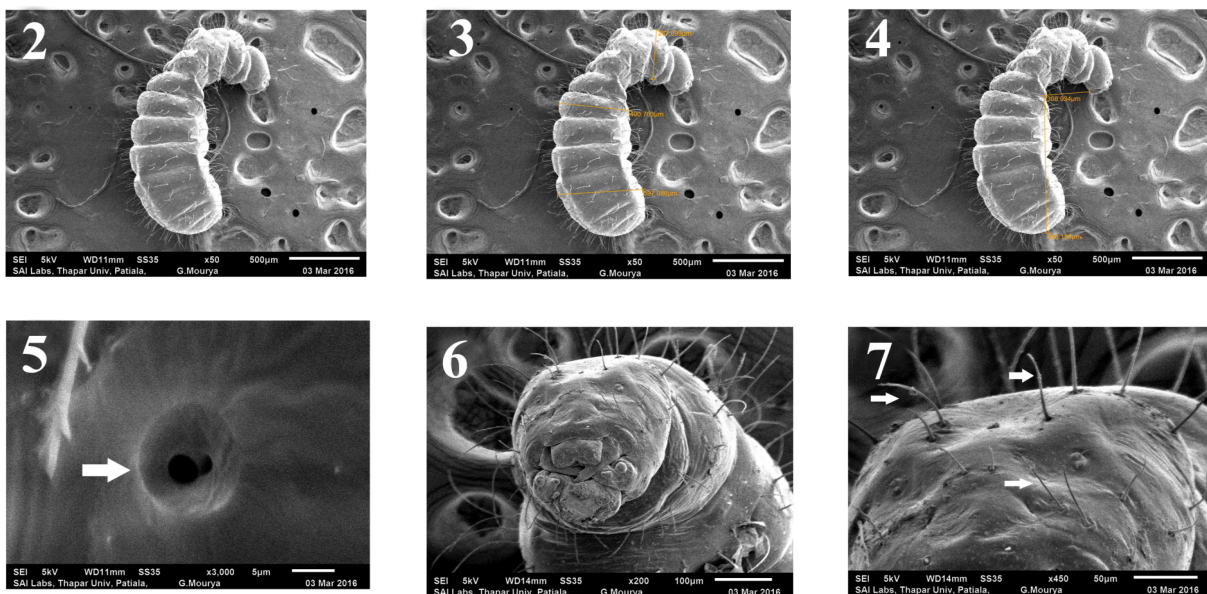


Fig 1. Frequency distribution of the maximum widths of head capsules of larvae of *Myrmica inezae*: (L1) first instar, (L2) second instar, and (L3) third instar.

Tentorial pits well- demarcated and distance between the two 76.96 μm ($n=1$). Six hairs observed on occipital border, three on vertex and two each on genae and frons region. Length of hair on the occipit, vertex, genae and frons 51.96 μm , 52.42 μm , 20.60 μm and 31.78 μm ($n=1$) respectively. Distance between two adjacent hairs on occipital 48.81 μm ; vertex 90.24 μm ; genal region 23.73 μm and frons 20.04 μm ($n=1$).

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,

width of clypeus 102.98 μm ($n=1$). Labrum bilobed, with six sensilla over the anterior surface, width of labrum 86.29-88.36 μm ($n=3$). Mandibles simple, roughly ectatommoid in shape, 101.34-105.42 μm long ($n=3$). Maxilla 74.62 μm long x 58.09 μm wide ($n=1$) and roughly conoidal in shape ($n=1$). Galea present as a small hump culminating with three sensilla, length of galea measures about 19.07 μm ($n=1$); maxillary palpus paxilliform. Labium hemispherical in shape, 120.59-123.51 μm wide ($n=3$). The medial opening of sericteries positioned at the base of hypopharynx. The hypopharynx is covered with spinules.



Figs 2-7: Fig 2. body profile of 1st larval instar; Fig 3. width of anterior, median & posterior end; Fig 4. length through spiracle; Fig 5. Unornamented mesothoracic spiracle; Fig 6. subpyriform shaped head; Fig 7. head hair.

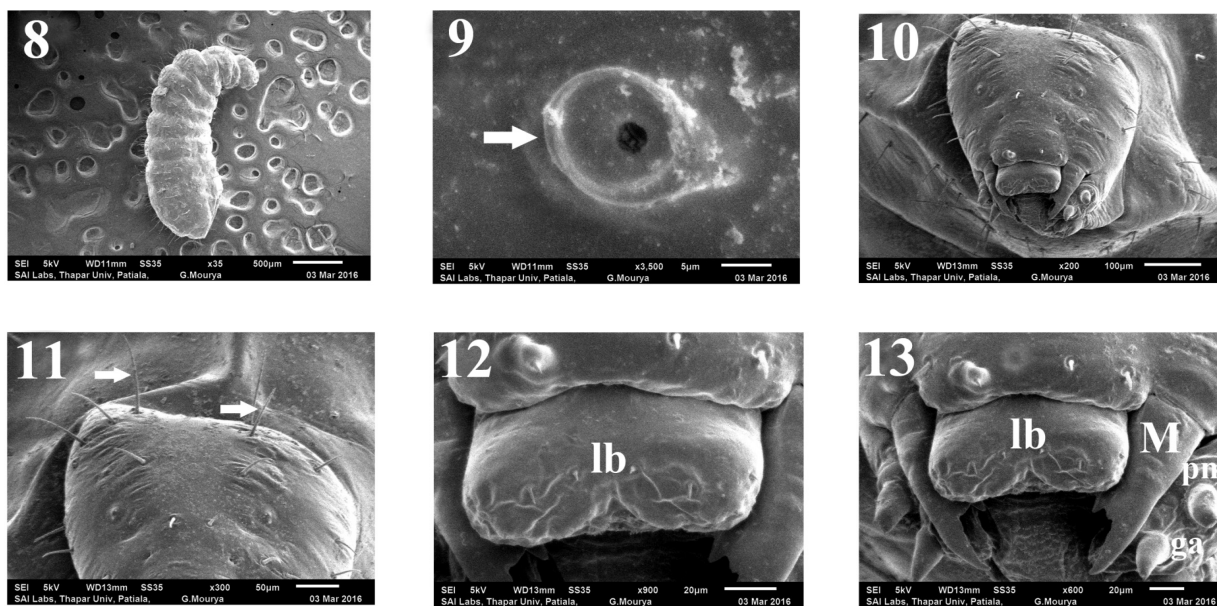
Second larval instar

Body: Dull whitish, body profile pheidoloid in shape (Fig 8), anus a ventral transverse slit. Body about 1.95-2.01 mm long (n=26) x 1.16 mm wide (n=1). Length through spiracles 1.70 mm (n=1). Width of anterior, median and posterior end of body was found to be 438.02 μ m, 552.75 μ m & 723.98 μ m (n=1) respectively. Unbranched denticulate (distal half) hair measures 98.52-110.94 μ m (n=10) in length and distance between two adjacent hairs 101.27-118.3 μ m (n=8). Body spiracle 10 in number, diameter of mesothoracic spiracle 14.00-15.18 μ m (n=3) (Fig 9), spiracle opening unornamented, diameter of opening of spiracle 3.09 μ m (n=1), distance between mesothoracic to metathoracic spiracle 235.94-248.69 μ m (n=5).

Head capsule: Head 0.36-0.37 mm long (n=4) x 0.34-0.36 mm wide (n=26) subpyriform in shape (Fig 10). Height of cranium measures 288.92 μ m (n=1). Elevated antennae positioned on the upper half of cranium with 3 sensilla and distance between antennae 178.03 μ m (n=1). Head surface smooth with unbranched denticulate (distal half) hairs (Fig 11).

Tentorial pits well-demarcated, and the distance between the two is 97.51 μ m (n=1). Six hairs observed on occipital border, three on vertex and two each on genae and frons. Length of hairs on occipital border, vertex, genae and frons 62.94 μ m, 86.69 μ m, 31.39 μ m, 52.69 μ m (n=1), respectively, and distance between two adjacent hairs on the occipital border 83.80 μ m, vertex 115.11 μ m, genal region 39.53 μ m and frons 40.01 μ m (n=1) respectively.

Mouthparts: Clypeus delimited from the cranium, upper surface of clypeus smooth, without sensilla; a distinct row of four simple hairs present along the distal clypeal border, width of clypeus about 134.03 μ m (n=1). Labrum bilobed, with six sensilla over the anterior surface, width of labrum 114.20-116.69 μ m (n=3) (Fig 12). Mandibles simple, sharp-pointed, ectatomoid in shape, 109.48-113.74 μ m long (n=3), two medial teeth present. Maxilla 79.51 μ m long x 62.59 μ m wide (n=1) and roughly conoidal in shape. Length of galea 27.75 μ m (n=1), skewed peg with three sensilla. Maxillary palpus paxilliform. Labium hemispherical in shape, 132.37-137.08 μ m wide (n=3) (Fig 13). The medial opening of sericteries positioned at the base of hypopharynx. The hypopharynx is covered with spinules.



Figs 8-13: Fig 8. body profile of 2nd instar; Fig 9. unornamented mesothoracic spiracle; Fig 10. subpyriform shaped head; Fig 11. head hair; Fig 12. labrum; Fig 13. mouthparts.

Third larval instar

Body: Brownish, body profile pheidoloid in shape same as a second instar (Fig 14); anus a ventral transverse slit. Body about 2.94-3.00 mm long (n=29) x 1.73 mm wide (n=1). Length through spiracles 1.84 mm (n=1). Width of anterior, median and posterior end of the body was found to be 698.81 μ m, 986.18 μ m, & 1.19 mm (n=1), respectively. Unbranched denticulate (distal half) hair (Fig 15) measures 120.37-142.30 μ m (n=11) in length and distance between two adjacent

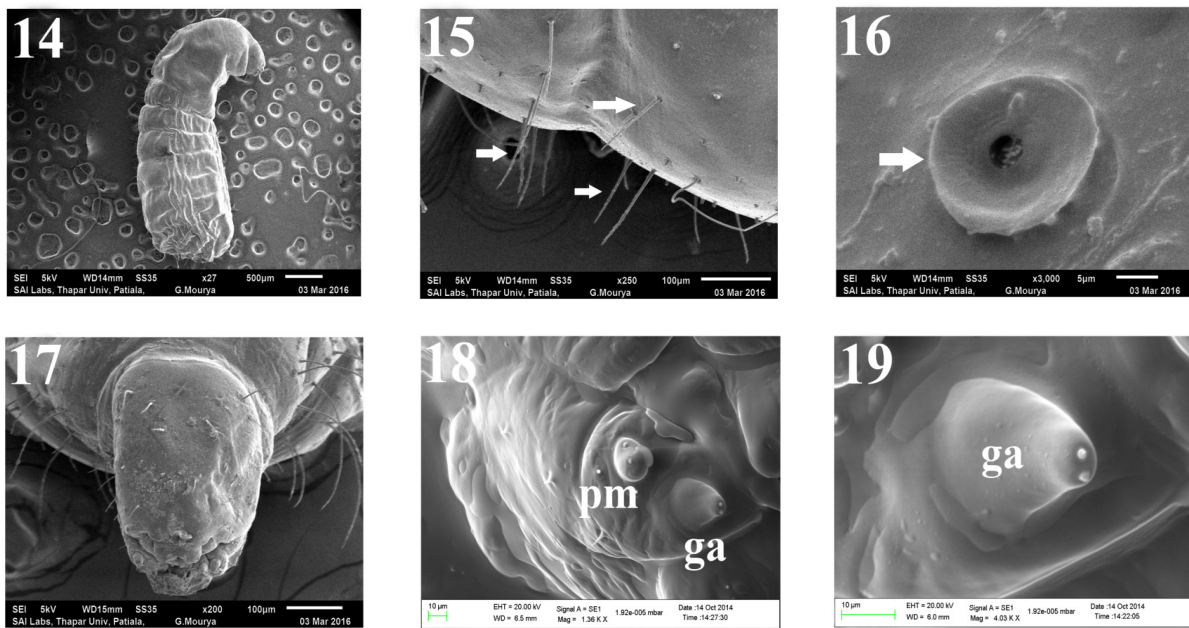
hairs 120.39-135.28 μ m (n=6). Body spiracles 10 in number, diameter of mesothoracic spiracle 19.37-21.73 μ m (n=3), spiracle opening unornamented (Fig 16), diameter of opening of spiracle 4.13 μ m (n=1), distance between mesothoracic to metathoracic spiracle 446.99-455.85 μ m (n=6).

Head capsule: Head 0.38-0.40 mm long (n=5) x 0.4-0.44 mm wide (n=29) subpyriform in shape (Fig 17). Height of cranium measures 304.41 μ m (n=1). Elevated antennae positioned on the upper half of cranium with three sensilla, and the distance between antennae measures about 252.0 μ m (n=1).

Head surface smooth with unbranched denticulate (distal half) hairs. Tentorial pits well-demarcated and distance between the two 113.62 μm (n=1). Six hairs observed on the occipital border, three on vertex, two each on genae and frons. Length of hairs on the occipital border, vertex, genae and frons 70.26 μm , 94.74 μm , 41.93 μm , 63.58 μm (n=1), respectively, and distance between two adjacent hairs on the occipital border 195.78 μm , vertex 123.05 μm , genal region 45.19 μm and frons 57.12 μm (n=1) respectively.

Mouthparts: Clypeus delimited from the cranium, upper surface of clypeus smooth, without sensilla; a distinct row

of four simple hairs present along the distal clypeal border, width of clypeus measures about 186 μm (n=1). Labrum bilobed, with six sensilla over the anterior surface, width of labrum 171.00-173.47 μm (n=3). Mandibles simple, ectatomoid in shape, 145.98-147.19 μm long (n=3), two medial teeth present. Maxilla 83.72 μm long x 69.43 μm wide (n=1) and conoidal in shape (Fig 18). Galea present as a simple small hump culminating with three sensilla, 29.99 μm (n=1) (Fig 19). Maxillary palpus paxilliform. Labium stout and strongly hemispherical in shape, 146.93-150.17 μm wide (n=4). The medial opening of sericteries positioned at the base of hypopharynx.



Figs 14-19: Fig 14. body profile of 3rd instar; Fig 15. unbranched denticulate hair; Fig 16. unornamented mesothoracic spiracle; Fig 17. subpyriform shaped head; Fig 18. conoidal shaped maxilla; Fig 19. galea.

Discussion

The first ever description of mature worker larva of *Myrmica* was provided by Wheeler and Wheeler (1976). However, the diagnosis was based on few larval forms of *Myrmica* species and without clear demarcation into larval instars. The generalized broad diagnostic features include: body pognomyrmecoid; denticulate on distal half hairs; mandibles ectatomoid; ten numbers of unornamented spiracles and bilobed labrum. Similar types of observations were made by Mizutani and Yamane (1978). Recently, Bharti and co-workers (2019) provided a detailed diagnosis of two Himalayan species (*Myrmica hecate* Weber, 1947 and *Myrmica rupestris* Forel, 1902). The adults of these two species belong to *rugosa* species group (Radchenko & Elmes, 2010), an informal clustering based on certain morphological resemblances. The third instar of these two species differed from one another based on few morphological characters (Table 3). Rest of the traits show similarity in morphology

hence appear constrained in the developmental stages of these two species. The species in question, *M. inezae* differs remarkably from the adults of *rugosa* species group, thus placed in the *inezae* species group. The females of the *inezae* share several features with females of the *ritae* species group, and it is assumed that the *inezae* group is a relict of ancient fauna closely related to the *ritae* species group. However, based on current distribution records, *M. inezae* is restricted to North-west Himalaya and in contrast *M. rugosa* and *M. rupestris* are widely distributed throughout the Himalayan range. Notably, *M. inezae* has been reported from a few nesting sites. The life cycle appears to be slow as compared to rest of the *Myrmica* species which mostly occupy open habitats (Bharti et al., 2016b).

Wheeler and Wheeler (1976) selected 46 morphological characters to larvae belonging to 156 ant genera to work out the 'specialization index' of each taxon. They compared each genus and family with a hypothetical ant larvae, possessing a combination of plesiomorphic morphological characters.

Table 2. Body and head capsule measurements of larvae of *Myrmica inezae*.

Character	1 st instar	2 nd instar	3 rd instar	
Profile	Aphaenogastroid	Pheidoloid	Pheidoloid	
BL	1.43 ± 0.02 mm (1.41-1.47) n = 23	1.97 ± 0.02 mm (1.95-2.01) n = 26	2.96 ± 0.02 mm (2.94-3) n = 29	
BLS	1.30 mm	1.70 mm	1.84 mm	
BW	490.76 µm	552.75 µm	986.18 µm	
LBH	72.91 ± 9.84 µm (60.39-86.50) n = 10	104.00 ± 4.24 µm (98.52-110.94) n = 10	134.04 ± 7.13 µm (120.37-142.30) n = 11	
DS	13.18 ± 0.44 µm (12.85-13.69) n = 3	14.58 ± 0.59 µm (14.00-15.18) n = 3	20.40 ± 1.20 µm (19.37-21.73) n = 3	
HW	0.25 ± 0.01 mm (0.24-0.28) n = 23	0.34 ± 0.01 mm (0.32-0.36) n = 26	0.42 ± 0.01 µm (0.4-0.44) n = 29	
ML	103.57 ± 2.06 µm (101.34-105.42) n = 3	112.02 ± 2.24 µm (109.48-113.74) n = 3	146.5 ± 0.62 µm (145.98-147.19) n = 3	
LM	74.62 µm n = 1	79.51 µm n = 1	83.72 µm n = 1	
WL	87.46 ± 1.06 µm (86.29-88.36) n = 3	115.55 ± 1.25 µm (114.20-116.69) n = 3	172.2 ± 1.23 µm (171.00-173.47) n = 3	
WA	122.32 ± 1.53 µm (120.59-123.51) n = 3	134.666 ± 2.35 µm (132.37-137.08) n = 3	148.565 ± 1.58 µm (146.93-150.17) n = 4	
NHH	B	6 n = 5	6 n = 8	6 n = 7
	C	4 n = 3	4 n = 2	4 n = 2
	F	2 n = 1	2 n = 1	2 n = 1
	G	4 n = 1	4 n = 1	4 n = 1
	V	3 n = 1	3 n = 1	3 n = 1
NSA	3 n = 8	3 n = 6	3 n = 5	
NSLP	3 n = 3	3 n = 4	3-4 n = 5	
NSMP	5-6 n = 3	5-7 n = 4	6-7 n = 5	

The family Myrmicinae is a heterogenous assemblage and different genera show varied adaptations according to their foraging behaviour and nesting habitats. Wheeler and Wheeler (1976) observed that Myrmicini is a generalised tribe encompassing both the plesiomorphic and apomorphic morphological characters. The ant larval characters are adaptive and differ within the genus as well. Based on available data on larval morphology in *Myrmica*, it was observed that the larval forms of *M. inezae* show significant differences from the earlier recorded larval forms of *M. hecate* and *M. rubepristis* (Bharti et al., 2019). The number of occipital hairs recorded in *M. inezae* are six while these are eight in *M. hecate* and

M. rubepristis; two mandibular teeth in *M. inezae* as compared to one in *M. hecate* and *M. rubepristis* and conoidal shape of maxilla has been observed in *M. inezae* while in *M. hecate* and *M. rubepristis* the shape is paraboloidal. Interestingly, the shape of maxilla and number of occipital hairs are plesiomorphic in *M. inezae* while the number of mandibular teeth represents an apomorphy. Also in the referred species, with every passing molt, all three larval forms exhibit similar morphological characteristics except the body profile in first larval instar is aphaenogastroid. However, in the case of second and third larval forms, the body profile shifts from aphaenogastroid to pheidoloid type.

Table 3. Comparison of larval morphology between *Myrmica hecate* Weber, 1947, *Myrmica rupestris* Forel, 1902 and *Myrmica inezae* Forel, 1902.

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

Authors' Contributions

HB: Conceptualization, investigation.

MB: formal analysis, writing-original draft, writing-review & editing.

PG: investigation.

JK: data curation, formal analysis.

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Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: Not applicable.

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