



# Ultrastructure of Larval Instars of *Artaxa vitellina* Kollar and *Maeoproctis latifascia* Walker (Insecta: Lepidoptera: Lymantriinae) by using Scanning Electron Microscopy from Himachal, India

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**Abstract:** The current study delves into comparing the ultrastructure of antennae and mouthparts of two moth larvae, *Artaxa vitellina* Kollar and *Maeoproctis latifascia* Walker, from the same subfamily, Lymantriinae (Family: Erebidae). It identifies distinct differences in their sensory structures, particularly in the distribution of medial sensilla of the maxillary palp and sensilla basiconica of the antenna across larval stages. Moreover, it underscores the significance of incorporating larval morphological characteristics to enhance taxonomic classification and offer insights for future electrophysiological studies on pest behaviour and phylogenetic analyses.

**Keywords:** Lepidoptera, Moths, Larvae, Caterpillars, Ultrastructure, Sensilla, Scanning electron microscopy, Statistical analysis, Taxonomy, Immature stages

Lepidoptera is the third-largest group of insects, mainly consisting of moths. These insects undergo complete metamorphosis, progressing through egg, larva, pupa, and adult stages. Larvae are particularly useful for taxonomic classification. This study focuses on using the distribution of sensilla (sensory organs) on larvae's antennae and mouthparts as a basis for classification. Sensilla are found on various body parts, but antennae and mouthparts are key sensory appendages responding to a range of stimuli. The family Erebidae is one of the most diverse families within the order Lepidoptera, comprising over 25,000 described species distributed worldwide (Zahiri et al., 2011) and includes a wide array of moths, ranging from large, robust species to smaller, cryptic forms. The current literature indicates a focus on studying the ultrastructure of only the final instars of larval stages, overlooking the initial stages. This study aims to utilize larval characteristics from all stages for identification purposes.

## MATERIAL AND METHODS

**Collection and identification:** The eggs and different instars were collected from different localities of district Kullu, Himachal Pradesh, India (31°57'36"N, 77°6'0"E, 1279m) and sample collection of lab was utilised for preparation for scanning electron microscopy.

**Scanning Electron Microscopy (SEM):** The head of each larval instar was separated and placed in Carnoy's fixative solution (95% ethanol and glacial acetic acid with ratio of 3:1) for 3 hours in case of first 3 instars and for 12 hours for later instars and then kept in 2% glutaraldehyde at 4°C for 2 hours

in case of initial three instars and overnight for later instars. The following day, the samples were cleaned twice with a phosphate buffer (each for 10 min) and washed in 100% ethanol solution (three times, each washing for 2 min). The antennae and mouthparts were then dehydrated through a graded ethanol series of 30, 50, 70, 80, and 90% (for 15 min each) and then fully dehydrated in 100% ethanol (three times for 30 min each), and lastly cleaned in isoamyl acetate (three times for 10 min each). Samples were air dried for 1 hour before performing Scanning Electron microscopy. Different aged instars were preserved in glass vials containing 70% alcohol and glycerol in the ratio of 8:2. Samples were examined and photographed for their ultrastructure studies under scanning electron microscope (JEOL) JSM-6100 in the Instrumentation Centre, Punjabi university, Patiala.

**Analysis of SEM micrographs:** The sensilla were identified from SEM micrographs based on terminologies proposed by Schneider (1964), Zacharuk (1980, 1991) and Grimes (1986 a, 1986b). Images were labelled using Adobe Photoshop CS4 software. Metric analyses were performed using ImageJ software (Li et al., 2018).

## RESULTS AND DISCUSSION

***Artaxa vitellina* Kollar (Figs. 1-5):** Five instars were observed in a single generation. Both the second and final segments of the antenna displayed the expected sensilla. The second segment featured a pair of sensilla chaetica (C1-C2) and three sensilla basiconica (B1-B3), while the final segment had three sensilla basiconica (B4-B6) along with a sensilla styloconica (Sty). However, sensilla B5 and B6 on

the final segment of the antenna were positioned together and not alternately arranged on either side of the sensilla styloconica. The mandibles consistently exhibited a pair of six sensilla chaetica (C1-C12) in all larval instars. Sensilla on the galea were complete. In the case of the maxillary palp, all sensilla basiconica (A1-A3, L1-L2, and M1-M2) were observed in all larval instars except for the medial sensilla basiconica (M1-M2), which were absent in the first instar. The labial palp maintained a pair of sensilla chaetica (C) and sensilla styloconica (Sty) along with a spindle-shaped spinneret across all instars.

***Maeoproctis latifascia* Walker (Figs. 6-10):** Five instars were observed within a single generation. Across all larval instars, the second segment of the antenna consistently exhibited a pair of sensilla chaetica (C1-C2). Furthermore, three sensilla basiconica (B1-B3) were consistently present on the antenna's second segment in all stages, except for sensillum B3, which was not observed in the third and fourth instar, possibly due to its concealed location. In the final segment of the antenna, sensillum B5 was not observed in first, third and fifth instars. Similarly, sensilla B6 was observed only in first two instars. Positions of sensilla B5 and B6 probably make it hard to observe them as large sensilla basiconica B4 is positioned just in front of them. Sensilla B5 and B6 were positioned close together in proximity, as observed in second instar. The mandibles consistently displayed a pair of six sensilla chaetica (C1-C12) in all larval instars. On the galea, three sensilla trichodea (ST1-ST3) and two sensilla styloconica (LSS-MSS) were observed. Similarly, the maxillary palp exhibited eight sensilla basiconica, including three apical (A1-A3), two lateral (L1-

L2), and two medial (M1-M2) sensilla in all larval instars, along with sensilla campaniformia (SC) and sensilla digitiformia (SD), which were observed in only two instars due to their visibility being angle-dependent. The labial palp consistently displayed a pair of sensilla chaetica (C) and sensilla styloconica (Sty) in addition to a spindle-shaped spinneret across all larval instars.

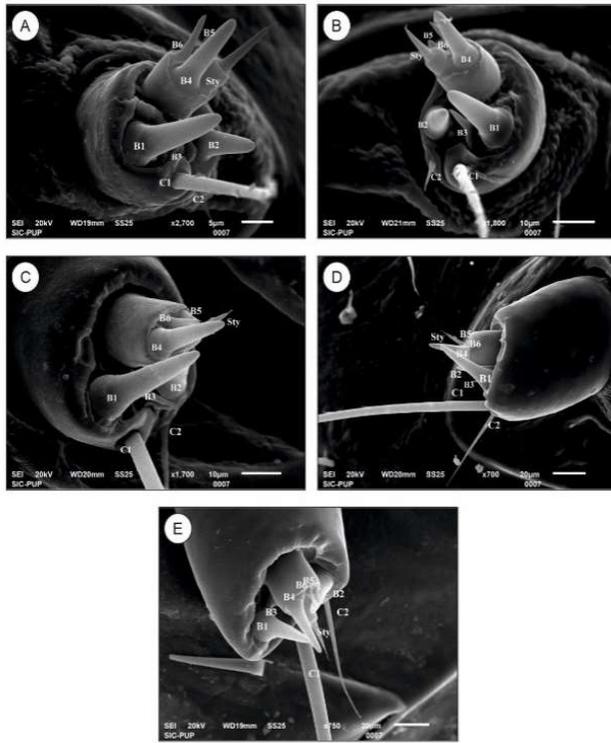
The present study provides a comparative account of the ultrastructural characteristics of the antennae and mouthparts of the larval instars of *Artaxa vitellina* and *Maeoproctis latifascia* using scanning electron microscopy. The observations in this study largely align with previous research on lepidopteran larvae, particularly concerning the general organization and presence of key sensilla types. The structural components of the antennae and mouthparts, including the presence of sensilla basiconica, sensilla chaetica, and sensilla styloconica, correspond with findings reported in other studies on lepidopteran larvae (Lin 1997, Fanger and Naumann 2001, Shields 2009, Song et al., 2014). However, species-specific differences in sensilla distribution and morphology were evident, emphasizing the importance of ultrastructural characteristics in taxonomic identification and ecological adaptation. The presence of sensilla campaniformia and sensilla digitiformia across all larval instars of *Artaxa vitellina*, compared to their occurrence in only select instars of *Maeoproctis latifascia*, suggests variations in mechanosensory adaptation. Previous studies have demonstrated the importance of such sensilla in detecting mechanical stimuli, particularly in larvae that engage in exploratory behaviours (Shields 2009). The consistent presence of these sensilla in *Artaxa vitellina* may

**Table 1.** Comparative account of observations made in all larval stages of the species studied:

Sensilla type	<i>Artaxa vitellina</i>	<i>Maeoproctis latifascia</i>
Sensilla campaniformia and sensilla digitiformia	Present in all instars	Observed only in two instars
Sensilla M1 and M2 of maxillary palp	Absent in first instar	Present in all instars
Sensilla B5 and B6	Observed in all instars	Observed in few instars
Shape of spinneret	Spinneret becomes elongated with a pointed tip	Spinneret remains broad till last instar

**Table 2.** Metrical analysis of sensilla of antennae of different aged instars of *Artaxa vitellina* (Mean length±SD micrometers)

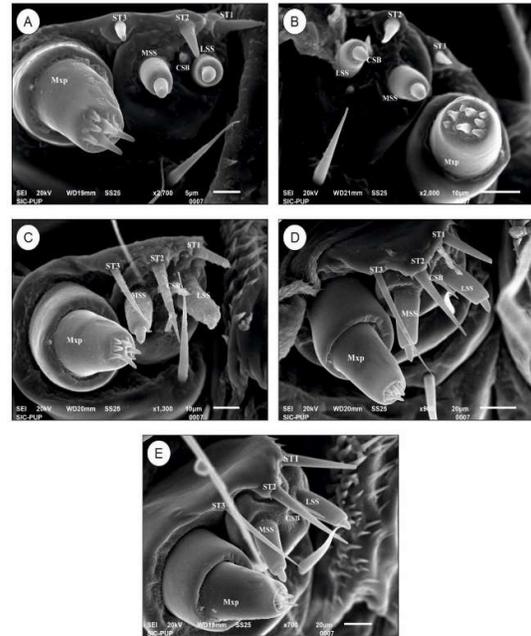
Sensilla type	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar
B1	15.81±0.64	23.76±0.21	29.55±0.59	38.62±0.22	33.40±0.35
B2	8.51±0.93	9.73±0.66	15.42±2.25	22.58±0.43	22.76±0.79
B3	1.89±0.16	1.89±0.33	8.11±0.18	10.88±0.83	9.20±0.68
B4	12.40±0.32	14.16± 1.05	19.93±0.10	24.60±0.13	33.45±0.29
B5	3.08±0.34	4.38±0.32	4.25±0.19	2.27±0.34	8.40±0.48
B6	7.23±0.24	7.18±0.21	8.53±0.34	6.31±0.16	11.25±0.37



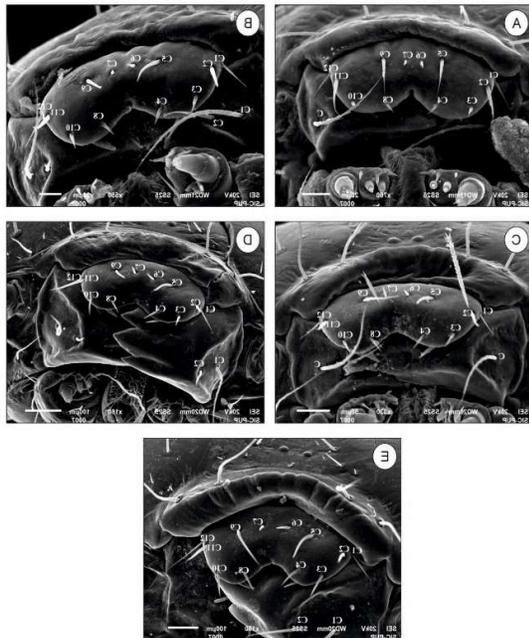
**Fig. 1.** Morphology and structure of sensilla on antenna in all larval instars of *A. vitellina*. (A) to (E) antenna of first to fifth instar respectively

indicate a more active role in environmental sensing across developmental stages.

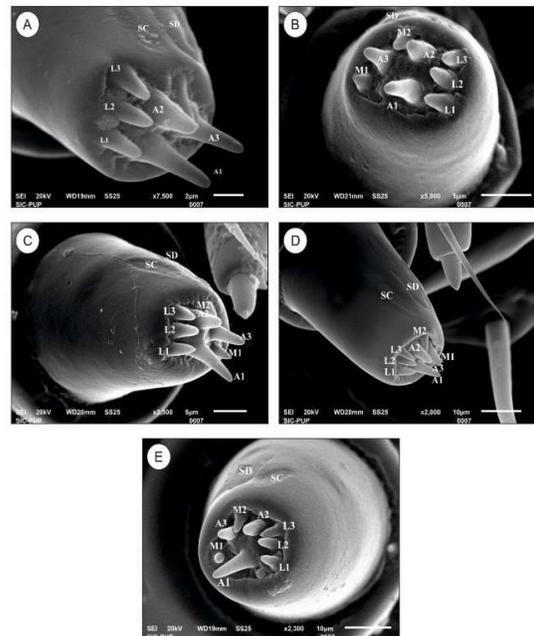
Further, the absence of sensilla M1 and M2 in the first instar of *Artaxa vitellina* but their presence in all instars of



**Fig. 3.** Morphology and structure of sensilla on galea in all larval instars of *A. vitellina*. (A) to (E) galea of first to fifth instar respectively



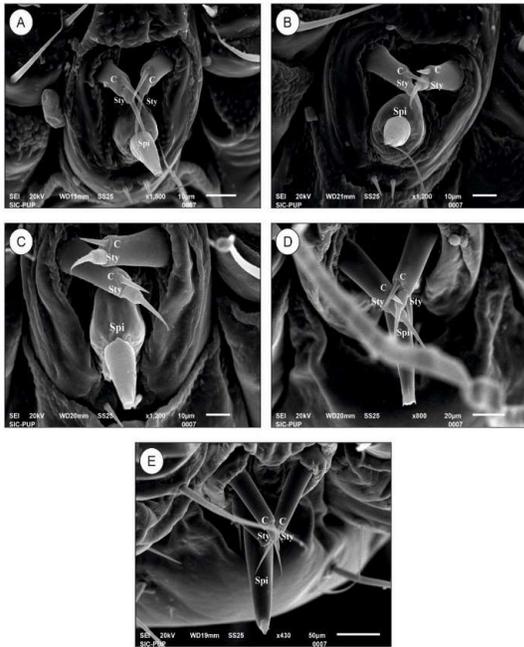
**Fig. 2.** Morphology and structure of sensilla on labrum and mandibles in all larval instars of *A. vitellina*. (A) to (E) labrum and mandibles of first to fifth instar respectively



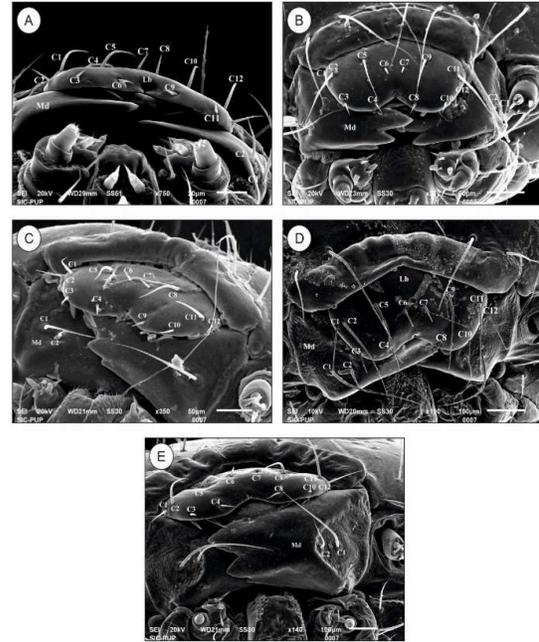
**Fig. 4.** Morphology and structure of sensilla on maxillary palp in all larval instars of *A. vitellina*. (A) to (E) maxillary palp of first to fifth instar respectively

*Maeoproctis latifascia* highlights interspecific differences in sensory organ development. Similar observations have been documented in other Lepidoptera, where the ontogeny of sensilla varies among species due to differences in feeding

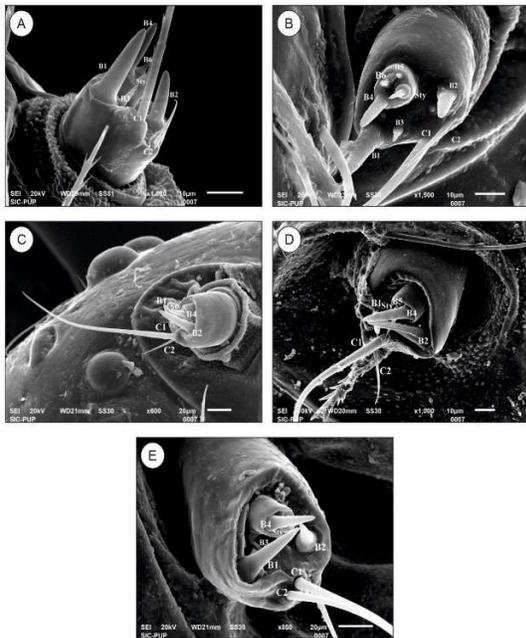
strategies and habitat preferences (Grimes and Neunzig 1986). The arrangement of sensilla basiconica on the antennae of both species also aligns with previous findings in related taxa. The alternate arrangement of sensilla B5 and



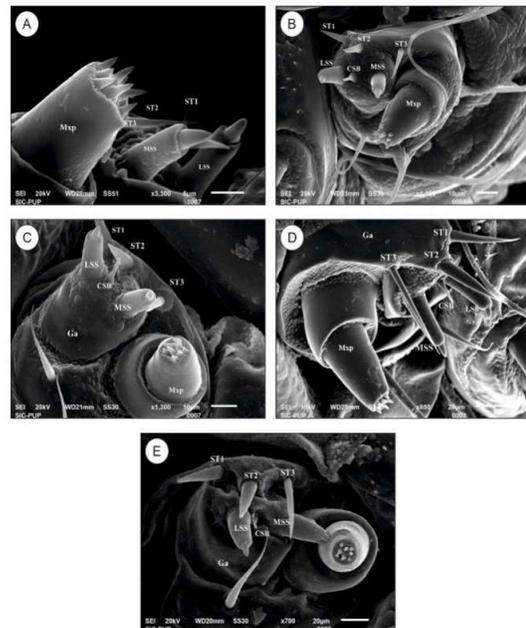
**Fig. 5.** Morphology and structure of sensilla on labial palp in all larval instars of *A. vitellina*. (A) to (E) labial palp of first to fifth instar respectively



**Fig. 7.** Morphology and structure of sensilla on labrum and mandibles in all larval instars of *M. latifascia* (A) to (E) labrum and mandibles of first to fifth instar respectively



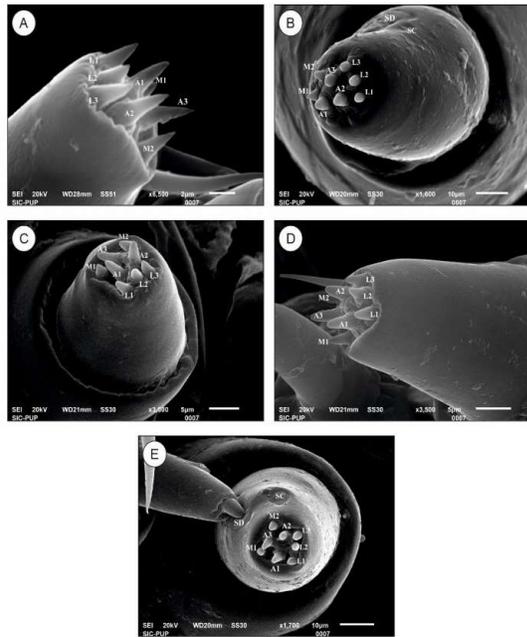
**Fig. 6.** Morphology and structure of sensilla on antenna in all larval instars of *M. latifascia*. (A) to (E) antenna of first to fifth instar respectively



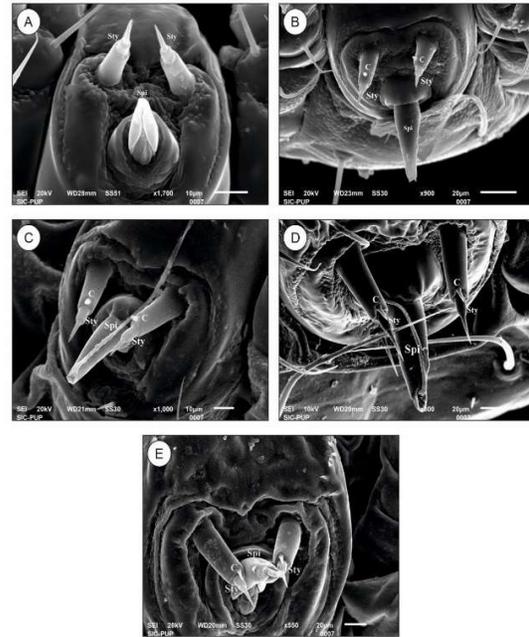
**Fig. 8.** Morphology and structure of sensilla on galea in all larval instars of *M. latifascia*. (A) to (E) galea of first to fifth instar respectively

B6 observed in some instars of *Artaxa vitellina* differs from *Maeoproctis latifascia*, where sensilla B5 was not observed in certain instars. This disparity suggests functional modifications in the sensory system, potentially linked to species-specific ecological interactions (Schneider 1964,

Zacharuk 1991). Another noteworthy observation is the elongation of the spinneret with a pointed tip in *Artaxa vitellina*, whereas in *Maeoproctis latifascia*, the spinneret remains broad until the final instar. Similar morphological differences in spinneret structure have been reported in other



**Fig. 9.** Morphology and structure of sensilla on maxillary palp in all larval instars of *M. latifascia*. (A) to (E) maxillary palp of first to fifth instar respectively



**Fig. 10.** Morphology and structure of sensilla on labial palp in all larval instars of *M. latifascia*. (A) to (E) labial palp of first to fifth instar respectively

**Table 3.** Metrical analysis of mouthparts and its sensilla of different aged instars of *A. vitellina* (Mean length±SD micrometers)

Name of structures	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar
Distal segment of Mxp	11.87±0.58	8.71±1.42	21.37±0.84	38.04±0.35	53.82±0.44
Galea					
ST1	8.67±0.16	16.74±1.60	14.38±0.84	30.64±0.58	54.28± 1.04
ST2	6.35±0.01	6.30±0.21	29.91± 0.15	42.28±0.39	65.54±1.07
ST3	2.98±0.48	4.47±0.03	24.83±0.57	62.85±0.46	73.60±1.38
Sensilla of maxillary palp					
A1	5.26±0.17	3.67±0.13	7.82±0.62	8.00± 0.45	9.81±0.41
A2	3.76±0.04	2.22±0.08	4.76±0.18	7.28±0.06	4.55±0.56
A3	4.84±0.15	2.94±0.034	5.44±0.05	6.94±0.41	3.27±0.29
L1	2.64±0.05	3.03±0.02	4.64±0.06	4.89±0.11	4.66±0.14
L2	2.64±0.02	4.15±0.25	3.32±0.10	5.46±0.18	4.08±0.29
L3	2.74±0.17	3.82±0.08	3.17±0.25	4.44±0.09	4.47±0.15
M1	Not visible	1.62±0.03	3.40±0.07	4.21±0.45	1.95±0.10
M2	Not visible	2.15±0.02	2.54±0.19	4.27±0.18	4.47±0.04
Sensilla of labial palp					
C	12.94±1.50	7.86±2.71	12.55±1.25	17.16±5.77	26.91±1.61
Sty	20.74±0.98	13.82±1.60	23.92±0.33	33.16±2.34	56.53±2.354

**Table 4.** Metrical analysis of sensilla of antennae of different aged instars of *M. latifascia* (Mean length±SD micrometers)

Sensilla type	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar
B1	19.65±1.04	16.95±1.64	19.87±0.37	9.83±0.14	38.29±1.98
B2	13.31±0.66	8.62±0.68	31.37±0.013	31.31±0.78	13.43±2.10
B3	7.38±0.13	4.38±0.26	Not visible	Not visible	7.22±0.19
B4	14.52±0.84	10.62±0.19	14.17±0.70	24.14±1.25	25.93±0.75
B5	Not visible	1.74±0.22	Not visible	2.78±0.30	Not visible
B6	5.96±0.06	2.64±0.015	Not visible	Not visible	Not visible

**Table 5.** Metrical analysis of mouthparts and its sensilla of different aged instars of *M. latifascia* (Mean length±SD micrometers)

Name of structures	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar
Distal segment of Mxp	16.00±0.78	21.98±0.20	14.04±1.56	45.23±4.45	16.46±2.61
Galea					
ST1	7.86±0.51	12.88±0.55	11.91±1.27	41.58±0.28	32.08±1.54
ST2	16.73±0.30	9.44±0.16	10.54±0.10	46.99±2.90	25.33±1.85
ST3	12.85±0.61	13.68±0.38	7.90±0.31	58.91±0.74	41.32±0.07
Sensilla of maxillary palp					
A1	3.18±0.15	3.66±0.37	3.90±0.21	3.69±0.20	3.05±0.25
A2	3.93±0.10	4.7±0.29	3.36±0.003	3.37±0.33	2.74±0.31
A3	4.30±0.39	4.17±0.54	4.33±0.19	4.06±0.15	3.47±0.31
L1	3.18±0.21	3.27±0.15	2.63±0.53	3.18±0.09	2.38±0.51
L2	2.26±0.26	3.37±0.40	1.66±0.07	2.24±0.01	1.88±0.10
L3	3.32±0.19	3.38±0.33	2.25±0.02	3.05±0.32	2.64±0.29
M1	1.42±0.05	3.58±0.29	1.86±0.02	3.02±0.05	1.80±0.13
M2	3.11±0.24	3.52±0.02	2.19±0.31	2.69±0.10	2.18±0.32
Sensilla of labial palp					
C	6.44±0.13	3.36±0.76	4.18±0.62	9.52±0.83	9.86±0.69
Sty	11.37±0.51	11.17±0.74	17.80±0.5	31.99±1.79	33.09±2.16

moth species, indicating potential differences in silk secretion and cocoon-spinning behaviour (Kaleka and Dulai 2024, Kaleka and Dulai 2025)..

The findings of this study underscore the importance of detailed ultrastructural analyses in lepidopteran taxonomy. The observed species-specific variations in sensilla distribution and morphology provide valuable insights into larval sensory adaptations, with implications for both phylogenetic studies and pest management strategies. Future research should focus on electrophysiological assessments to better understand the functional significance of these sensilla in the feeding and behavioural ecology of these species.

### CONCLUSION

The presence of Sensillum B5 across all larval stages of *Artaxa vitellina* Kollar, in contrast to its absence in some instars of *Maeoproctis latifascia* Walker, indicates species-specific developmental trajectories and sensory adaptations.

The consistent presence of sensilla campaniformia and sensilla digitiformia in *Artaxa vitellina* Kollar across all instars, versus their occurrence only in later instars of *Maeoproctis latifascia* Walker, highlights significant interspecific differences in sensory organ development and ecological adaptations.

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