

Comparative Ultrastructure of Antennal and Mouthpart Sensilla in Larval Instars of *Amata bicincta* (Kollar, [1844]) and *Areas galactina* (Hoeven, 1840) (Lepidoptera: Erebidae)

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Abstract

This study compares the ultrastructural development of antennal and mouthpart sensilla across larval instars of *Amata bicincta* (Kollar, [1840]) and *Areas galactina* (van der Hoeven, 1840) (Lepidoptera: Erebidae) using scanning electron microscopy (SEM). *A. bicincta* exhibits five larval instars, while *A. galactina* has eight. Key findings include species-specific sensilla reductions and modifications: *A. bicincta* loses antennal sensillum B3 after the first instar, and its spinneret transitions to a broad, leaf-like structure. *A. galactina* retains B5 in later stages but loses B3 entirely, and its spinneret becomes conical in the final instar. Additional variations include transient appearances and disappearances of galeal sensilla (MSB, LSB/CSB) in *A. galactina*. These results underscore the utility of larval ultrastructure in taxonomic and sensory ecology studies, as well as the importance of examining all larval stages.

Keywords: Lepidoptera, Erebidae, *Amata*, *Areas*, larvae, ultrastructure, sensilla, SEM, taxonomy, India.

Ultraestructura comparativa de las sensillas antenales y bucales en los estadios larvarios de *Amata bicincta* (Kollar, [1844]) y *Areas galactina* (Hoeven, 1840) (Lepidoptera: Erebidae)

Resumen

Este estudio compara el desarrollo ultraestructural de las sensillas antenales y bucales a lo largo de los estadios larvarios de *Amata bicincta* (Kollar, [1844]) y *Areas galactina* (Hoeven, 1840) (Lepidoptera: Erebidae) mediante microscopía electrónica de barrido (SEM). *A. bicincta* presenta cinco estadios larvarios, mientras que *A. galactina* tiene ocho. Entre los hallazgos más destacados se encuentran reducciones y modificaciones de las sensillas específicas de cada especie: *A. bicincta* pierde la sensilla antenal B3 después del primer estadio, y su hilera se transforma en una estructura ancha similar a una hoja. *A. galactina* conserva la B5 en estadios posteriores, pero pierde la B3 por completo, y su hilera se vuelve cónica en el estadio final. Otras variaciones incluyen la aparición y desaparición transitorias de sensillas galeales (MSB, LSB/CSB) en *A. galactina*. Estos resultados subrayan la utilidad de la ultraestructura larvaria en los estudios taxonómicos y de ecología sensorial, así como la importancia de examinar todas las etapas larvarias.

Palabras clave: Lepidoptera, Erebidae, *Amata*, *Areas*, larvas, ultraestructura, sensillas, SEM, taxonomía, India.

Introduction

Lepidoptera larvae exhibit diverse morphological adaptations, with sensilla on antennae and mouthparts playing crucial roles in sensory ecology. Detailed studies across larval instars are essential for understanding species-specific adaptations and refining taxonomic classifications (Kaleka & Dulai, 2024). This study examines *Amata bicincta* (Kollar, 1840) and *Areas galactina* (van der Hoeven, 1840), two Erebidæ with poorly documented larval stages. By comparing sensilla development across instars, we aim to:

1. Characterize the ontogenetic trajectories of antennal and mouthpart sensilla in both species.
2. Identify species-specific adaptations linked to ecological niches and feeding strategies.
3. Evaluate spinneret morphology as a taxonomic marker, contributing to ongoing phylogenetic studies.

By integrating SEM-based analysis, we extend the work of Kaleka & Dulai (2024) on Lymantriinae larvae, emphasizing the utility of larval ultrastructure in taxonomic identification.

Materials and Methods

Collection and Rearing: Larvae of *A. bicincta* (five instars) and *A. galactina* (eight instars) were collected from localities Kullu, Himachal Pradesh and reared on host plants under controlled conditions (25°C, 70% humidity, 14:10 light-dark cycle). Identification was done on basis of adult morphological characters.

SEM Preparation: Heads of each instar were fixed in Carnoy's solution (3:1 ethanol: acetic acid) for 3–12 hours, post-fixed in 2% glutaraldehyde (4°C, overnight), and dehydrated through a graded ethanol series (30–100%). Samples were critical-point dried, gold-coated, and imaged using a scanning electron microscope (JEOL) JSM-6100 in the Instrumentation Centre, Punjabi university, Patiala, India.

Sensilla Analysis: Sensilla were identified using Schneider (1964) and Zacharuk (1985, 1991). Metric data (length, distribution) were extracted using ImageJ software. Statistical analyses were conducted using ANOVA F test to record the level of significance.

Developmental Trajectory of Sensilla in *Amata bicincta*: Larvae (Figures 1–5) across five larval instars, *Amata bicincta* exhibits dynamic sensilla modifications on antennae and mouthparts, reflecting ontogenetic adaptations. Antennal sensilla progress from an initial eight sensilla in the first instar (B1–B6, C1–C2, Sty) to a reduced count in later stages: sensillum B3 disappears by the fifth instar, likely due to compensatory elongation of B1–B2, while B6 becomes obscured by B4's growth (Figures 1A–B). The terminal segment retains four sensilla (B4–B6, Sty) in early instars but loses B6 by the fourth instar, mirroring *Maeoproctis latifascia*'s regression of B5–B6 in later stages¹. Mouthpart sensilla show stability in labral (C1–C12) and mandibular (C1–C2) chaetia but dynamic maxillary palp patterns: medial sensillum M2 disappears by the fourth instar, and apical sensilla (A2–A3) shorten progressively, contrasting with *Artaxa vitellina*'s retention of all maxillary sensilla¹. The spinneret transitions from spindle-shaped in early instars to a broad, leaf-like structure in the fifth, akin to *A. vitellina*'s elongation¹.

These shifts align with lepidopteran trends where sensilla reductions correlate with niche-specific foraging, e.g., loss of M2 may reflect tactile over chemosensory reliance in later instars, as seen in foliage-feeding *M. latifascia*¹. The conserved labral chaetia (C1–C12) supports Erebidæ taxonomy, while spinneret divergence (leaf-like vs. spindle-shaped) underscores species-level adaptations in silk-spinning behavior¹. Such ontogenetic patterns provide taxonomic markers and ecological insights, paralleling findings in *A. vitellina* and *M. latifascia*.

Developmental Patterns in *Areas galactina* Larvae (Figures 6–10): Across eight larval instars, *Areas galactina* exhibits dynamic sensilla modifications on antennae and mouthparts, reflecting ontogenetic and ecological adaptations. Antennal sensilla show progressive reductions: sensillum B3 is absent after the first instar, while B6 is not observed in the first and seventh instars but present in others. In the final instar, sensillum B5 reappears. Mouthpart sensilla display dynamic galeal patterns, including the transient presence of medial sensillum basiconicum (MSB) in the third instar. Additionally, sensilla LSB/CSB on the galea are present only in the first four instars. The labrum and mandibles have stable chaetia (C1–C12) and (C1–C2) respectively, and the maxillary palp maintains similar sensilla counts across instars. The spinneret transitions from spindle-shaped to a conical, prominent structure in the final stage.

These shifts align with lepidopteran trends where sensilla reductions correlate with niche-specific foraging. The transient presence of MSB and the loss of LSB/CSB may indicate a shift in feeding strategies. The conserved labral chaetika (C1-C12) supports erebid taxonomy, while spinneret divergence underscores species-level adaptations in silk-spinning behavior. Such ontogenetic patterns provide taxonomic markers and ecological insights.

Discussion

Antennal Sensilla Development: *A. bicincta*: Antennal sensilla show progressive reductions: sensillum B3 disappears after the first instar, likely due to compensatory elongation of B1-B2, while B6 is obscured in later stages (e.g., seventh instar) by positional shifts of B4. *A. galactina*: B3 is absent after the first instar, while B6 is not observed in the first and seventh instars but present in others. Sensillum B5 reappears in the final instar (Table 1).

Table 1. Metrical analysis of sensilla of antennae of different aged instars of *Amata bicincta* (represented as mean length $\pm$ SD micrometers)

Sensilla type	1st instar	2nd instar	3rd instar	4th instar	5th instar
B1	6.69 1.18	10.66 $\pm$ 0.55	16.26 0.70	35.5 $\pm$ 1.24	49.11 $\pm$ 0.30
B2	6.54 0.21	9.09 $\pm$ 0.84	12.97 1.09	30.15 $\pm$ 1.07	289.30 $\pm$ 14.28
B3	2.51 0.36	4.47 $\pm$ 0.16	5.95 0.29	9.08 $\pm$ 0.42	Not seen
B4	4.97 0.19	10.68 $\pm$ 0.73	18.23 0.39	23.21 $\pm$ 1.03	413.08 $\pm$ 5.98
B5	3.17 0.41	7.09 $\pm$ 0.12	4.20 $\pm$ 0.23	5.03 $\pm$ 0.33	14.09 $\pm$ 0.23
B6	2.21 0.13	3.78 $\pm$ 0.15	3.61 $\pm$ 0.17	Not seen	5.22 $\pm$ 0.86

Mouthpart Sensilla Development: *A. bicincta*: Medial sensillum basiconicum (MSB) appears transiently in the third instar, while LSB/CSB on the galea diminish by the fourth instar. The maxillary palp loses M2 by the fourth instar *A. galactina*: Transient presence of MSB in the third instar. The spinneret transitions from spindle-shaped to a conical, prominent structure in the final stage (Table 2).

Table 2. Metrical analysis of mouthparts and its sensilla of different aged instars of *Amata bicinata* (represented as mean length $\pm$ SD micrometers).

Name of structures	1 st instar	2 nd instar	3 rd instar	4 th instar	5 th instar
Distal segment of Mxp	8.55 $\pm$ 0.57	10.11 $\pm$ 0.43	16.28 $\pm$ 0.42	16.09 $\pm$ 2.60	18.18 $\pm$ 2.23
Galea					
ST1	7.46 $\pm$ 0.51	9.37 $\pm$ 0.25	10.74 $\pm$ 0.64	33.61 $\pm$ 0.90	22.40 $\pm$ 1.39
ST2	3.07 $\pm$ 0.48	6.91 $\pm$ 0.45	7.79 $\pm$ 1.14	32.87 $\pm$ 0.96	15.20 $\pm$ 0.51
ST3	8.46 $\pm$ 0.53	3.55 $\pm$ 0.82	12.01 $\pm$ 0.15	14.60 $\pm$ 0.13	23.24 $\pm$ 0.92
Sensilla of Maxillary Palp					
A1	1.93 $\pm$ 0.09	2.13 $\pm$ 0.13	2.94 $\pm$ 0.18	2.51 $\pm$ 0.15	4.00 $\pm$ 0.2
A2	2.09 $\pm$ 0.05	1.90 $\pm$ 0.24	2.56 $\pm$ 0.15	2.08 $\pm$ 0.38	0.97 $\pm$ 0.09

A3	1.35±0.05	1.73±0.23	2.46±0.23	2.07±0.19	2.75±0.07
L1	2.34±0.08	1.34±0.14	2.55±0.06	2.59±0.08	1.54±0.24
L2	1.05±0.09	1.36±0.03	2.60±0.09	3.07±0.26	1.47±0.005
L3	1.02±0.03	1.14±0.02	2.06±0.17	3.63±0.36	2.21±0.05
M1	1.29±0.19	1.47±0.14	1.62±0.23	2.28±0.40	1.22±0.22
M2	1.14±0.13	1.98±0.29	1.16±0.28	Not seen	Not seen
Sensilla of Labial palp					
C	4.84±0.61	2.57±0.19	5.62±0.80	7.27±0.22	7.37±0.46
Sty	15.37±0.41	11.26±1.27	9.99±0.99	12.23±0.56	11.26±1.27

Taxonomic Implications: Consistent sensilla chaetica (C1-C12) on both species' labrums support their Erebiidae classification. However, *A. galactina*'s missing galeal sensilla basiconica distinguish it from *A. bi-cincta*, echoing subfamily-level differences in Lymantriinae (Grimes & Neunzig, 1986a).

Table 3. Metrical analysis of sensilla of antennae of different aged instars of *Areas galactina* (represented as mean length ± SD micrometers).

Sensilla type	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar	7th instar	8th instar
B1	28.19±1.64	31.68±1.92	48.96±0.61	44.45±2.60	46.91±2.14	44.76±5.46	39.47±0.87	43.82±9.16
B2	8.47±0.81	25.04±2.26	19.12±0.27	48.95±4.07	25.82±1.72	26.50±3.62	47.39±0.24	24.16±0.73
B3	3.36±0.31	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen
B4	15.64± 0.29	21.96±2.00	24.69±0.80	26.25±1.31	36.24±2.64	34.23±0.45	42.30±1.35	35.47±0.61
B5	5.47±0.83	10.24±0.83	7.46±0.37	8.61±0.17	7.33±0.16	6.82±0.78	9.91±1.48	5.02±0.11
B6	Not seen	7.69±0.49	5.77±0.75	3.90±0.67	5.20±3.07	5.28±0.25	Not seen	3.32±0.99

Table 4. Metrical analysis of mouthparts and its sensilla of different aged instars of *Areas galactina* (represented as mean length ± SD micrometers).

Name of structures	1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar	7th instar	8th instar
Distal segment of Mxp	9.43±1.39	8.54±0.27	16.10±0.16	34.74± 2.13	48.74±2.94	92.48±5.48	98.83±1.90	126.92±0.56
Galea								
ST1	11.97±0.13	11.61±0.51	62.47±0.15	86.43±3.45	126.78±0.41	152.54±0.25	202.79±0.74	187.94±2.89

ST2	9.69±0.55	13.29±1.81	56.30±1.69	93.63± 2.93	117.27± 1.79	149.59± 1.13	206.39± 0.41	347.71± 12.93
ST3	14.73±0.3	16.55±1.95	38.62± 0.21	29.54± 2.06	141.71± 1.27	Not seen	197.13± 0.22	255.93± 5.74
Sensilla of Maxillary Palp								
A1	2.13±0.28	3.48±0.37	5.43± 0.34	5.24±0.20	5.88± 0.39	7.88±1.07	8.41± 0.65	8.47± 1.44
A2	1.55±0.10	2.93±0.22	4.80± 0.09	5.14±0.17	4.55± 0.17	5.64±0.22	4.45± 0.60	5.99± 0.42
A3	1.66±0.34	3.49±0.28	3.71±0.11	3.76±0.11	4.13± 0.01	7.2± 0.19	5.85± 0.71	7.25± 1.53
L1	1.15±0.05	1.36±0.32	4.23±0.31	3.49± 0.22±	4.42±0.41	6.96± 0.79	4.36± 0.91	4.68± 0.49
L2	1.09±0.10	1.64±0.16	2.78± 0.42	2.61±0.53	2.92± 0.17	8.81± 2.23	5.30± 0.11	4.63±0.40
L3	1.32±0.07	1.68±0.05	4.64±0.46	2.68±0.45	4.11±0.64	5.08±0.10	5.52±1.26	8.39± 0.57
M1	1.20±0.10	1.95±0.22	2.47±0.28	3.29±0.03	2.13± 0.01	5.70± 0.21	5.77± 0.29	5.13± 0.48
M2	0.77±0.04	2.54±0.15	2.39±0.64	4.46± 0.57	2.46± 0.62	5.19± 0.41	4.14 0.42	8.82± 0.66
Sensilla of Labial palp								
C	5.93±0.34	8.83±0.04	12.59± 0.82	10.03± 1.05	19.19± 0.82	16.74± 0.11	19.68± 0.44	11.56± 1.09
Sty	11.37±1.04	14.21± 0.02	29.97± 0.22	37.82± 1.98	41.82± 1.63	57.34± 2.61	59.33± 1.46	23.07± 0.155

Conclusion

Ontogenetic sensilla patterns in *A. bicincta* and *A. galactina* reveal species-specific adaptations, with B3 loss and spinneret divergence serving as taxonomic markers. These findings align with Lepidoptera trends, underscoring larval morphology's utility in sensory ecology and systematics. Further studies are needed to determine any phylogenetic implications or relationships (Kaleka & Dulai, 2024).

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Conflict of Interest

The authors declare that there is no known financial interest or personal relationship that could have influenced the work presented in this article.

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Figure 1. Morphology and structure of sensilla on antennae in all larval instars of *Amata bicincta*.

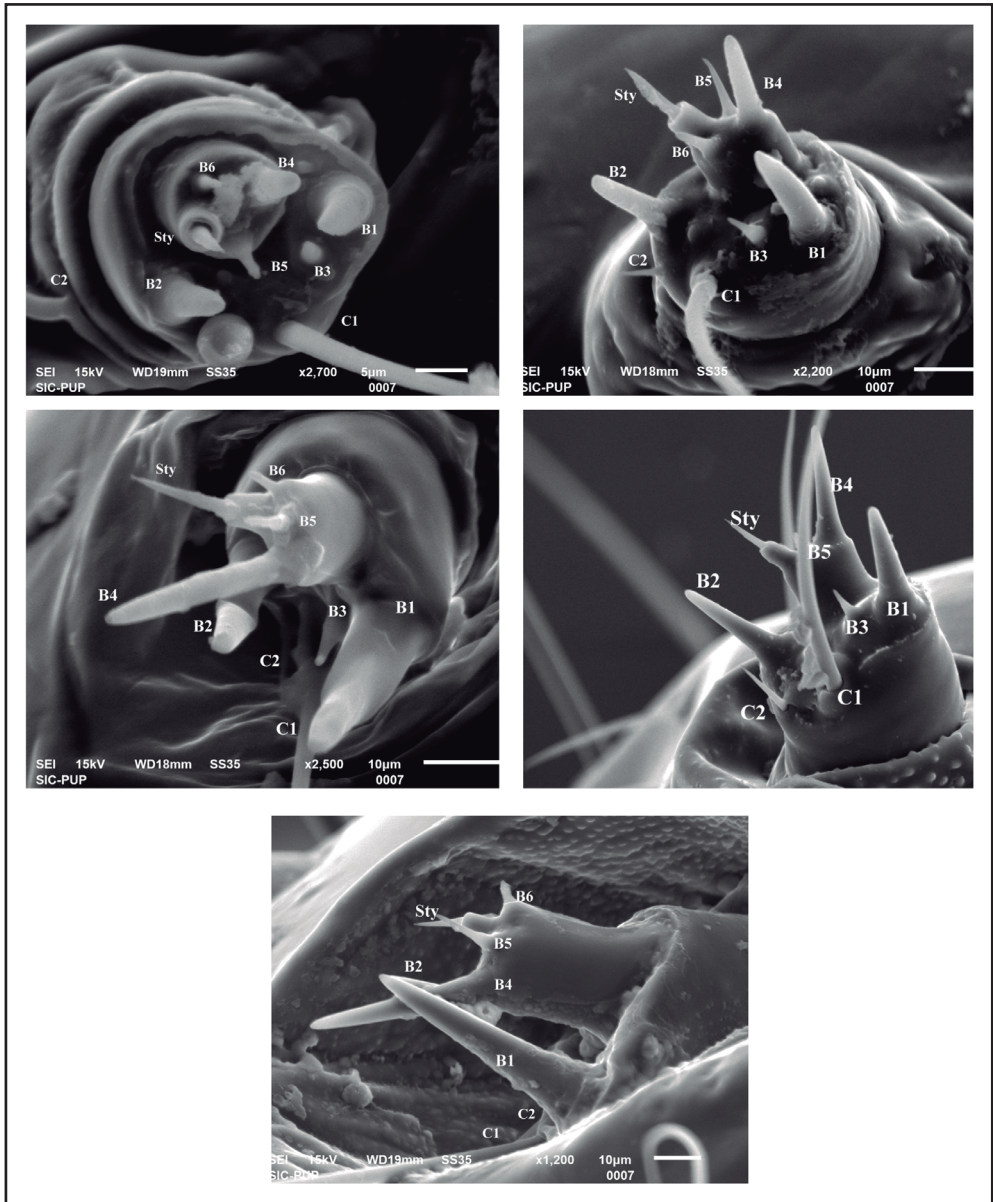


Figure 2. Morphology and structure of sensilla on labrum and mandibles in all larval instars of *Amata bicincta*.

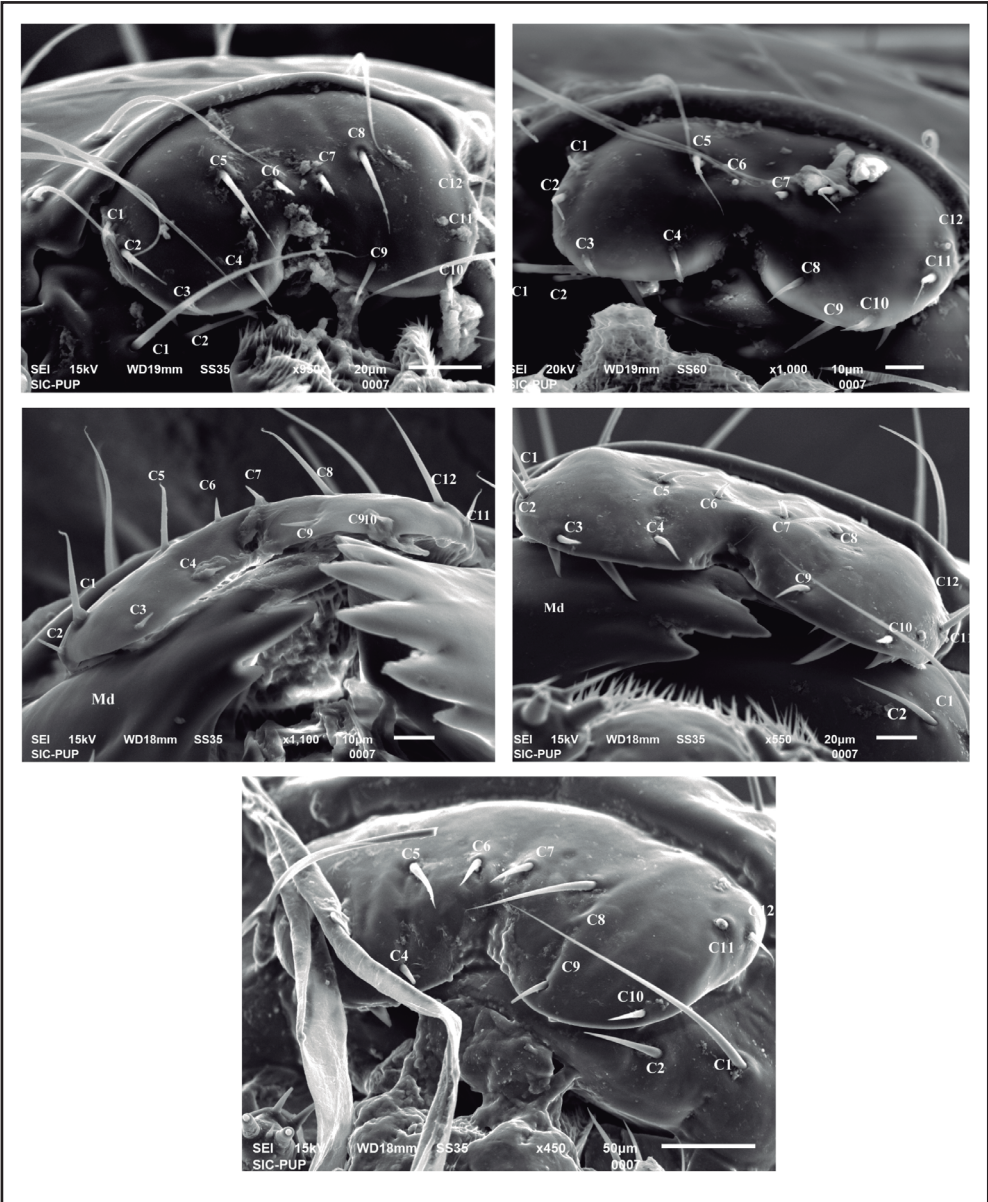


Figure 3. Morphology and structure of sensilla on galea in all larval instars of *Amata bicincta*.

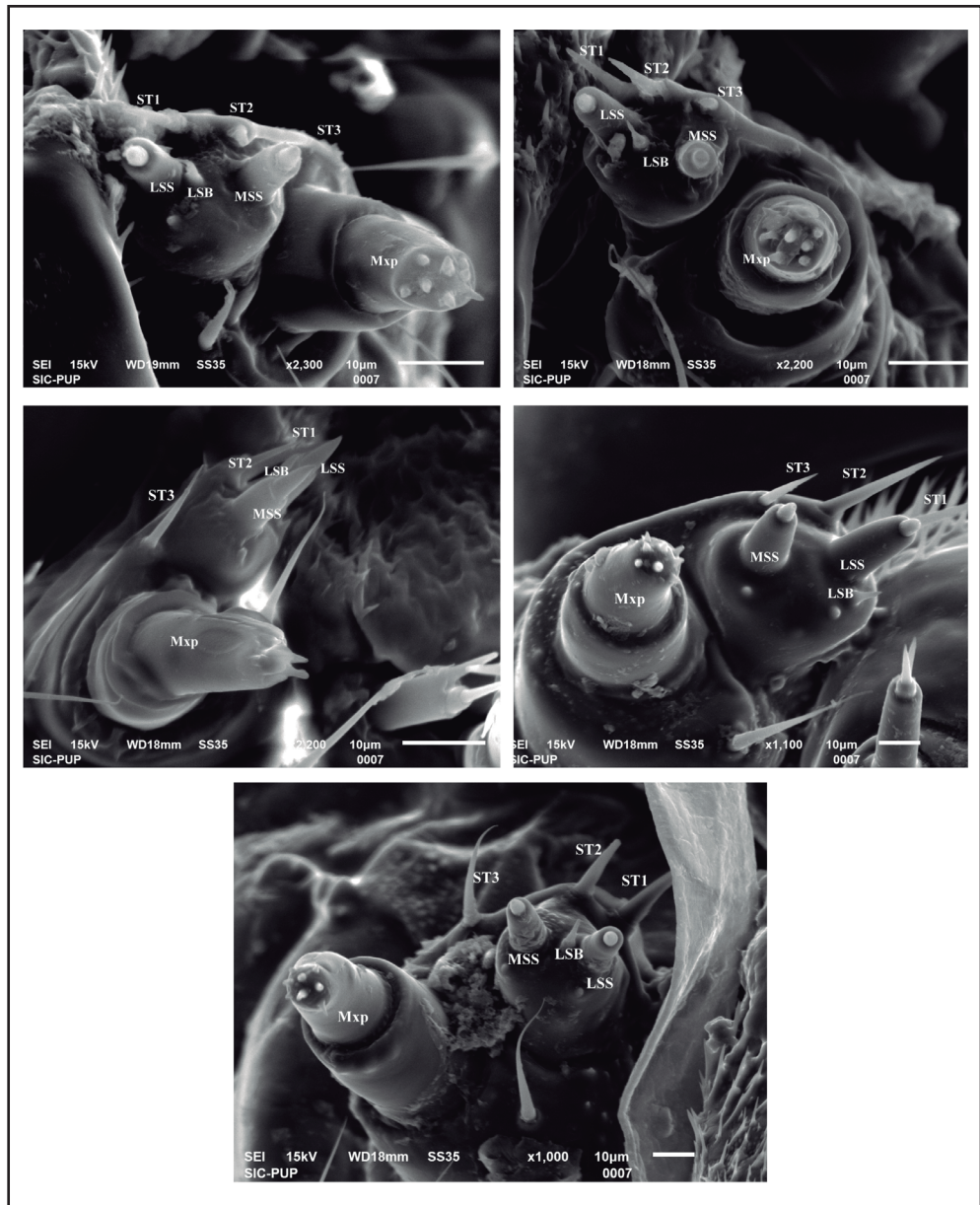


Figure 4. Morphology and structure of sensilla on maxillary palps in all larval instars of *Amata bicincta*.

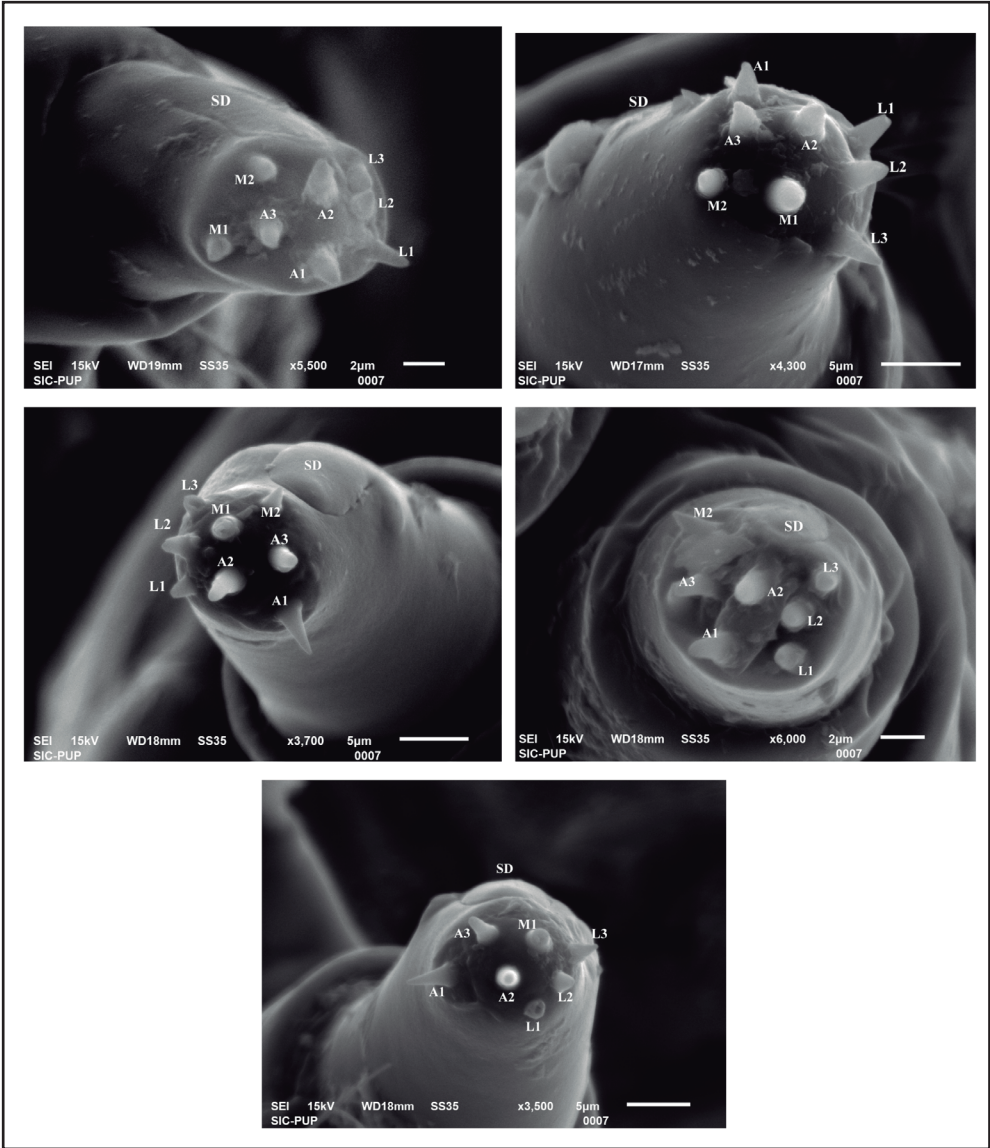


Figure 5. Morphology and structure of sensilla on labial palps in all larval instars of *Amata bicincta*.

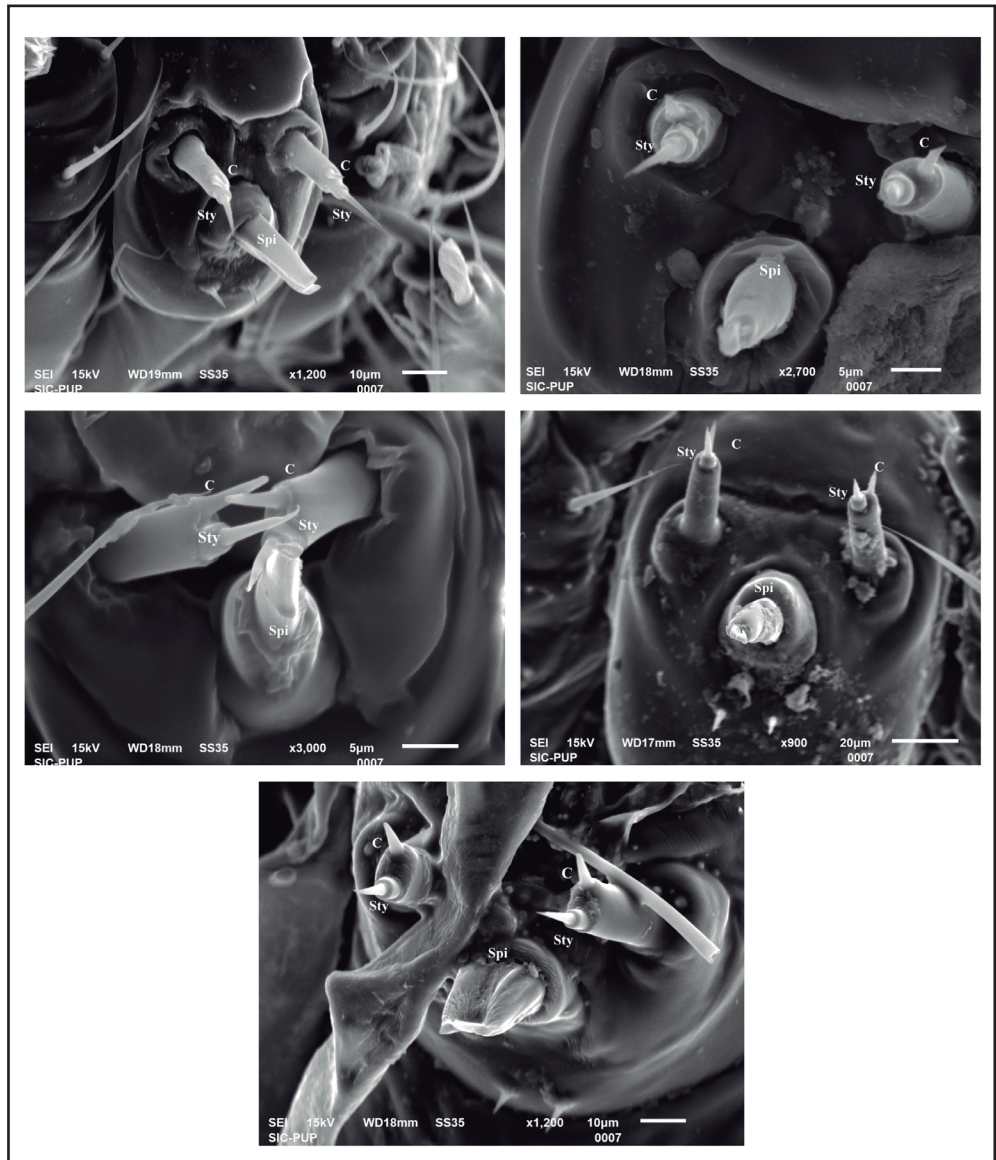


Figure 6. Morphology and structure of sensilla on antennae in all larval instars of *Areas galactina*.

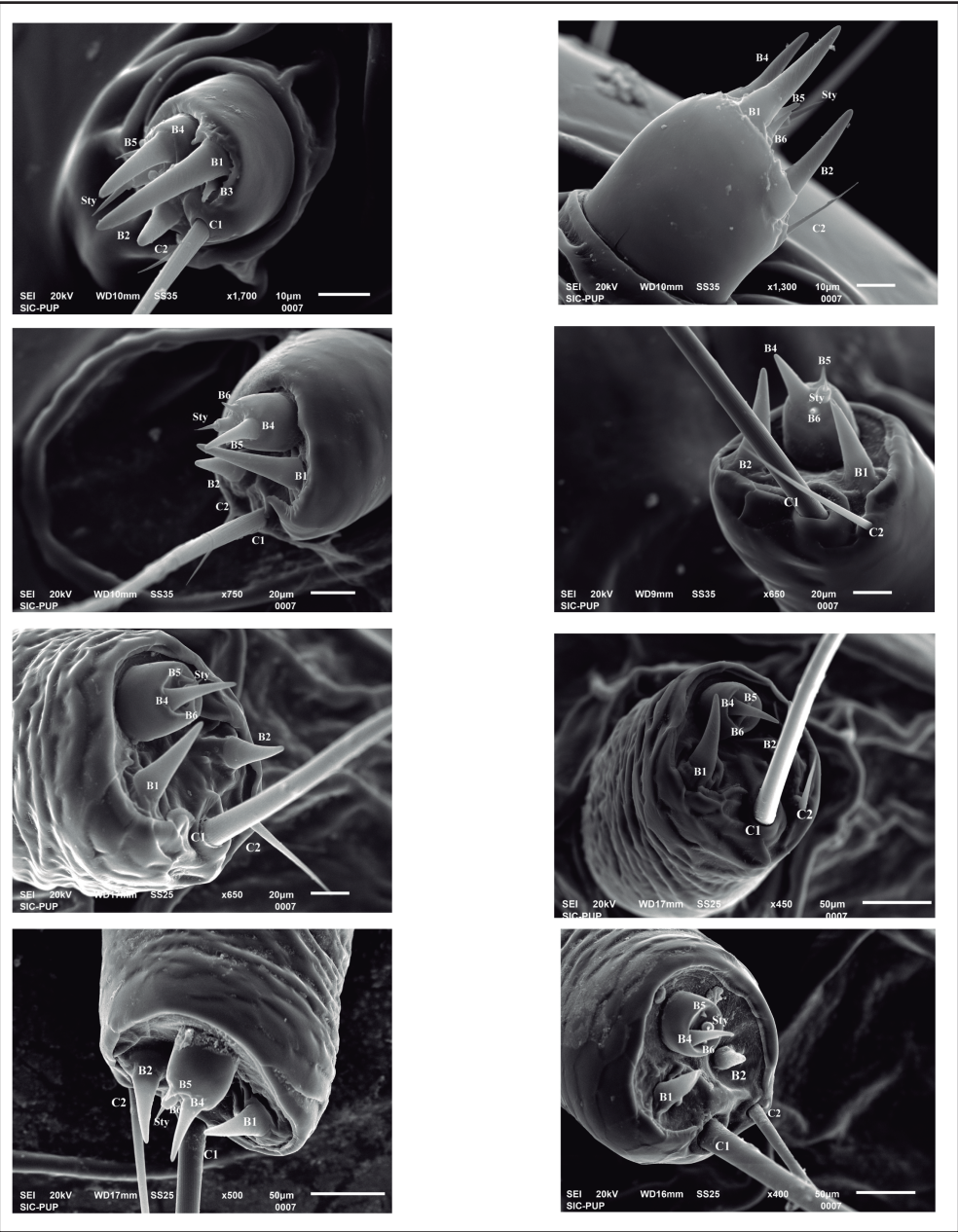


Figure 7. Morphology and structure of sensilla on labrum and mandibles in all larval instars of *Areas galactina*.

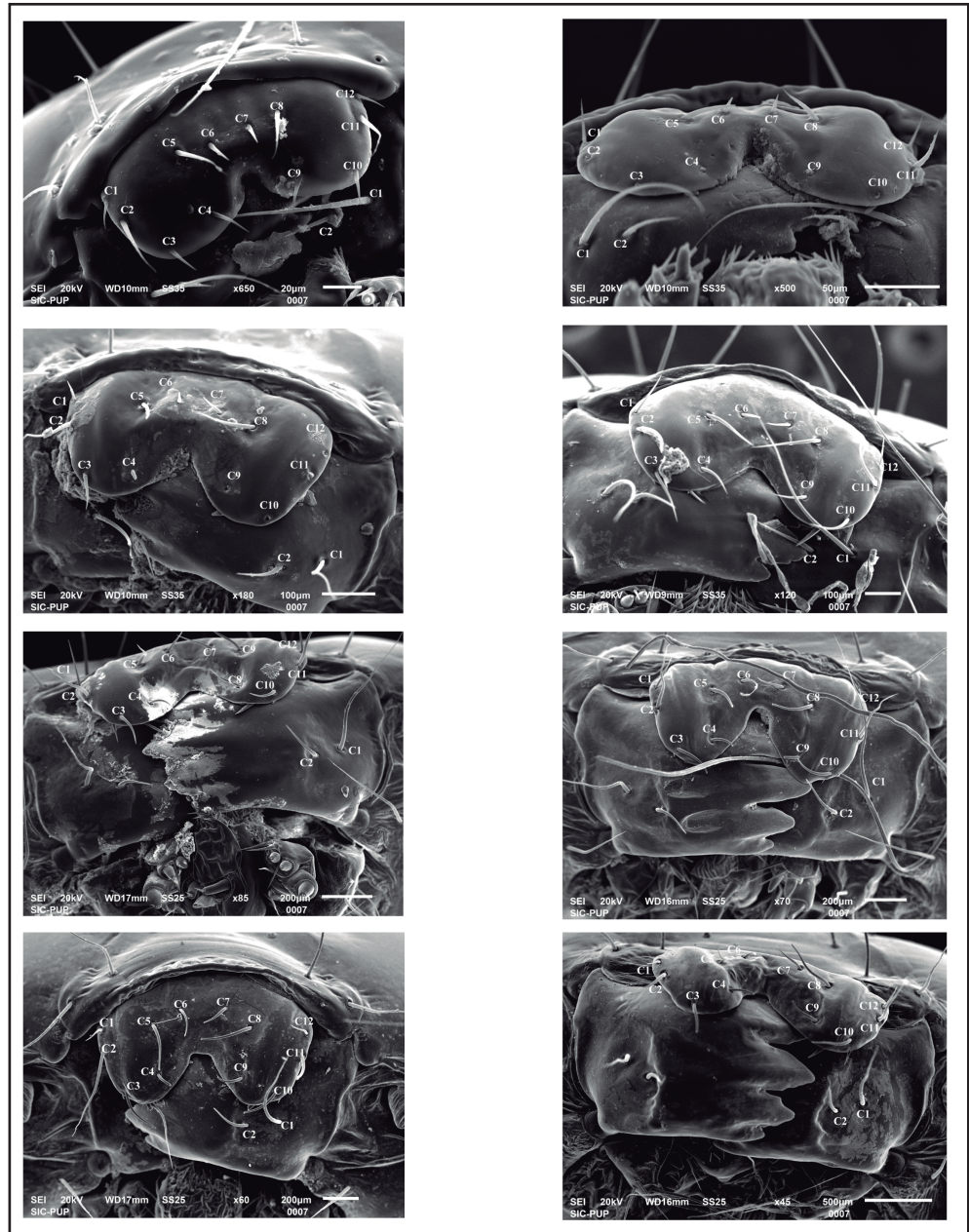


Figure 8. Morphology and structure of sensilla on galea in all larval instars of *Areas galactina*.

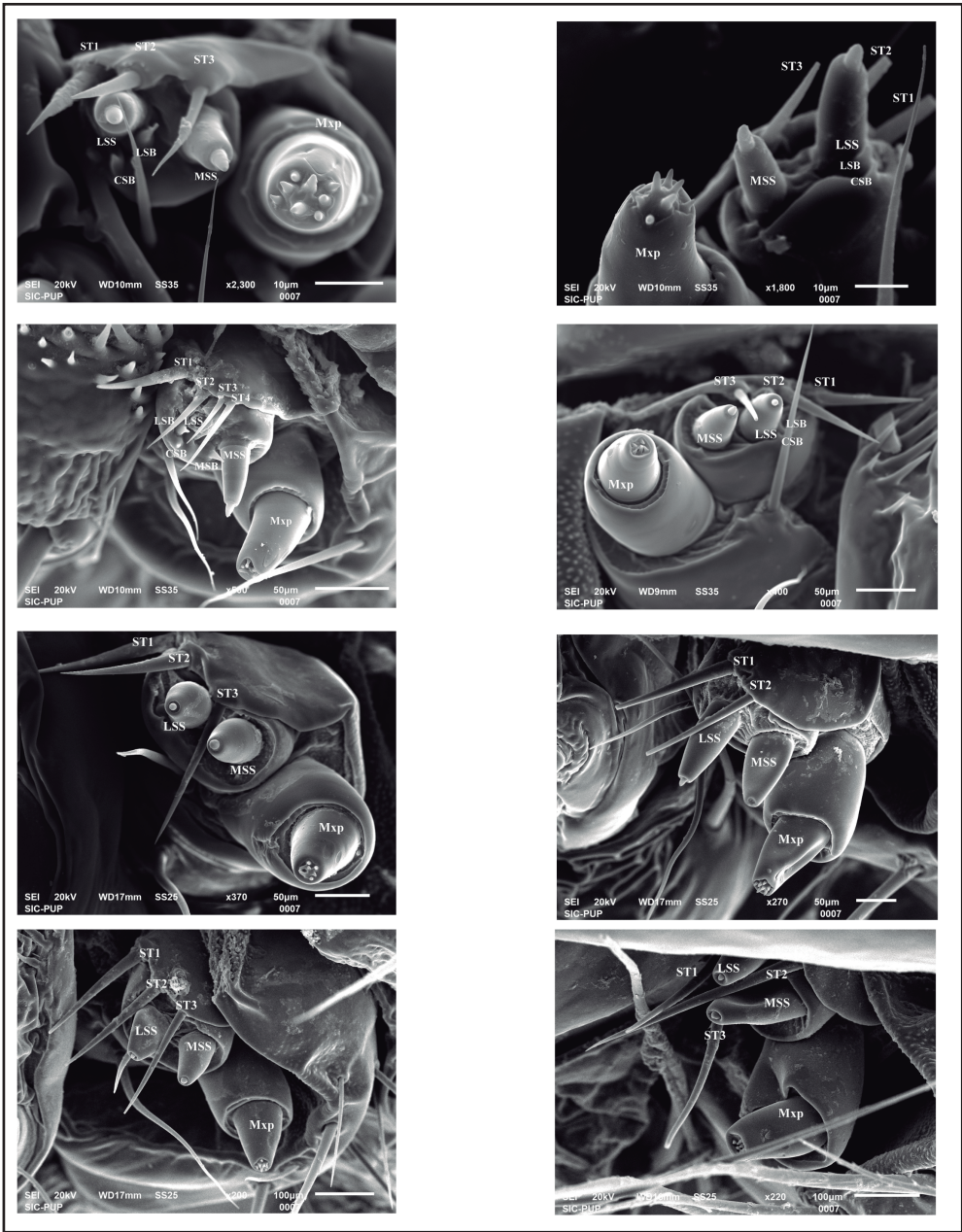


Figure 9. Morphology and structure of sensilla on maxillary palps in all larval instars of *Areas galactina*.

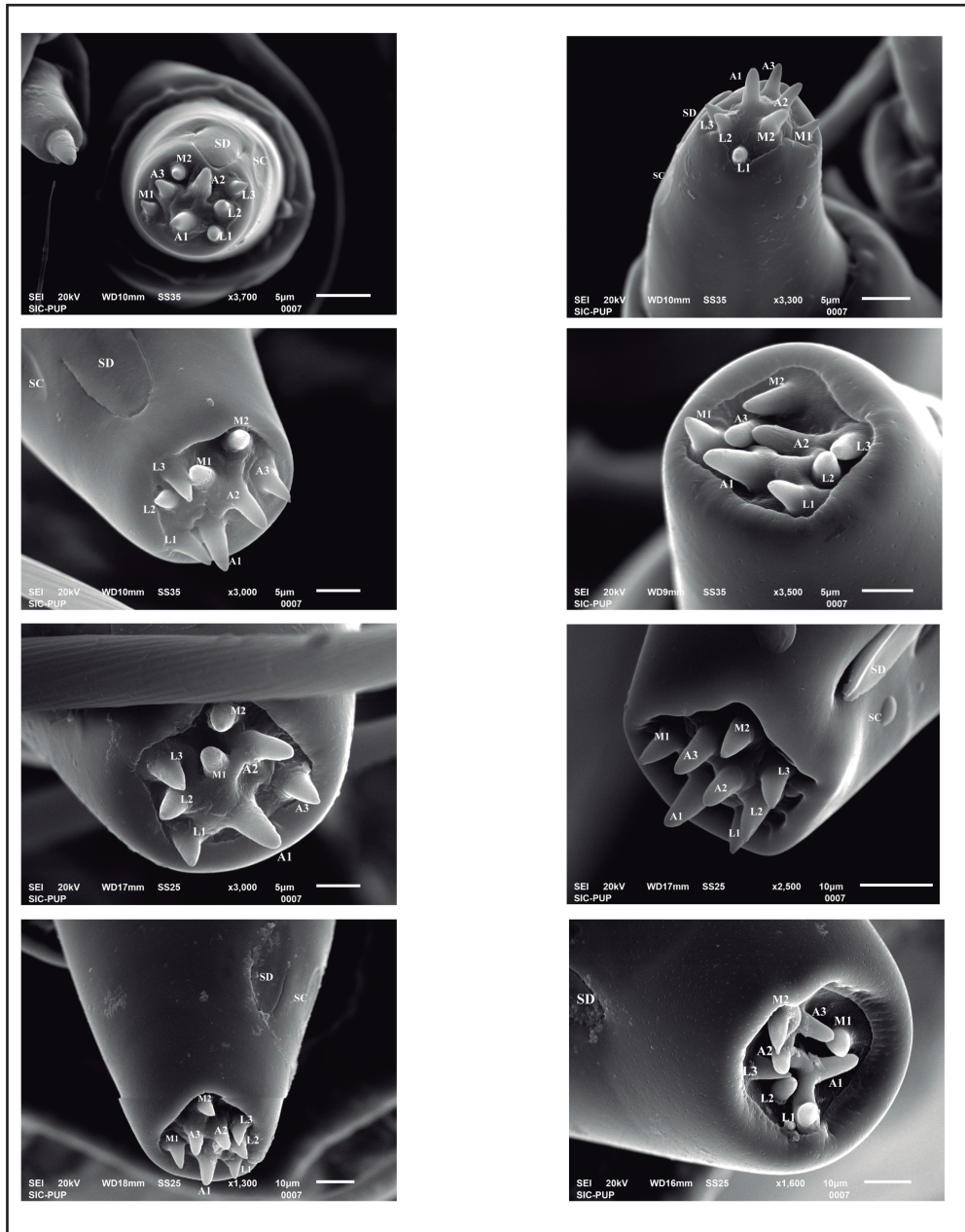


Figure 10. Morphology and structure of sensilla on labial palps in all larval instars of *Areas galactina*.

