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Comparison of sensory structures present on larval antennae and mouthparts of lepidopteran crop pests

Seema Rana¹ and Subbarayalu Mohankumar^{1,*}

Abstract

Lepidopteran crop pests like *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and *Maruca vitrata* (F.) (Lepidoptera: Pyralidae) are the major constraints for crop production and productivity. They are polyphagous and feed on tobacco, millets, cruciferous crops, and leguminous crops, respectively. Sensory structures and sensory genes are the key players responsible for their preference for particular crop species. Sensory genes code for the chemoreceptors present in the dendritic membrane of the neuron, which interpret the signal perceived by the insect nervous system that is responsible for insect behavior. In this study, we examined the distribution and morphology of sensory structures present on larval antennae and mouthparts of 4 lepidopteran crop pest species using scanning electron microscopy. We found that the mouthparts of lepidopteran larvae all possess sensory structures and organs such as sensilla chaetica, sensilla basiconica, and sensilla styloconica involved in gustatory and olfactory functions. The only difference between species lies in the external appearance, position, distribution, and number of sensory organs. Sensilla chaetica alone were observed on the labrum and mandibles, whereas sensilla styloconica and sensilla chaetica were present on the labium. All 3 types of sensilla were observed on the maxilla of *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* with varied numbers and morphology. Mainly, sensilla basiconica were present on antennae and apical maxillary palps, which are involved in olfactory and gustatory functions, respectively. In addition, we compared the sensory gene odorant receptor co-receptor (*Or83b*), which is pivotal in olfactory reception, among the 4 lepidopteran species and found that *Or83b* is conserved, showing the ancestral relationship among the 4 species.

Key Words: *Spodoptera litura*; *Chilo partellus*; *Plutella xylostella*; *Maruca vitrata*; sensillum; odorant receptor co-receptor

Resumen

Las plagas lepidópteros como *Spodoptera litura* (F.) (Noctuidae); *Chilo partellus* (Swinhoe) (Crambidae); *Plutella xylostella* (L.) (Plutellidae) y *Maruca vitrata* (F.) (Pyralidae) son las principales restricciones para la producción y productividad de los cultivos. Estas especies son polífagas y se alimentan de tabaco, mijo, cultivos crucíferos y leguminosas, respectivamente. Las estructuras sensoriales y los genes sensoriales son los principales factores responsables para su preferencia hacia ciertas especies de cultivos. Los genes sensoriales codifican los quimiorreceptores presentes en la membrana dendrítica de las neuronas, que interpretan la señal percibida por el sistema nervioso del insecto que es responsable del comportamiento del insecto. En este estudio, se examinó la distribución y la morfología de las estructuras sensoriales presentes en las antenas y partes bucales de la larva de 4 especies de plagas lepidópteros de cultivo mediante microscopía electrónica de barrido (SEM). Encontramos que las partes bucales de las larvas de los lepidópteros poseen todas las estructuras sensoriales y órganos como las sensillas chaéticas, sensillas basiconicas y sensillas estiloconicas involucradas en las funciones gustativas y olfativas. La única diferencia entre las especies radica en la apariencia externa, posición, distribución y número de órganos sensoriales. Se observaron las sensillas chaéticas solamente en el labrum y las mandíbulas, mientras que las sensillas estiloconicas y sensillas chaéticas estaban presentes en el labio. Se observaron las tres clases de sensillas en el maxilar de *S. litura*, *C. partellus*, *P. xylostella* y *M. vitrata* con números y morfología variadas. Principalmente, las sensillas basiconicas estaban presentes en las antenas y el apice de los palpos maxilares, que están implicadas en las funciones olfatoria y gustativa, respectivamente. Además, se comparó el co-receptor de receptores odorantes del gen sensorial (*Or83b*), que es fundamental en la recepción olfativa, entre las 4 especies de lepidópteros y se encontró que *Or83b* se conserva, mostrando la relación ancestral entre las 4 especies.

Palabras Clave: *Spodoptera litura*; *Chilo partellus*; *Plutella xylostella*; *Maruca vitrata*; sensilla; receptor y co-receptor de olores

Lepidoptera is the second largest insect pest order after Coleoptera affecting almost all the cultivated plants in the world. Major pests of this order include *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and *Maruca vitrata* (F.) (Lepidoptera: Pyralidae), which cause deteriorating effects on the quality and quantity of produce in field crops. All 4 species are polyphagous in nature and present in many parts of the world. *Spodoptera litura*, commonly known as tobacco cutworm, feeds on foliage of major crops like castor (*Ricinus*

communis L.; Euphorbiaceae), cotton (*Gossypium* species; Malvaceae), flax (*Linum usitatissimum* L.; Linaceae), groundnut (*Arachis hypogaea* L.; Fabaceae), jute (*Corchorus* species; Malvaceae), lucerne (*Medicago sativa* L.; Fabaceae), maize (*Zea mays* L.; Poaceae), rice (*Oryza sativa* L.; Poaceae), soybean (*Glycine max* [L.] Merrill; Fabaceae), tea (*Camellia sinensis* L.; Theaceae), tobacco (*Nicotiana tabacum* L.; Solanaceae), and tomato (*Solanum lycopersicum* L.; Solanaceae). *Chilo partellus*, a predominant shoot borer species, feeds on maize and sorghum (*Sorghum bicolor* [L.] Moench; Poaceae). *Plutella xylostella*, known as

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diamondback moth, feeds only on foliar tissues of cruciferous crops, especially *Brassica* species (Brassicaceae) including broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, mustard, and radish. *Maruca vitrata*, the legume pod borer, feeds on flowers and pods of grain legumes, including black gram (*Vigna mungo* [L.] Hepper; Fabaceae), cowpea (*Vigna unguiculata* [L.] Walpers; Fabaceae), pigeon pea (*Cajanus cajan* [L.] Millsp.; Fabaceae), and soybean. The damaging potential of these crop pests lies in their feeding behavior.

Feeding behavior or preference for particular host species depends on the various sensilla present on the insects' mouthparts, including olfactory, gustatory, and mechanosensory ones, which are involved in sensory perception via chemoreceptors (Dey et al. 2011). Sensilla are the organs or appendages present on the mouthparts, i.e., labrum, labium, maxillae, mandibles, and hypopharynx, that can detect smell (olfaction) or taste (gustation) and can recognize behavioral activities such as locomotion, oviposition, pupation, feeding, and orientation through tactile sensation (Lewis 1970). In physiological studies, sensilla possessed neurons that responded to plant odors (Anderson et al. 2000; Zheng et al. 2014).

Chemoreception plays a role in almost every aspect of insect life and represents a key interface between insects and the environment. It is used by insects to recognize a great variety of airborne molecules that provide information about food, predators, and potential mates (Engsontia et al. 2008). The olfactory system is the most widely used sensory detection method in insects and as such is highly specific and receptive to chemical cues in the environment (Hildebrand & Shepherd 1997). Chemoreceptors of insects mainly consist of olfactory receptors (ORs) and gustatory receptors (GRs), which are located in the dendritic membrane of neurons. The ORs belong to the large superfamily of G protein-coupled receptors (GPCRs), which detect chemicals in the environment (Clyne et al. 1999). Odorant-binding proteins surround OR neurons in insect antennae and are involved in the first step of olfactory transduction. Odorant-binding proteins are small, globular, water-soluble proteins that carry airborne semiochemicals to the chemoreceptors in insect antennae (Pophof 2004; Grosse-Wilde et al. 2006; Pelosi et al. 2006). The gene encoding odorant receptor *Or83b* is co-receptor for most of the odor recognition in insects and is extremely well conserved in distant insect species (Jones et al. 2005). The unique expression of specific members of olfactory gene families plays a role in determining the phenotype of a sensillum and what odors can be detected (Vogt et al. 2002).

Several studies were conducted to investigate the morphology of the sensory structures and their role in feeding behavior in lepidopteran insect pests through scanning electron microscopy (SEM) analysis. The high resolution and large depth of field make SEM vital for such studies in insects (Dey et al. 2011). SEM studies revealed the role and function of sensilla of *Philosamia ricini* (Drury) (Saturniidae) in various behaviors during the larval life (Barsagade et al. 2013). The sensilla basiconica present on the maxillary palp of *P. ricini* larvae respond to gustatory, mechanical, and olfactory stimuli (Barsagade et al. 2013). In many lepidopteran larvae, the chemosensilla or chemoreceptors present on galea, maxillary palp, and the inner surface of the labrum are involved in determining food preferences (Ishikawa 1963; Ma 1972; Dethier 1973; Stadler & Hanson 1975; De Boer et al. 1977; Albert 1980; Devitt & Smith 1982; Wazalwar & Khurad 2009). In larvae of *C. partellus*, the antennae contain sensilla basiconica and sensilla coeloconica that have olfactory function, and the maxillary palp contains sensilla styloconica that detect the difference between saps from susceptible and resistant host plants (Waladde & Kahoro 1990). Li et al. (2008) documented the morphology and distribution of sensilla present on larval antennae and mouthparts of *Spodoptera exigua* Hübner (Noctuidae).

Other reports of sensory structures in lepidopterans document the distribution, morphology, and number of sensory receptors on the larval maxillae and labia of *Heliothis virescens* (F.) and *Helicoverpa zea* Boddie (Noctuidae) (Baker et al. 1986); the type and distribution of antennal sensilla in *Manduca sexta* L. (Sphingidae) (Sanes & Hildebrand 1976), *C. partellus* (Waladde & Kahoro 1990), *Helicoverpa assulta* Guenée (Noctuidae) (Koh et al. 1995), *Spodoptera frugiperda* (J. E. Smith) (Noctuidae) (Malo et al. 2004), *Maruca testulalis* (Geyer) (Pyralidae) (Wang et al. 2008), and *Catocala remissa* Staudinger (Noctuidae) (Zheng et al. 2014); and the sensory structures present on the maxillary and labial palps of *Helicoverpa armigera* (Hübner) (Noctuidae), which are responsible for chemoreception (Keil 1996; Zhao et al. 2013).

However, very few studies are available that compare the sensory structures and sensory genes present on the mouthparts among the larvae of lepidopteran crop pests. Hence, we here compared the odorant gene *Or83b* and the distribution and morphology of sensilla present in sensory organs of 4 species in order to better understand the feeding behavior in insects.

Materials and Methods

INSECTS STUDIED

Larvae of tobacco cutworm, *S. litura*, diamondback moth, *P. xylostella*, and legume pod borer, *M. vitrata*, were collected from research fields of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, and larvae of maize shoot borer, *C. partellus*, were obtained from the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India. Larvae of *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* were reared on castor leaves (*R. communis*), baby corn (*Z. mays*), cauliflower leaves (*Brassica oleracea* L.; Brassicaceae), and lablab pods (*Lablab purpureus* [L.] Sweet; Fabaceae), respectively, in the Molecular Ecology Laboratory, Department of Plant Biotechnology, Tamil Nadu Agricultural University.

Mass Culturing of *S. litura* and *P. xylostella*

The larvae of *S. litura* and *P. xylostella* were reared on castor leaves and cauliflower leaves, respectively, in plastic buckets (22.5 cm diameter, 25 cm height) until pupation. The leaves were changed once in 2 d for earlier instars and daily for later instars. The pupae from the culture were collected, surface sterilized with 0.5% sodium hypochlorite, rinsed with distilled water, and kept in adult emergence cages. Newly emerged adults were transferred to plastic buckets for mating and oviposition. Adults were fed with 10% sugar solution enriched with vitamin E. The top of the plastic bucket was covered with a muslin cloth, which served as an oviposition substrate. The temperature and relative humidity were maintained at 25 ± 1 °C and 75%, respectively, inside the culture room. Muslin cloths with eggs were collected from the 3rd day onwards, replaced with fresh cloth daily, and placed in plastic cups covered with a lid. Twenty-four hours after the collection of egg cloths, eggs were observed for the development of embryos. Egg cloths were placed in a humidifying chamber for hatching. Newly hatched larvae were transferred with a camel hair brush to a plastic bucket containing fresh leaves. The hatched larvae were used for experiments.

Mass Culturing of *C. partellus*

The rearing of *C. partellus* was identical to that of *S. litura* and *P. xylostella* with the following modifications: Larvae were reared on baby corn, and folded wax paper was provided as oviposition substrate to female moths.

Mass Culturing of *M. vitrata*

The larvae of *M. vitrata* were reared in groups in the tender pods of lablab in plastic rearing trays (30 × 10 cm) until pupation. The 5th instar larvae were carefully examined for any disease incidence. Four days after pupation, the pupae were collected and all malformed or undersized pupae were discarded. The healthy pupae were surface sterilized by immersion in 0.5% sodium hypochlorite solution, washed thoroughly with water, and spread on filter paper to dry. The surface sterilized pupae from the parent colony were kept in a plastic bucket (22.5 cm diameter, 25 cm height) for adult emergence. Ten pairs of healthy adults were maintained in each bucket. Fresh lablab pods were provided for egg laying. The plastic bucket was covered with black colored sterile muslin cloth, which also served as an oviposition substrate. Sugar solution (10%) with a drop of vitamin E was provided as adult feed in sterile glass vials with sterile absorbent cotton. The sterile muslin cloth and sugar solution were removed periodically and fresh lablab pods provided daily. The eggs were stored in a humidified (95% relative humidity) plastic container. After hatching, larvae were transferred to the rearing trays.

SCANNING ELECTRON MICROSCOPY

The heads from larvae of *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* were used to observe and document the sensilla structures with SEM. Two individual larvae of each species were used. The larvae were anesthetized using CO₂ for about 10 min before their heads were dissected and mounted on stubs with double-sided adhesive tape. Micrographs of the antennae, mouthparts, and sensilla were taken from individuals with a FEI Quanta 250 (Netherlands) at the Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore. The abundance and distribution of the sensilla types were compared between the 4 lepidopteran species. Sensilla present on the mouthparts of larvae were characterized based on the criteria of Li et al. (2008).

ISOLATION OF TOTAL RNA

Total RNA was extracted by homogenizing antennae and the head portion of larvae of *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* individually by using the Trizol method (Chomczynski & Mackey 1995). Antennae and head were collected from 4th instar larvae and immediately transferred into 2.0 mL microcentrifuge tubes immersed in liquid nitrogen. The RNA isolated was converted into cDNA. First-strand cDNA synthesis was performed using the cDNA Synthesis Kit (Cat # K1622, Thermo Scientific, Waltham, Massachusetts). The synthesized cDNA was used as template for polymerase chain reaction (PCR) to amplify the *Or83b* gene using degenerate primers (*Or83b* F: 5'-GCCAACACTATCACC-3'; *Or83b* R: 5'-TCGAGGGGCTACTTC-3'; amplicon size: 312 bp). Then, the PCR product was column purified as per the manufacture's instruction provided by a purification spin kit (Bio Basic Inc., Ontario, Canada). The final concentration of the purified product was determined by spectrophotometry (Nanodrop ND-1000, Thermo Scientific, Waltham, Massachusetts). The quality of the purified PCR product was checked by electrophoresis in a 1.5% agarose gel.

CLONING OF THE *OR83B* GENE

The column-purified, PCR-amplified DNA fragments were used for cloning (InsTAclone PCR Cloning Kit, Cat #K1213, Thermo Scientific, Waltham, Massachusetts) and bacterial transformation. Further validation of recombinant colonies was done by colony PCR and restriction

digestion analysis. Plasmid DNA was isolated using EZ-10 Spin Column Plasmid DNA MiniPreps Kit (Cat #BS414, Bio Basic Inc., Ontario, Canada,) as per the manufacturer's instruction. Small aliquots of plasmid DNA were analyzed on a 0.8% agarose gel and sent for sequencing to SciGenom Labs Pvt. Ltd., Cochin, Kerala, India.

SEQUENCING OF CLONED FRAGMENT AND ANALYSIS

The samples were sequenced through single pass analysis from forward and reverse direction. Sequence data were compared with available gene sequences in the National Center for Biotechnology Information (NCBI) sequence data bank (<http://www.ncbi.nih.gov>) by BLASTn analysis. Sequence alignment and phylogenetic analysis in different species for the *Or83b* gene was done by the ClustalW version 2.0 online tool (www.ebi.ac.uk/Tools/msa/clustalw2/). The edited sequences were aligned with reference sequences of *Or83b* (Accession No: JQ811935). The phylogenetic tree was constructed for the partial amplified *Or83b* gene in the 4 lepidopteran species using MEGA v 6.0 software (Tamura et al. 2013).

The *Or83b* nucleotide sequence data obtained by cloning were deduced into amino acid sequences via EMBOSS Transeq (http://www.ebi.ac.uk/Tools/st/emboss_transeq). The amino acid sequences from various lepidopteran species were collected from the NCBI database, and a phylogenetic tree was constructed for available sequences of *Or83b* protein sequences from the following lepidopteran species: *Heliothis virescens* (Hufnagel) (Noctuidae) (Accession No: AF125169), *H. assulta* (Accession No: ABU45983), *H. armigera* (Accession No: ADQ13177), *H. zea* (Accession No: AAX14773), *P. xylostella* (Accession No: ACX54944), *Chilo suppressalis* Walker (Crambidae) (Accession No: ACJ07125), and from *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Accession No: AAT71306). The deduced amino acid sequence of *Or83b* from *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* were aligned with the amino acid sequences of *Or83b* (Accession No: AFN22085) collected from the NCBI database. Multiple sequence alignment was carried out using the ClustalW version 2.0 online tool (www.ebi.ac.uk/Tools/msa/clustalw2/). A phylogenetic analysis was conducted to identify the relatedness of the *Or83b* gene among the lepidopteran species using MEGA v 6.0 software (Tamura et al. 2013).

Results

The sensory structures, i.e., sensilla basiconica, sensilla styloconica, and sensilla chaetica present on antennae and mouthparts of *S. litura*, *M. vitrata*, *C. partellus*, and *P. xylostella* larvae were documented and are presented in Figs. 1 to 13 and Table 1. The larval heads of *S. litura*, *M. vitrata*, and *C. partellus* are smooth, oval with a small number of setae whereas that of *P. xylostella* has sharp, rough and dense setae, including long tactile setae (LTS) and short tactile setae (STS) (Fig. 1).

ANTENNA

The antennal development on the larval head progressed from the 1st to the last instar in *S. litura* (Fig. 2), *C. partellus* (Fig. 3), *P. xylostella* (Fig. 4), and *M. vitrata* (Fig. 5). The antenna was 3-segmented in all 4 species with variation in the distribution of sensilla. The antenna of *C. partellus* and *M. vitrata* possessed 3 sensilla basiconica (B1, B2, B3), 2 sensilla styloconica (S1, S2), and 1 sensillum chaeticum (C), whereas in *P. xylostella* and *S. litura*, 3 sensilla basiconica, 1 sensillum styloconicum, and 1 sensillum chaeticum in lateral position were observed and dense projections were seen at the basal segment (Fig. 6). Sensilla basiconica were peg shaped, slender, thick walled, and located at lateral

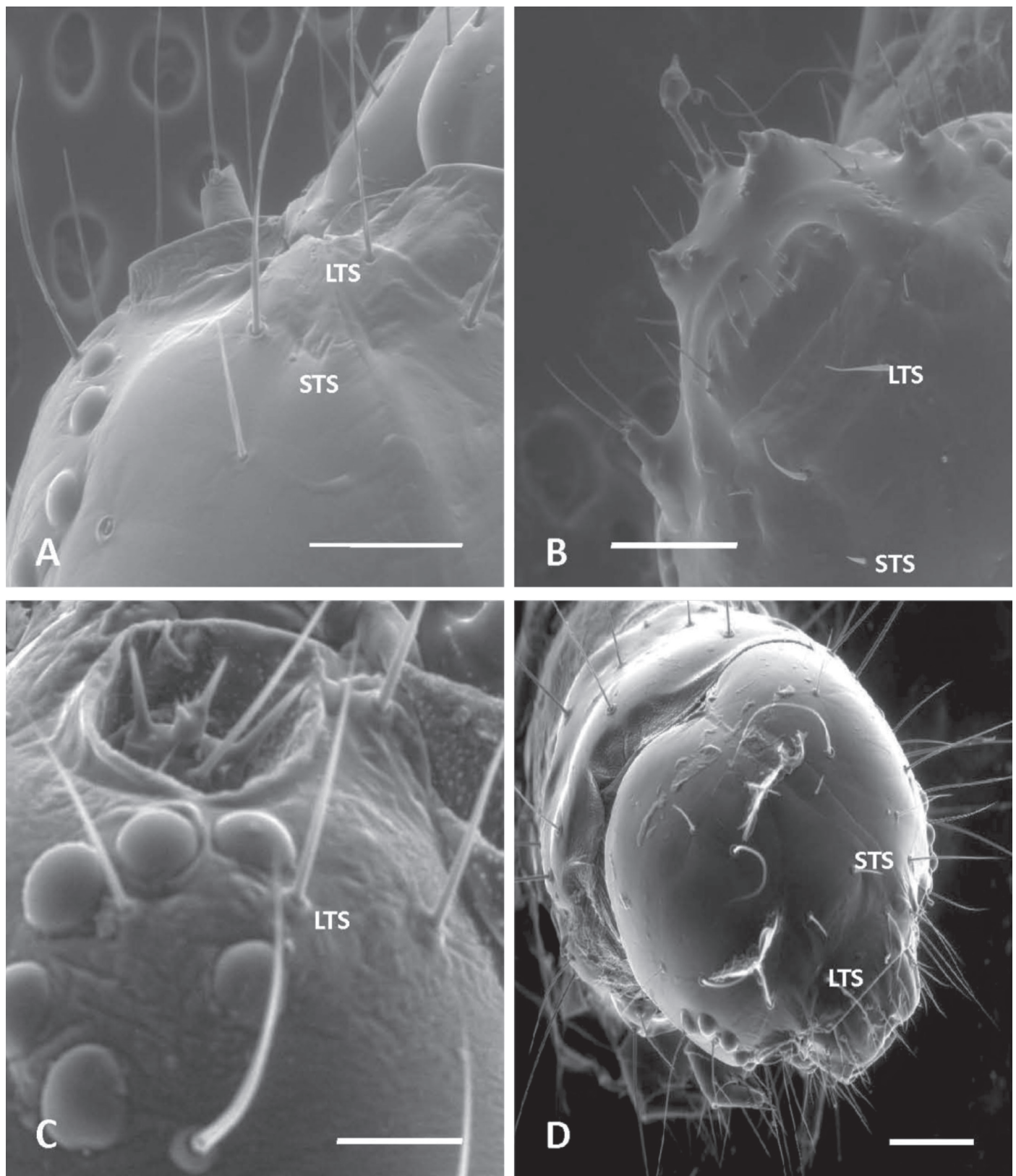


Fig. 1. Dorsal surface of the larval head showing short tactile setae (STS) and long tactile setae (LTS) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

position whereas sensilla chaetica were long and tapered towards the end. Sensilla styloconica (S1, S2) were 2-segmented, cone shaped, and embedded inside a stout peg-like structure.

MOUTHPARTS

Larval mouthparts consisted of labrum (L), mandible (M), maxilla (MX), and labium (LI) (Fig. 7).

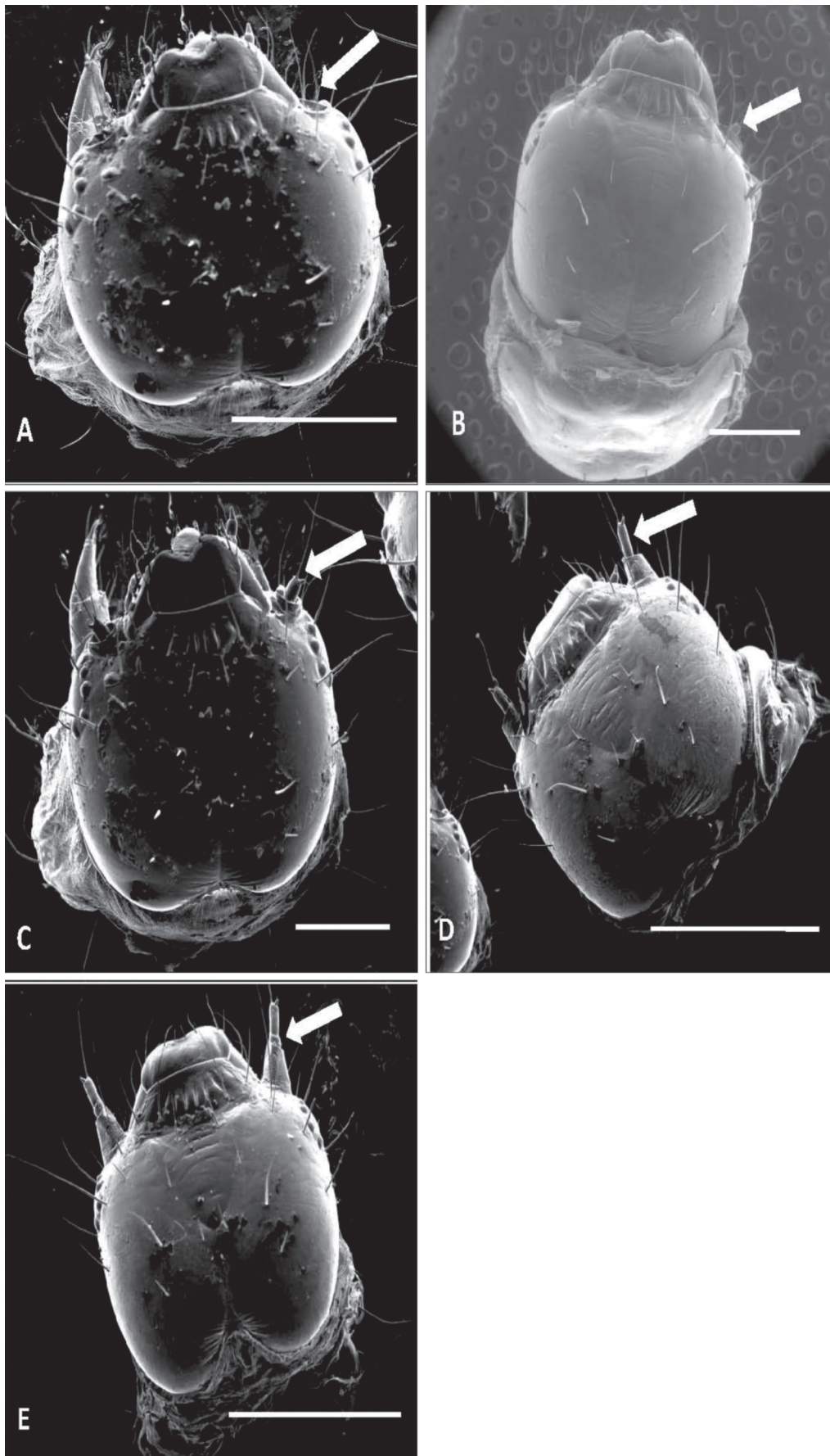


Fig. 2. SEM images of larval antennae of *Spodoptera litura* at different instars: A) 1st instar, B) 2nd instar, C) 3rd instar, D) 4th instar, and E) 5th instar.

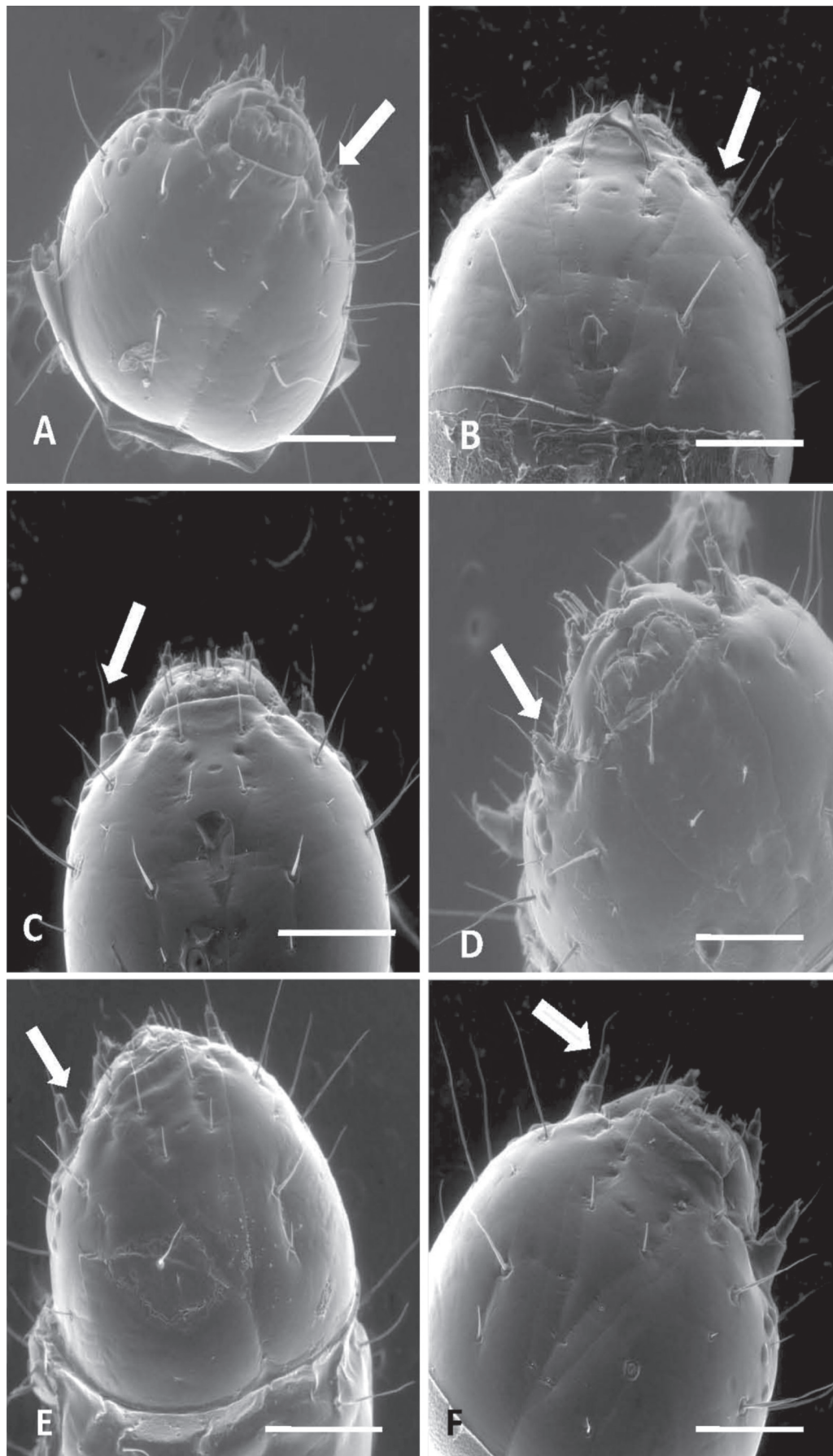


Fig. 3. SEM images of larval antennae of *Chilo partellus* at different instars: A) 1st instar, B) 2nd instar, C) 3rd instar, D) 4th instar, E) 5th instar, and F) 6th instar.

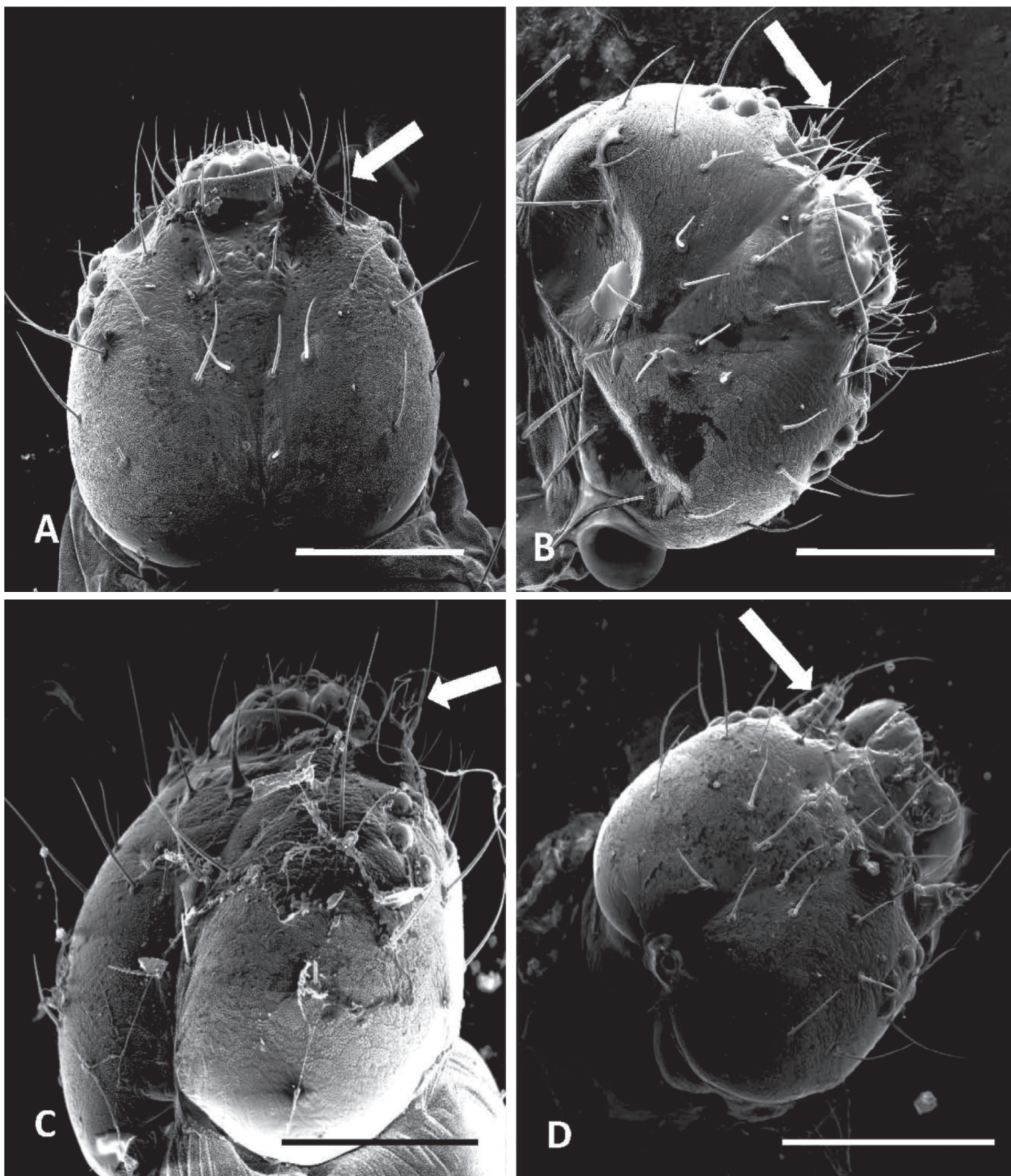


Fig. 4. SEM images of larval antennae of *Plutella xylostella* at different instars: A) 1st instar, B) 2nd instar, C) 3rd instar, and D) 4th instar.

Labrum

Sensilla chaetica were widely distributed throughout the surface of the labrum. In *P. xylostella*, they were more abundant than in *C. partellus*, *M. vitrata*, and *S. litura* (Fig. 8). The maximum number of sensilla

chaetica (15–16) was observed in *P. xylostella* followed by *C. partellus* (10–11). The smallest number of sensilla chaetica (9–10) was recorded in *S. litura* and *M. vitrata* (Fig. 8). STS and LTS were observed both on the labrum and the head portion of larvae.

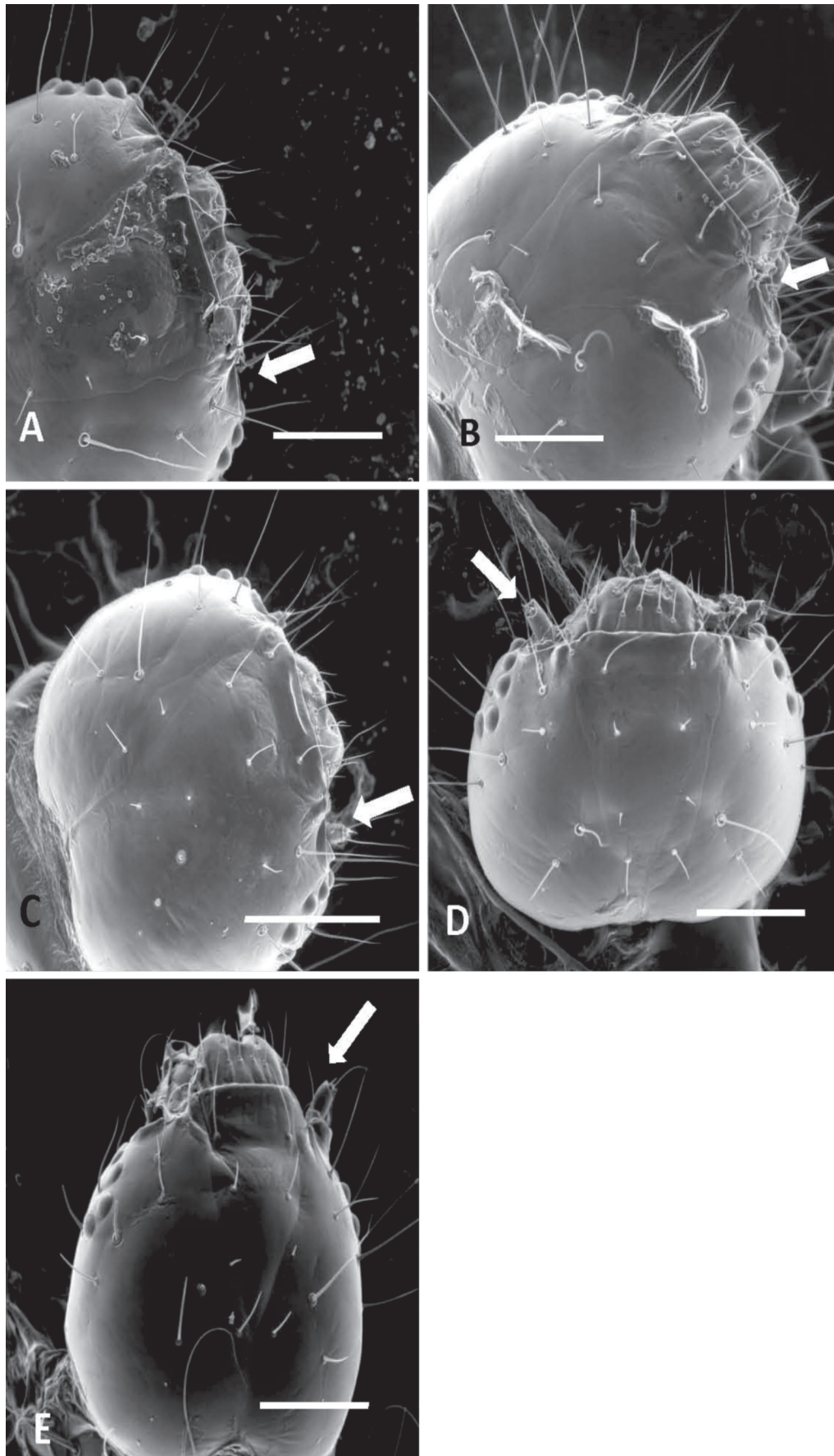


Fig. 5. SEM images of larval antennae of *Maruca vitrata* at different instars: A) 1st instar, B) 2nd instar, C) 3rd instar, D) 4th instar, and E) 5th instar.

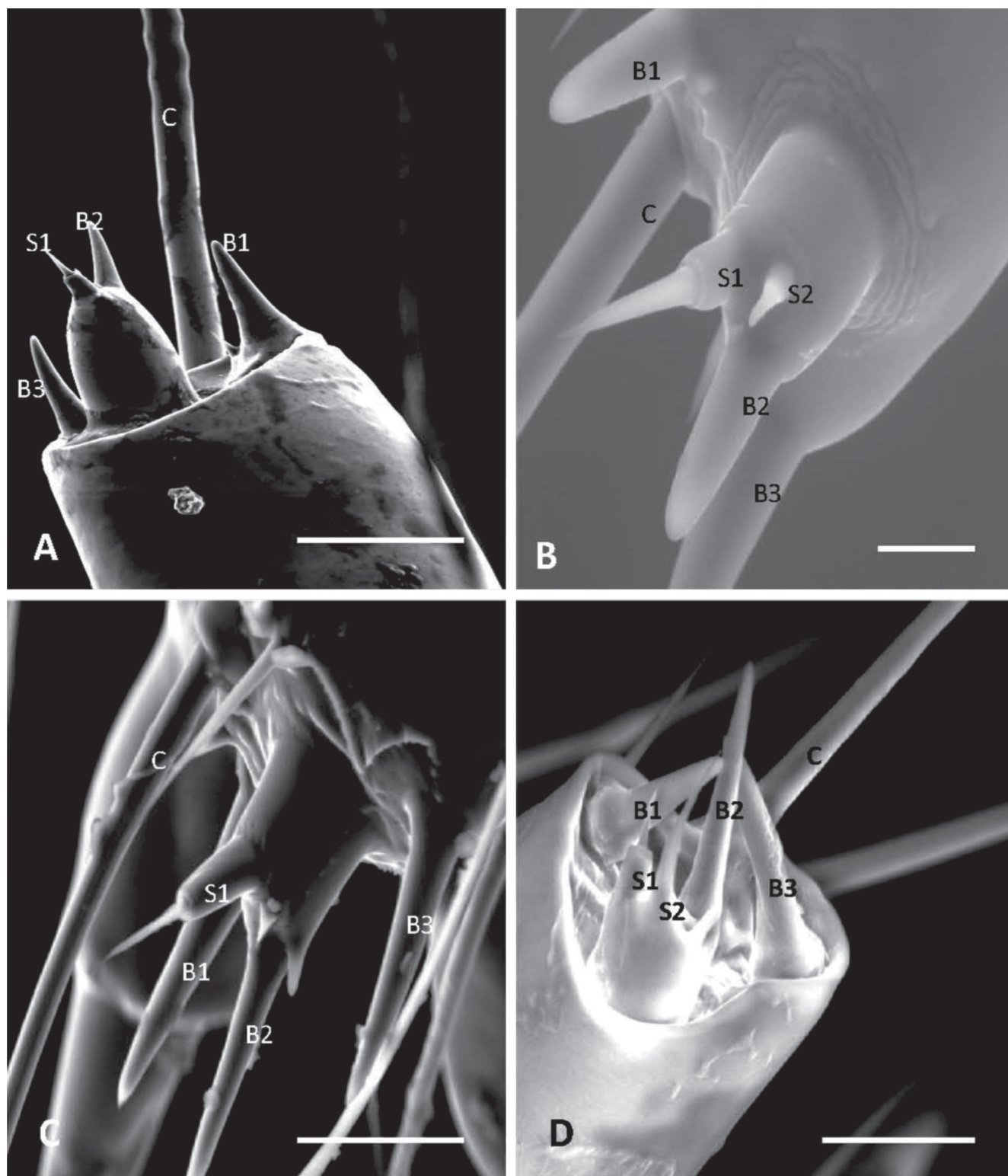


Fig. 6. Antenna is segmented (I–III), showing the presence of sensilla styloconica (S1, S2), sensilla basiconica (B1, B2, B3), and sensillum chaeticum (C) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

Mandible

A heavily sclerotized mandible was present in all 4 species, carrying 2 sensilla chaetica (C1, C2) in *S. litura* and *C. partellus* and 4 sensilla chaetica (C1, C2, C3, C4) in *P. xylostella* and *M. vitrata*. They had a corn-cob like structure, wide at the base and curved apically (Fig. 9).

Maxilla

The maxilla was composed of 4 parts (Fig. 10), i.e., maxillary palp, stipes, cardo, and galea. The maxillary palp was 2-segmented with cardo and stipes at the basal portion. The stipes had 1 sensillum chaeticum. On the distal end of the stipes, the galea had 2 sensilla stylo-

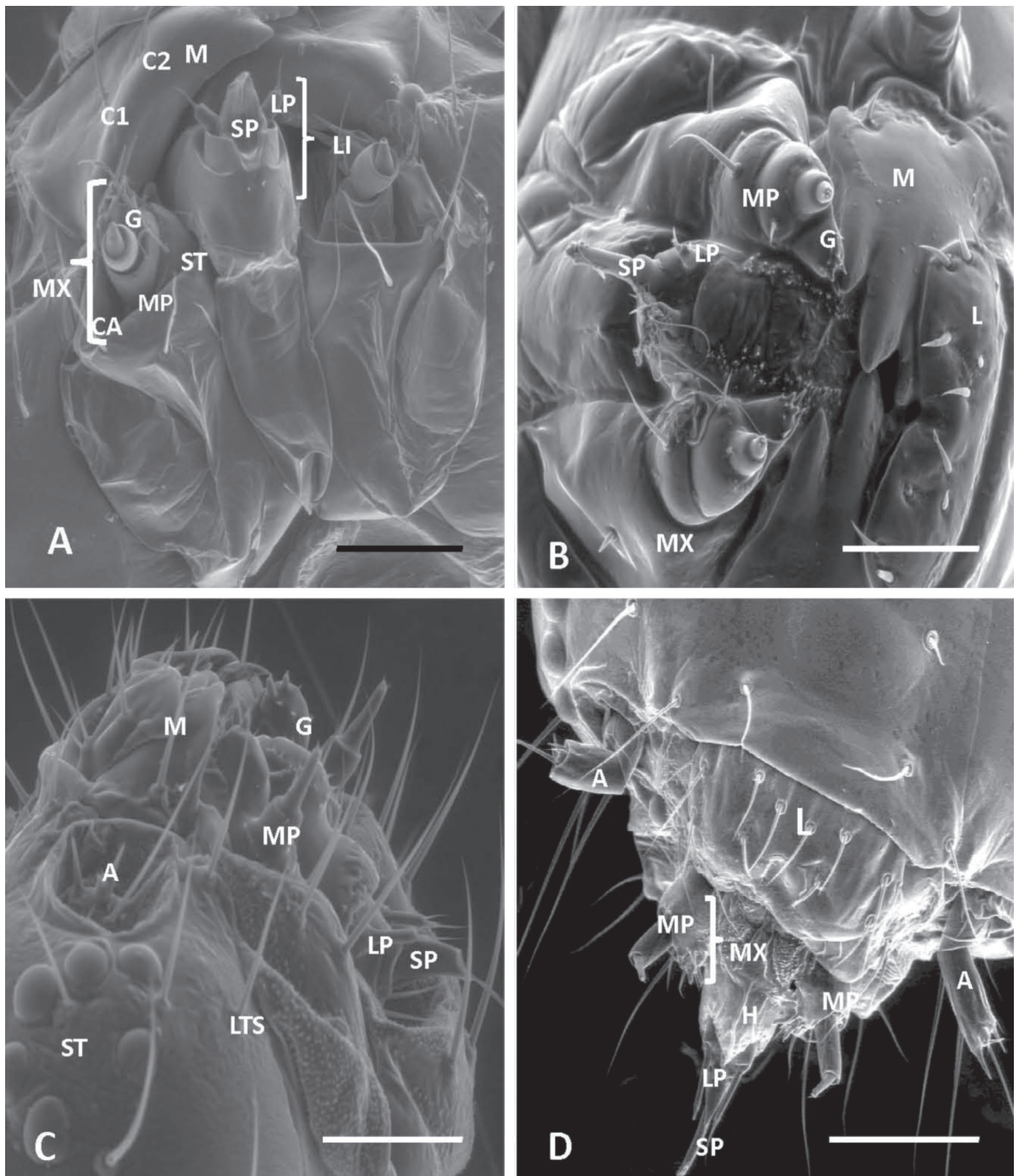


Fig. 7. Ventral and lateral view of larval mouthparts showing labrum (L), mandible (M), maxillae (MX), labium (LI), antennae (A), stemma (ST), and long tactile setae (LTS) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

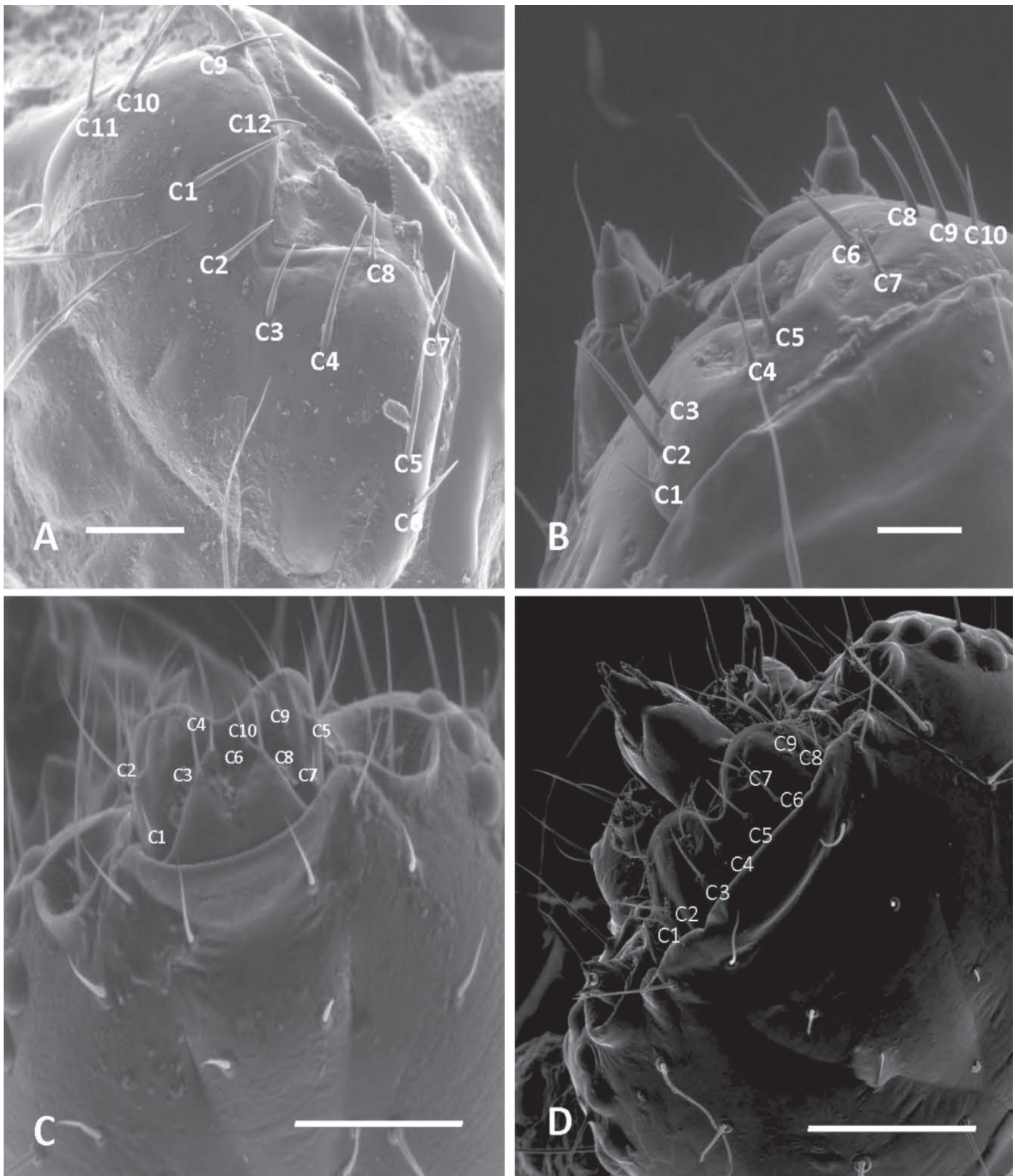


Fig. 8. Labrum (L) showing distribution and number of sensilla chaetica (C1–C15) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

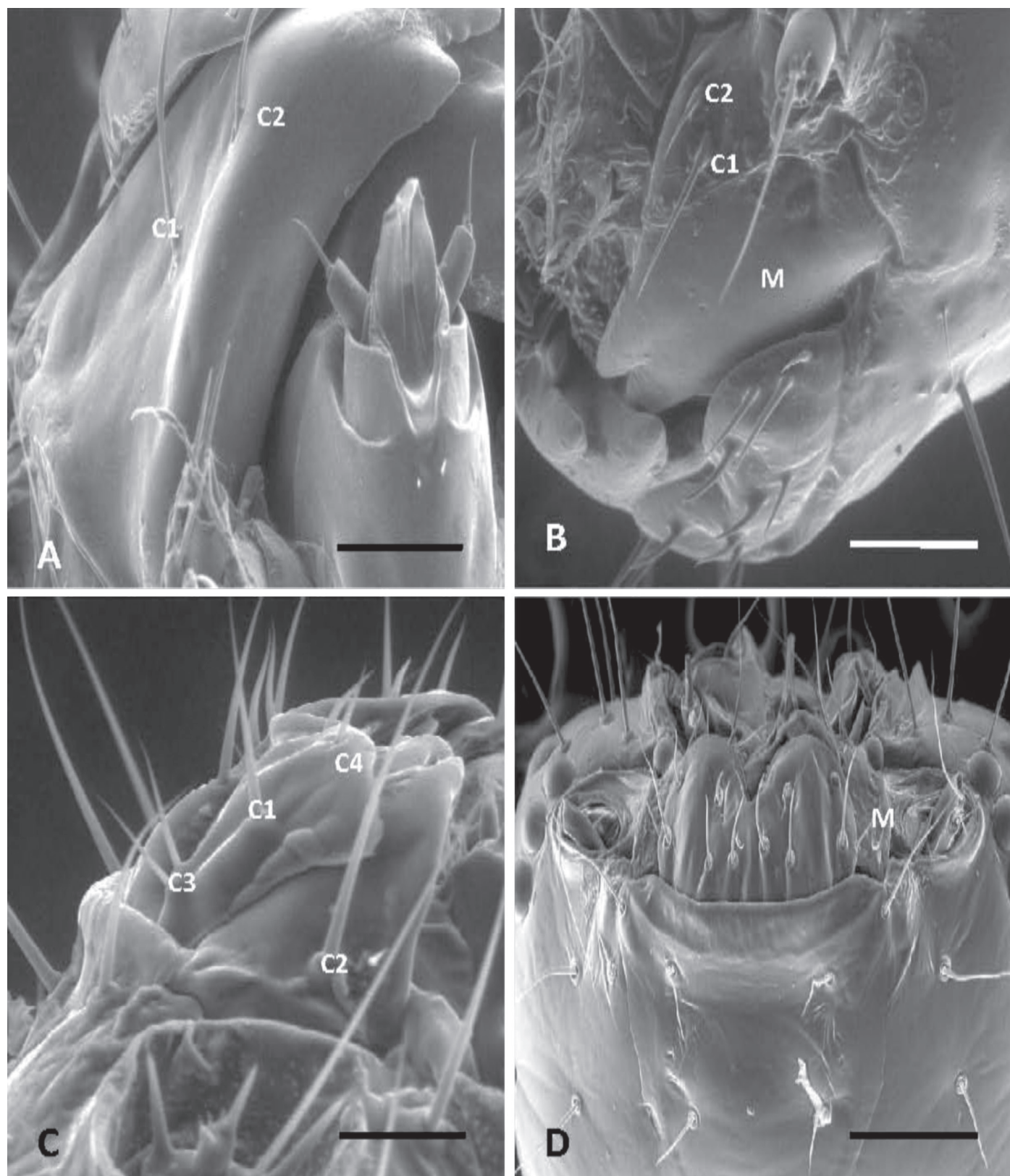


Fig. 9. Mandible (M) showing sensilla chaetica (C1, C2) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

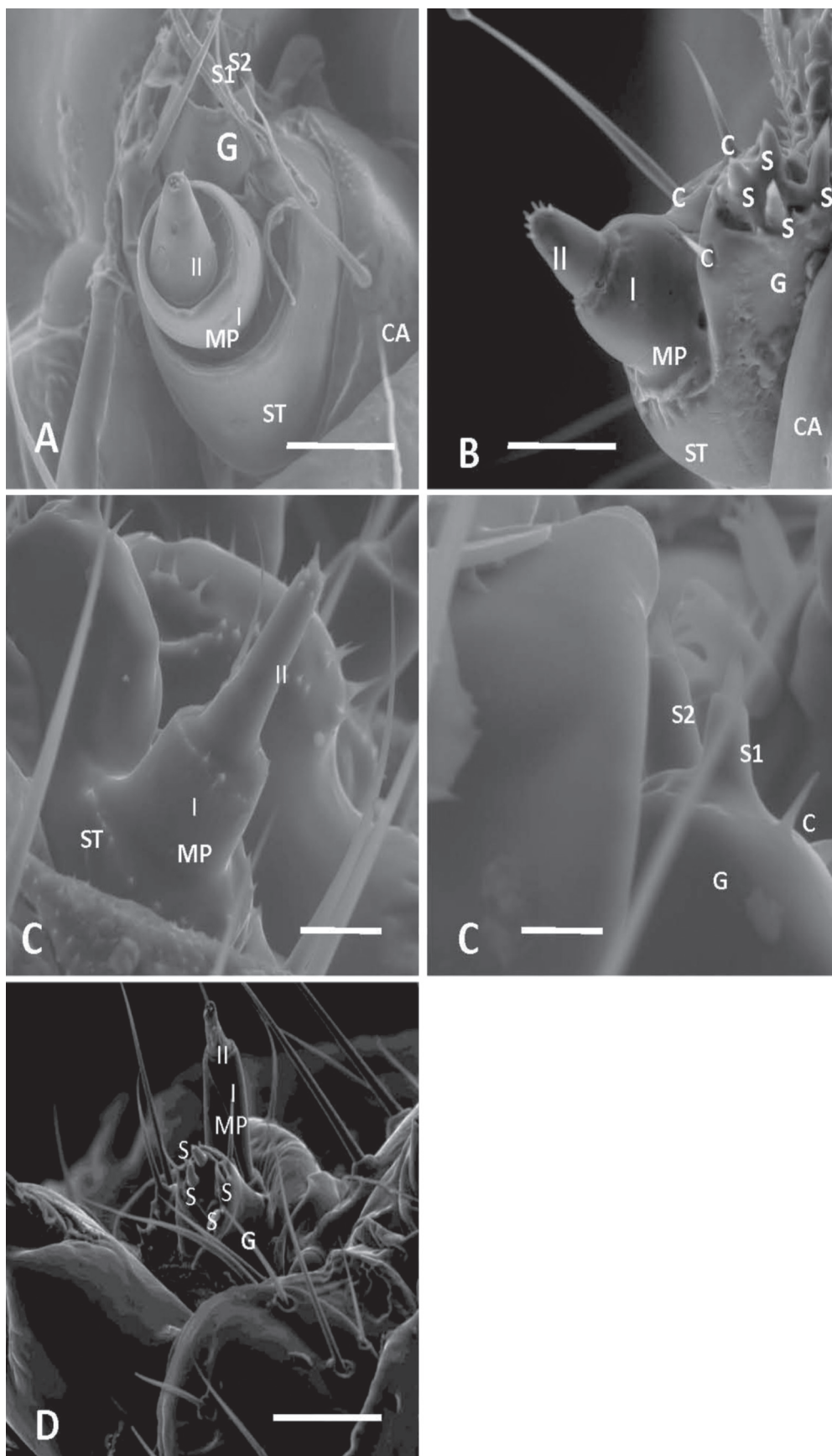


Fig. 10. Enlarged maxillae (MX) showing maxillary palp (MP), galea (G), stipes (ST), and cardo (CA), with galea showing the distribution of sensilla styloconica (S1–S4) and sensilla chaetica (C) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

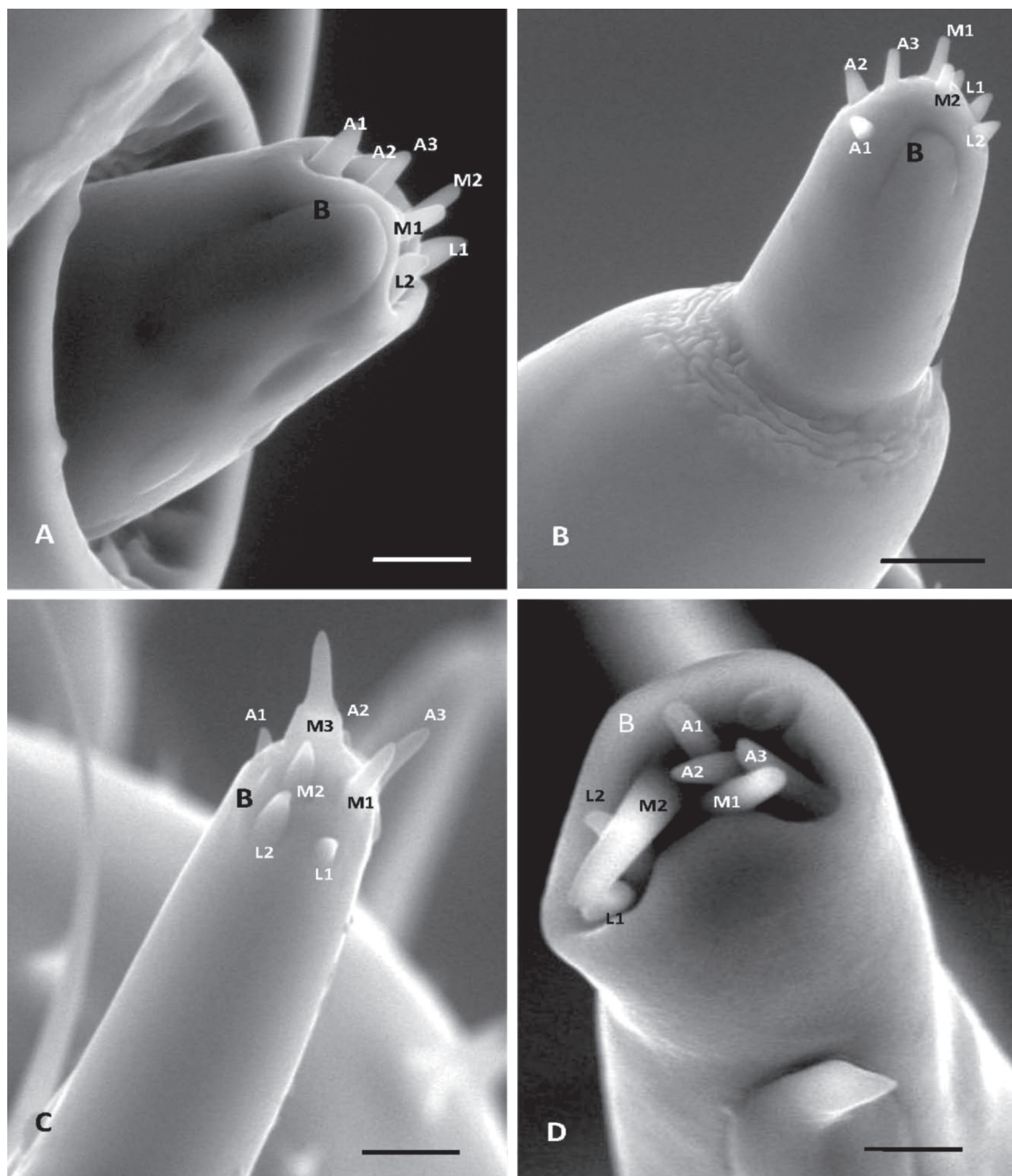


Fig. 11. Apical maxillary palp showing apical sensilla basiconica (A1, A2, A3), medial sensilla basiconica (M1, M2, M3), and lateral sensilla basiconica (L1, L2) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

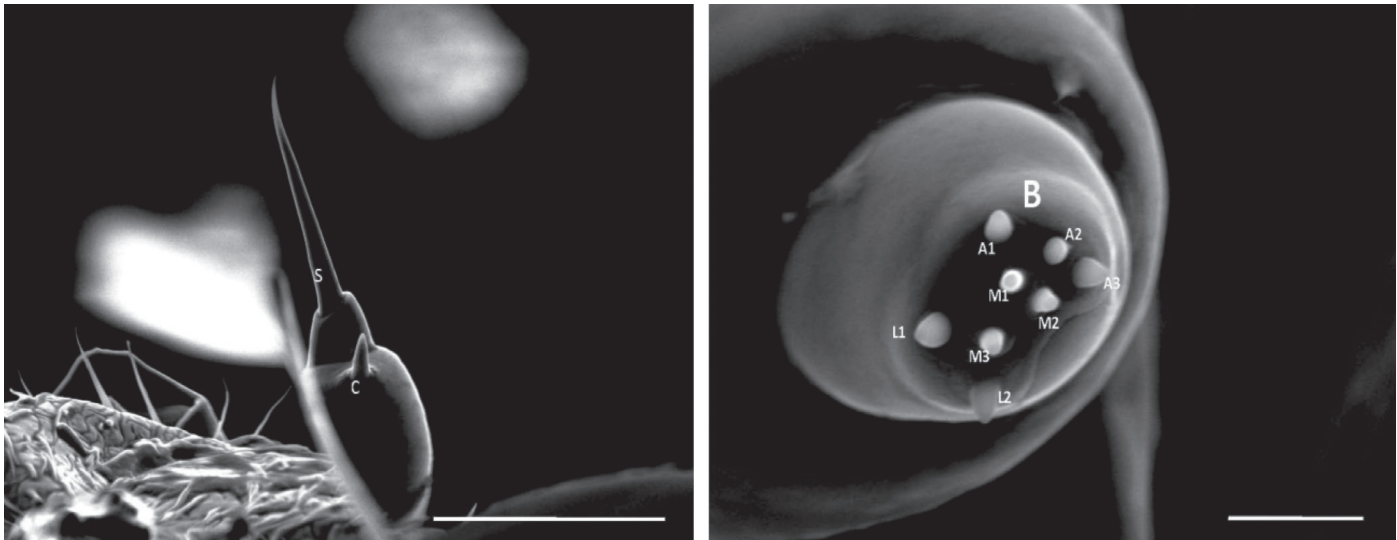


Fig. 12. Enlarged labial palp (left) showing sensillum chaeticum (C) and sensillum styloconicum (S); and enlarged apical maxillary palp (right) showing apical sensilla basiconica (A1, A2, A3), medial sensilla basiconica (M1, M2, M3), and lateral sensilla basiconica (L1, L2).

conica (S1, S2) in *S. litura* and *P. xylostella* and 4 sensilla styloconica in *C. partellus* and *M. vitrata*. The numbers of sensilla chaetica present on the maxilla of *S. litura* (1), *C. partellus* (3), *P. xylostella* (1), and *M. vitrata* (3) could also be determined (Fig. 10). The apical maxillary palp of *C. partellus* and *P. xylostella* had 8 sensilla basiconica arranged as 3-3-2, i.e., 3 apical sensilla (A1, A2, A3), 3 medial sensilla (M1, M2, M3), and 2 lateral sensilla (L1, L2). All these sensilla possessed an apical pore (Fig. 11).

Labium

The labium consisted of 2 labial palps with a sensillum styloconicum at the apex, a sensillum chaeticum in the center (Fig. 12), and a long spinneret (SP) for secreting silk (Fig. 13). The labial palps of *S. litura* and *M. vitrata* larvae were broad and flat with smooth texture whereas those of *P. xylostella* larvae were sharp and hairy (Fig. 13). There were no variations in the numbers and morphological structures of sensilla styloconica and sensilla chaetica in all 4 species.

PHYLOGENETIC ANALYSIS OF THE *OR83B* GENE

The phylogenetic tree showed that the reference amino acid sequence of the *Or83b* gene from *S. litura* (Accession No. AFN22085) formed a cluster with the amplified amino acid sequences of the *Or83b* gene from *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* (Fig. 14). Also, a close evolutionary association between available lepidopteran *Or83b* amino acid sequences was found, whereas no association existed with the *Or83b* amino acid sequence from *D. melanogaster*.

The multiple sequence alignment of the cloned partial sequence of the *Or83b* gene from *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* with the reference sequence of *Or83b* from *S. litura* showed that there was a match of 312 bp according to the expected size. There were nucleotide variations at the 347, 391, 435, 440, and 606 bp positions among the amplified sequences of *Or83b* from *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata*, and all sequences were similar to the reference sequence (Fig. 15). The multiple sequence alignment of the deduced amino acid sequences of the amplified *Or83b* gene from the 4 species with the reference sequence showed that most of the residues were conserved with 98.08 % identity (Fig. 16).

In summary, the sequence analysis revealed that the partial sequences of the *Or83b* gene cloned in the present study were conserved among the 4 lepidopteran species.

Discussion

The larval stage of lepidopteran pests is the most damaging stage to host plants, and thus is the major target stage in pest management. Particularly, from the 3rd instar onwards, the larva enters into the period of maximal food searching and feeding activity. The larval antenna is the principle olfactory organ that senses host volatiles, allowing the larva to locate host plants for feeding. The functional studies of larval antenna-specific or high-expression olfactory genes will be of significance for larva-based pest control. These larvae depend largely on their gustatory and olfactory sensory structures (sensilla) to find food sources. Their feeding behavior is controlled by input from the gustatory sensilla on the mouthparts.

The use of electron microscopy enables researchers to detect sensory structures and infer their function in many biological processes. With the aid of SEM, we recorded and illustrated the external morphology, distribution, and types of sensory structures on the antennae and mouthparts of 4 lepidopteran species, with a particular focus on the sensilla. Insect sensilla play an important role in olfactory and gustatory sensation. The majority of lepidopteran larvae possess 4 types of bilateral gustatory sensilla. One type, the lateral and medial sensilla styloconica, is thought to play a decisive role in host plant selection behavior. These sensilla are in continuous contact with plant sap during feeding and are capable of detecting different phytochemicals. The structures of the sensilla chaetica, sensilla styloconica, and sensilla basiconica have been established. The external morphology, distribution, and types of sensilla on antennae and mouthparts in the 4 species we studied largely agree with those reported for other lepidopteran species (Sanes & Hildebrand 1976; Baker et al. 1986; Waladde & Kahoro 1990; Koh et al. 1995; Keil 1996; Lin 1997; Malo et al. 2004; Li et al. 2008; Wang et al. 2008; Dey et al. 2011; Barsagade et al. 2013; Zhao et al. 2013; Zheng et al. 2014).

On the antennae of *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* larvae, we found that 3 multiporous sensilla basiconica were

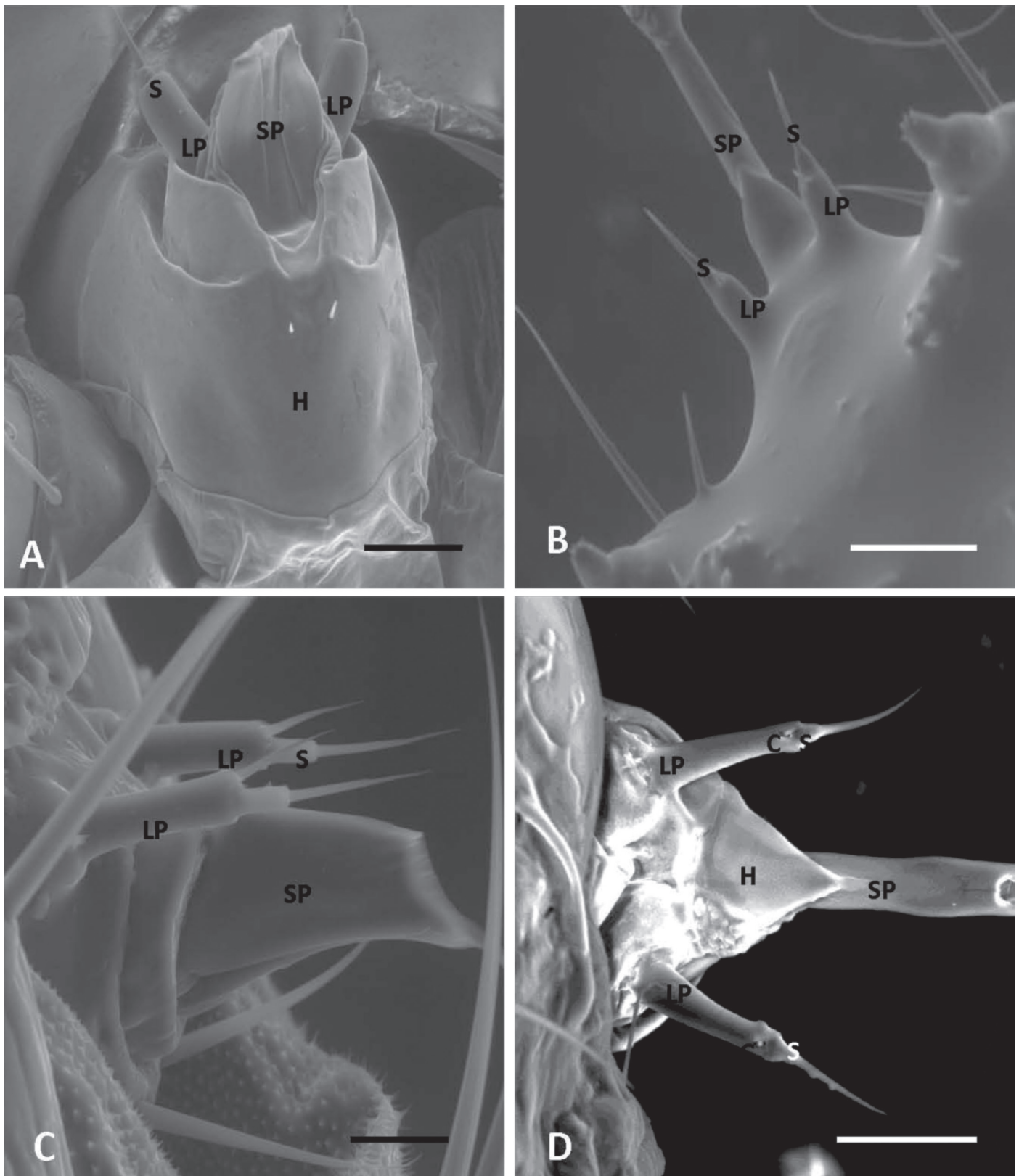


Fig. 13. Ventral labium (LI) surface of mouthparts showing hypopharynx (H), labial palp (LP), spinneret (SP), sensilla chaetica (C), and sensilla styloconica (S) in 4 species of Lepidoptera: A) *Spodoptera litura*, B) *Chilo partellus*, C) *Plutella xylostella*, and D) *Maruca vitrata*.

present and had olfactory receptors similar to those described from other lepidopteran species (Schoonhoven & Dethier 1966; Dethier

1973; Lin 1997; Li et al. 2008). Klowden (2007) and Palma et al. (2013) described sensilla basiconica as compact, thick walled and

Table 1. Hypothesized function, abundance, and external morphology of sensory structures present on larval antennae and mouthparts of *Spodoptera litura*, *Chilo partellus*, *Plutella xylostella*, and *Maruca vitrata*.

Sensory organ	Hypothesized function	Number, location, structure, and surface			
		<i>Spodoptera litura</i> (leaf feeder)	<i>Chilo partellus</i> (shoot borer)	<i>Plutella xylostella</i> (leaf feeder)	<i>Maruca vitrata</i> (pod borer)
Antenna (Fig. 6)					
Sensillum basiconicum	Olfaction				
Sensillum styloconicum	Olfaction, thermoreception	3, apical segment, corncob like 1, apical segment, cone shaped with broad base	3, apical segment, corncob like 2, apical segment, cone shaped with broad base	3, apical segment, corncob like 1, apical segment, cone shaped with broad base	3, apical segment, corncob like 2, apical segment, cone shaped with broad base
Sensillum chaeticum	Mechanosensory	1, apical segment at lateral position, long, tapered towards end	1, apical segment at lateral position, long, tapered towards end	1, apical segment at lateral position, long, tapered towards end	1, apical segment at lateral position, long, tapered towards end
Labrum (Fig. 8)					
Sensillum chaeticum	Mechanosensory, chemoreception (Shields 2010)	9–10, sparsely spread over the surface, short hair like projection	10–11, spread over the surface, short hair like projection	15–16, excessively spread over the surface, long hair like projection	9–10, sparsely spread over the surface, short hair like projection
Mandible (Fig. 9)					
Sensillum chaeticum	Mechanosensory (Albert 1980)	2, C1 long and C2 short, present at center, smooth surface	2, C1 long and C2 short, present at distal end, smooth surface	4–6, abundant, spread all over the surface	4, spread all over the surface
Maxilla (Fig. 10)					
Stipes					
Sensillum chaeticum	Mechanosensory	1, present at cardo, tapered towards end	3, base of maxilla, lateral to sensilla styloconica	1, present at galea, tapered towards end	3, base of maxilla, lateral to sensilla styloconica
Galea					
Sensillum styloconicum	Olfaction, gustation (Ishikawa 1963; Albert 1980; Barsagade et al. 2013)	2, side of maxillary palp, bulbous base and cone-shaped top, smooth	4, side of maxillary palp, irregular shape, protrusion	2, side of maxillary palp, bulbous base and cone-shaped top, smooth	4, side of maxillary palp, irregular shape, protrusion
Apical maxillary palp (Figs. 11, 12)					
Sensillum basiconicum	Gustation, olfaction (Ma 1972; Albert 1980; Li et al. 2008; Wazalwar & Khurad 2009; Barsagade et al. 2013)	7, apex, protuberant, thick walled, compact, 3 apical, 2 medial, 2 lateral	8, apex, protuberant, thick walled, compact, 3 apical, 3 medial, 2 lateral	8, apex, protuberant, thick walled, compact, 3 apical, 3 medial, 2 lateral	7, apex, protuberant, thick walled, compact, 3 apical, 2 medial, 2 lateral
Labium (Figs. 12, 13)					
Sensillum styloconicum	Thermoreception	2, apex, bulbous base with pointed end, smooth	2, apex, bulbous base with pointed end, smooth	2, apex, bulbous base with pointed end, smooth	2, apex, bulbous base with pointed end, smooth
Sensillum chaeticum	Mechanosensory, chemoreception (Shields 2010)	2, subapical segment, pointed tooth like	2, subapical segment, pointed tooth like	2, subapical segment, pointed tooth like	2, subapical segment, pointed tooth like

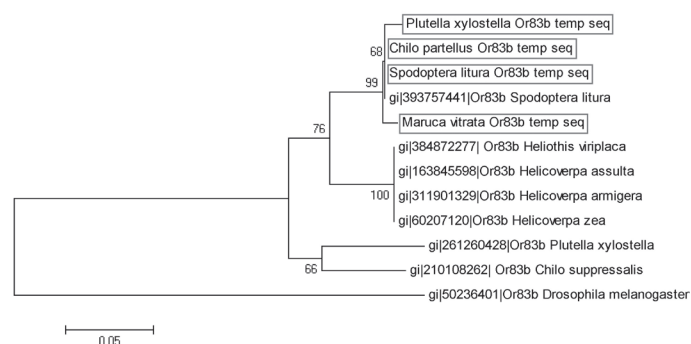


Fig. 14. Phylogenetic tree produced for the Or83b amino acid sequences of lepidopteran species collected from the NCBI database and Or83b amino acid sequences of *Spodoptera litura*, *Chilo partellus*, *Plutella xylostella*, and *Maruca vitrata* generated in this study, analyzed using MEGA6 software. The rectangular boxes indicate the template sequences generated in this study.

arranged radially in the cuticular wall, and suggested olfaction as the likely function of this type of sensillum. This description is consistent with our study and agrees with previous findings in Lepidoptera, such as *C. partellus* (Waladde & Kahoro 1990) and *M. sexta* (Shields 2010), and Coleoptera, such as *Dendroctonus valens* Leconte (Curculionidae) (Chen et al. 2010) and *Tomicus* sp. (Curculionidae) (Wang et al. 2012).

Sensilla styloconica were present in the apical antennal segment of larvae of all 4 examined species. Sensilla styloconica have food-recognition and biting-related sensory organs (Ishikawa 1963; Fauchaux 1995) and may have olfactory capacity as reported for *M. sexta* (Lin 1997). In the present study, in addition to their presence on antennae, variation in their number was recorded. In *S. litura* and *P. xylostella*, 1 sensillum styloconicum was present whereas in *C. partellus* and *M. vitrata*, 2 sensilla styloconica were recorded. The former species are leaf feeders whereas the latter are shoot and pod borers, which could be the reason for the numerical variation. Similarly, different apical structures of sensilla styloconica were observed in noctuids, e.g., *Trichoplusia ni* Hübner), *H. zea*, *Prodenia ornithogalli* (Guenée), and *S. exigua* (Jefferson et al. 1970; Zheng et al. 2014).

On the labrum of all 4 examined species, only sensilla chaetica were present. Their number varied with species but their morphology was similar to that described in other lepidopterans (Lin 1997; Li et al. 2008; Zheng et al. 2014). Sensilla chaetica were also present on the mandibles of all 4 species. Mandibles are mechanosensory and meant for determining the hardness of the food (Zacharuk 1980). Several studies suggested that sensilla chaetica function as contact chemoreceptors and mechanoreceptors because they arise from a socket and possess a terminal pore (Altner & Prillinger 1980; Van der Pers et al. 1980; Fauchaux 1990). Sensilla chaetica on the mandibles regulate the movement of abductor muscles during feeding, aid in the inspection of potential food material, and possibly have a defensive function. In addition, sensilla chaetica present at the distal part of the cardo and stipes come in contact with plant sap during feeding and respond to gustatory stimuli, such as sugars, glycosides, or water (Li et al. 2008).

The most notable variation in the larval mouthparts of *S. litura*, *C. partellus*, *P. xylostella*, and *M. vitrata* existed on the maxilla. As the maxillary palps are involved in both olfaction and gustation, it is possible that some of the receptors expressed in these organs have a gustatory function. The external morphology of the maxillary palps observed in the present study revealed that segments were curved in *S. litura* and *C. partellus* but not in *P. xylostella* and *M. vi-*

trata. In all 4 species, the apical portion of the maxillary palp had 7 to 8 sensilla basiconica, with a difference in their arrangement. For example, the sensilla basiconica in *C. partellus* and *P. xylostella* were arranged in a 3-3-2 pattern whereas those in *S. litura* and *M. vitrata* were arranged in a 3-2-2 pattern. This is in contrast to the 3-2-3 pattern reported for *S. exigua* (Li et al. 2008) and *Haritalodes derogates* F. (Lepidoptera: Crambidae) (Lin 1997). The sensilla basiconica on maxillary palps have been shown to have gustatory function and perceive plant stimulants or deterrents (Chen et al. 1994; Xie et al. 2006; Dey et al. 2011). On the galea, we recorded 2 or 4 sensilla styloconica with a stalk at the basal portion and being cone shaped at the top. In *S. litura* and *P. xylostella*, 2 well-developed, long sensilla styloconica were observed, whereas 4 short ones were seen in *C. partellus* and *M. vitrata*. Similar variations were previously observed in *S. exigua* (Li et al. 2008), *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) (Dey et al. 2011), and *Bombyx mori* L. (Lepidoptera: Bombycidae) (Asaoka 2003).

Although the sensory structures had similar morphology among the 4 examined lepidopteran species, they varied in numbers. Notably, sensilla styloconica were more numerous in *C. partellus* and *M. vitrata* (borers) than in *S. litura* and *P. xylostella* (leaf feeders) both on the antenna and the maxilla. These body parts play a major role in discriminating between host species and subsequently in the biting response (Li et al. 2008).

Several studies have suggested that the morphological phenotype of sensilla is influenced by sensory genes that are expressed in different development stages of the insect (Vogt et al. 2002). Sensory genes include ORs involved in the first step of the physiological processes that lead to olfaction, and the ORs constitute superfamilies in the insect genome. Of the 62 OR genes identified in *Drosophila*, the *Or83b* gene was found to be co-expressed with other ORs in nearly all olfactory neurons (Larsson et al. 2004). The remaining 61 OR genes were individually expressed in small non-overlapping subpopulations of olfactory sensory neurons of the antennae and maxillary palps (Robertson et al. 2003). The odorant co-receptor *Or83b* gene is thus a useful target gene for analyzing ancestral relationships of ORs between insect species on a molecular level. Here, we compared a partial sequence of the *Or83b* gene among 4 lepidopteran species and revealed its ancestral relationship and conservative nature. The phylogenetic analysis showed that the *Or83b* gene of *S. litura* and *C. partellus* formed one cluster and that of *P. xylostella* and *M. vitrata* formed a separate cluster, but that both originated from the same ancestor. This phylogenetic relationship reflects the results from the SEM study of the sensory structures, as sensilla chaetica on the mandibles and setae present on the head along with dense spines on the mouthparts of *P. xylostella* and *M. vitrata* were different from those found in *S. litura* and *C. partellus*.

Furthermore, the phylogenetic tree constructed using amino acid sequences of *Or83b* from larvae of various lepidopteran species was consistent with the inferred phylogeny of these larvae based on DNA sequences. In addition, multiple nucleotide sequence alignment and multiple amino acid sequence alignment validated the sequence similarity for the *Or83b* gene among lepidopteran species. Unlike conventional ORs, whose inter-species sequence diversity may reflect the distinct food preferences and ecological niches used by insects, the remarkable sequence conservation of *Or83b* suggests a unique and essential function in insect olfaction that has been conserved through insect evolution (Krieger et al. 2003). The strong selective pressure on *Or83b* orthologs clearly demonstrates the critical importance of this gene for insect olfaction. This conservation of function may reflect a requirement for *Or83b* to interact with other conserved cellular mechanisms. The results further

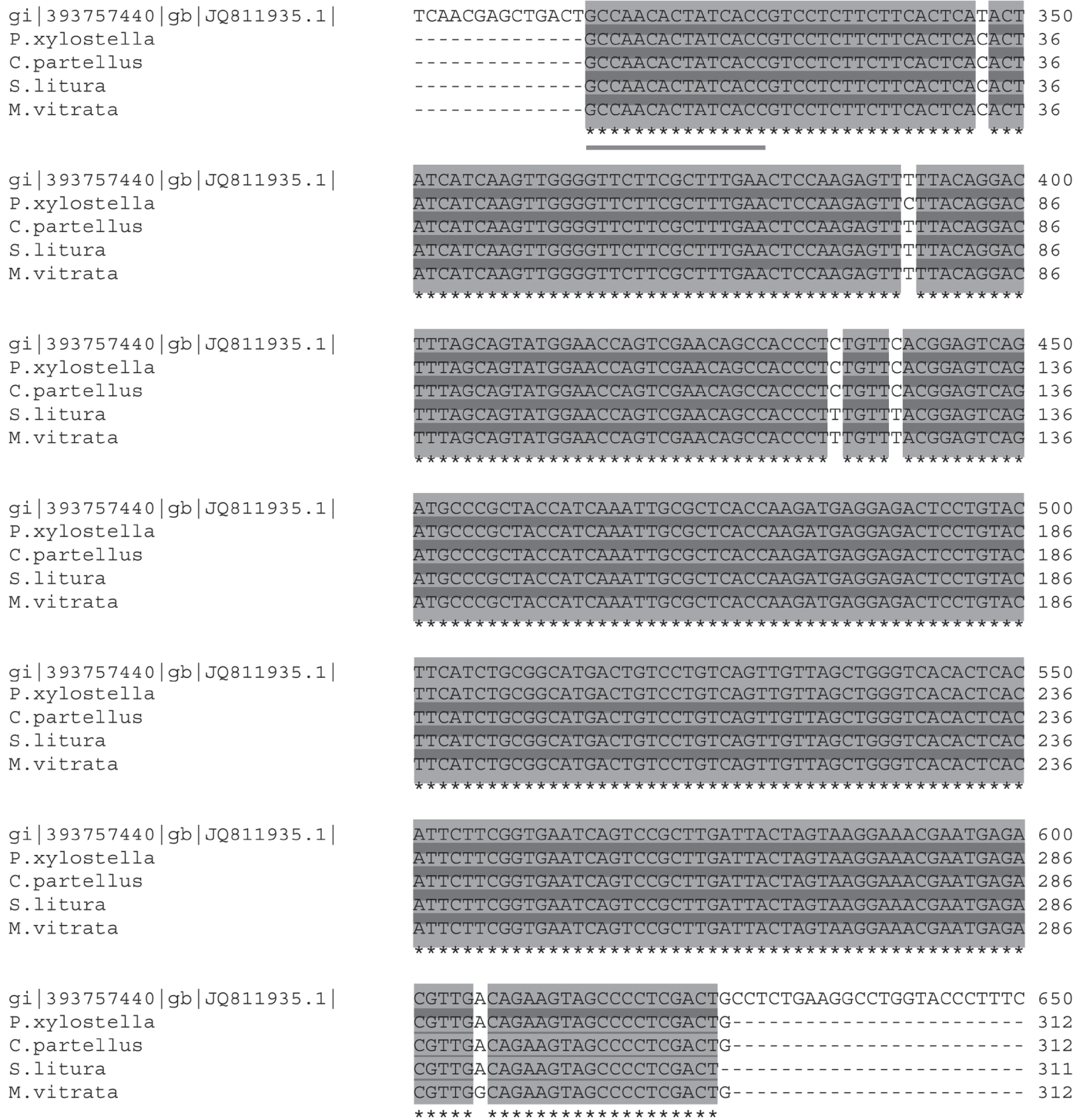


Fig. 15. Multiple sequence alignment of the partial sequence of the *Or83b* gene in *Spodoptera litura*, *Chilo partellus*, *Plutella xylostella*, and *Maruca vitrata* with the reference sequence of *Spodoptera litura Or83b* (Accession No. JQ811935) using ClustalW software. Grey shades represent completely conserved bases. Primer sequences used for amplification of the partial *Or83b* gene are underlined.

suggest that *Or83b* might be an “Achilles heel” for insects; rational design of novel insect control strategies targeted against *Or83b* orthologs might be effective in blocking host-seeking behavior in diverse insect pests.

Larvae feed, grow, and accumulate energy in order to perform metamorphosis whereas adults usually feed on different substrates

and have reproductive responsibilities. Accordingly, both stages are sensitive to different chemical cues. Thus, it is expected that their molecular equipment required for odor detection should be different. The description of sensilla types in the larvae of 4 lepidopteran species will serve as a basis for future electrophysiological and molecular studies aiming to understand feeding preferences for par-



Fig. 16. Multiple sequence alignment of the deduced amino acid sequence of the partial *Or83b* gene of *Spodoptera litura*, *Chilo partellus*, *Plutella xylostella*, and *Maruca vitrata* with the reference sequence of *Spodoptera litura Or83b* (Accession No. AFN22085) using ClustalW software. Grey shades represent completely conserved residues.

ticular host species. Also, the olfactory gene *Or83b* may prove as a promising target for the suppression of insect responses with a perspective to develop future control methods.

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