

**ULTRASTRUCTURAL STUDIES ON THE SENSILLA OF THE  
MOUTH PARTS AND ANTENNAE OF THE PRE-ADULT STAGES OF  
*SITOPHILUS GRANARIUS* (COLEOPTERA: CURCULIONIDAE)**

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**Received:** 22/7/2019 **Accepted:** 29/11/2019 **Available Online:** 1/12/2019

The granary weevil *S. granarius* is one of the most important stored product pests on the world, because of its damage. This pest is the main reason of the post harvest losses in world production; as a result of adults and larvae infesting and feeding on stored grains. The morphology and ultrstructure of the mouth parts and antennae of the pre-adult stage of *S. granarius* was investigated using scanning electron microscopy. The antennae and mouth parts of the granary weevil larvae and pre-pupal stage have a number of very interesting sensillae. The antennae have one large sensillum with different morphological appearance. Pores have been noticed on both sides. This one is known as sensilla basiconica. The pores may have an auditory function. The 2<sup>nd</sup>, 4<sup>th</sup>, and prepupal stages have a very strong mandibles each of them have two long sensillae. On the lateral view of the distal part of maxillary palp there was a digitiform sensillum. The digitiform organ is considered as a hygrothermo-receptor and was located on the lateral surface of the last segment of the larval maxillary palp. Another type of sensillae have been present and called chaetica sensillae, sensillae like a peg (sensilla twig basiconica) and campaniform sensilla which are located on the upper surface of the antennae of the 2<sup>nd</sup>, 4<sup>th</sup> larval instar and prepupal stages.

**Key words:** *Sitophilus*; Sensillae; SEM; Thermohygroreceptors; Olfactory receptors.

## INTRODUCTION

The mouth parts of insects are included in the mechanic of feeding, processing and catching food. Mouth parts have evolved into a great diversity of forms and functions to achieve the developmental changes mainly the finding of new food sources (Jervis 1998). Different forms of mouth parts are adapted to the type of feeding. Krenn (2007) reported that studying on the mouth parts morphology is require to know the feeding mechanisms and give the morphological characters for using in taxonomic studies ( Bae 2000; Morimoto and Kojima 2003; Krenn 2007; Brozek and Bourgoïn 2012).

*Sitophilus granarius* (Linnaeus), is a weevil species belonging to order Coleoptera and family Curculionidae, is one of the most destructive stored grain insect pests, that cause extensive loss of stored grains and decrease viability of seeds. The major effort of *S. granarius* is destroying the grain making holes on it. The adults may feed on grinding products for short time, but the larvae require hard masses for completion of their development as the wheat kernel. Small holes are making by the females of granary weevils in the kernel to lay their eggs. The larvae hatch and staying there live inside the kernels as grubs, and feeding on its interior contents. Only a very thin sheath remains when the insects reach maturity and also, weevils feeding decrease the quality of the grains by the frass and dust (Longstaff, 1981; Anonymous, 2008).

The morphology and the microscopical structure of the mouth parts, antennae and the distribution of their sensillae on the larval stage of *S. oryzae* using scanning electron microscopy have been studied by Stickney, 1923; Ting, 1936; Williams, 1938; DuPorte, 1960; Morimoto, 1962; Speirs *et al.*, (1986); Lyal, 1995; Boe, 1996; Morimoto and Kojima, 2003; Morimoto *et al.*, 2006. Zacharuk and shields, (1991) have been detected the structure of the sensillae on the mouth parts of insect and have described more than ten basic sensillum types in adult insects, all are found on the larvae except sensillae squamiformia. The common sensory structure that are found on Coleopteran and Lepidoptera larvae

are known as placoid which located on the apical antennal segments and maxillary palps. The digitiform organs have been found on maxillary palps and the peg like sensillae on the apical surface and on the antennal sides ( Faucheux, 1995; Guse and Honomichi. 1980; Honomichi and Guse, 1981; Doane and Klingler, 1978; Shield,2009). Giglio *et al.*, 2003 reported that the pore plates on the larval antennae which may be have an olfactory function have been found in Carabidae. Peg-like sensillae of unclear function have been observed on apices of antennae, labial and maxillary palp in Scarabidae, Chrysomelidae, Curculionidae and Elateridae (Devitt and Smith (1982); Renou *et al.*, 1998; Kim and leal, 2000; Giglio *et al.*, 2003; Dossall and McFarlane (2004). The digitiform organs described on the larvae of the same family which their function is hygrthermoreceptors or CO<sub>2</sub> reception (Honomichi and Guse, 1981) but in the larvae of Lepidoptera its function was mechanoreception (Devitt and Smith 1982).

More complete knowledge of the requirements of the insect and the means by which its functions to survive and multiply may be useful in improving control measures. So, the aim of the present work was to illustrate the structure of the mouth parts and the sensillae located on the antennae of 2<sup>nd</sup> and 4<sup>th</sup> larval instars, pre-pupal using the scanning electron microscopic observations and investigations.

## MATERIALS AND METHODS

### **Insect**

Insects used in this study were obtained from laboratory colonies reared for several generations on wheat, at the Entomology laboratory Faculty of Science, Sohag University, Egypt. Insect were kept at a constant temperature of 27±2 and 70±5 R.H.

### **Scanning electron microscopy**

For the SEM observations, infested kernels of hard wheat was taken from laboratory cultures and then dissected to expose the insect in various stages

of development. The egg, young larvae, (2<sup>nd</sup>, 4<sup>th</sup> instars), prepupa, pupa were removed from the kernel. Specimens were prepared for examination by subjecting them to dehydration series of 70%, 90%, 95% and absolute alcohol. Specimen were then critical point dried and coated with gold using Sputter coating for examination using a scanning electron microscope (SEM), JEOL., JSM 5300 in Assiut University .

## RESULTS

### Scanning electron microscopy

The result showed that the egg, larva, pre-pupa, and the pupa are included within the kernel until the adult emergence (plate1). Plate (2A) shows the larval head from a view exposing the mouth parts, antennae, and single ocelli (Plate 2B). Long curved hairs (setae) are dispersed on the surfaces of the epicranium, frons, gena, Labrum, mandibles, maxillae and labium (plate 2A). The antennae of all pre-adult stages have the largest sensillum occurs in the center of the apical antennal side. This sensillum is bifurcated with pores on the sides called basiconica sensillum (plates 2C, 3B, 5A and 5B). The antennae of the different pre-adult stage of *S. granarius* consists of one segment (plate 2C and 3B); while the maxillary and labial palps consists of two segments (plate 2E and F). The number of sensillae found on the apices of the maxillary and labial palps were 12 and 8, respectively. As all appendages shows conspicuous crown-like apical sensillum fields have been noticed on (plate 2, 3, 4, 5 and 6). Maxillary palps and antennae carry additional apical sensillae, called pore plates on the apical surfaces of the 2<sup>nd</sup> larval instar (plate 6C), and a digitiform organ on the maxillary palps (plate 6 A and B). In total, there are different types of sensillae are found on the larval head appendages.

### Types and the morphology of sensillae:

The SEM studies revealed four different types of sensillae which were observed in the different pre-adult stages (2<sup>nd</sup>, 4<sup>th</sup> larval instar and prepupal

stages) on the antennae, maxillae and labium of *S. granaries*. There are sensillae twig basiconica (plate 3 and 4), sensilla basiconica (plate 5A and B), sensilla digitiformia (plate 6A and B) , sensillae chaetica (plate 5C) and pore plates (plate 6C).

### **Sensilla twig basiconica**

The sensillae twig basiconica were peg like, short, straight and standing upright with the presence of furrow on their tips (plate 4). According to their morphological structure, the presence of grooves on their tips and the presence of pores on the apex are divided into different types:

The S.t.b.1 sensillum on the antennae is relatively large and conical in shape. It measures about 7.5  $\mu\text{m}$  in length. Also, the sensillum surface is slightly sculptured (plate 4A).

All sensillae classified as S.t.b.2 have smooth wall sensilla, terminal pore and a porless shaft. The terminal pore is formed by densely arranged finger-like cuticular protuberances (plate 4B). The sensillum measures about 4.5  $\mu\text{m}$  in length.

The S.t.b.3 sensillum is relatively large and exclusively located in the center of the antennal apex. Its blunt tip bears a laterally shifted sub-terminal pore which is surrounded by furrow like projection, it measures about 4  $\mu\text{m}$  in length (plate 4B).

The S.t.b.4 found in the maxillae: a specialized tip formed by newly spherical apex which is surrounded by cuticular collar characterized this sensillum. In addition of inconspicuous terminal pore surrounded by finger-like protrusions (plate 4C). Smooth wall sensillum set on an area of the cuticle and were surrounded by thin circular socket (plate 4C). The S.t.b.4 measures about 3 $\mu\text{m}$  in length.

The S.t.b.5 is found on the antenna of the 4<sup>th</sup> larval instar and measures about 1.8  $\mu\text{m}$  in length this sensillum is short with a cuticular socket (plate 4D).

Also, have smooth wall and set on an area of the cuticle and were surrounded by thick circular socket (plate 4D).

The S.t.b.6 were found on the labial palp, these sensilla were cylindrical in shape with smooth walls and show terminal pores on the rounded apex and inserted directly into the cuticle (plate 4E). It measures about 2.5  $\mu\text{m}$  in length.

### **Sensilla basiconica:**

The sensillae basiconica had hard, strong bases and blunt tips, may be inserted directly into the cuticle (plate 5A) or surrounded by thin cuticular sockets (plate 5B). These sensillae had two types of cuticular pores; large cuticular pores and very small cuticular pores on the surface of the sensillae. The sensilla basiconica were divided into two type, sensillae basiconica type 1 and the sensillae basiconica type 2, according to the presence of the two type of cuticular pores (plate 5A and B). Sensillae basiconica type 1 had large number of tiny pores dispersed on the surface of the sensillae, smooth wall, inserted directly to the cuticle without cuticular socket and measures about 10  $\mu\text{m}$  in length (plate 5A). Sensillae basiconica type 2 had only one large cuticular pore in every sensillum which was on the tip of the sensillae, its wall is not smooth, the sensillum surrounded by thin cuticular socket and measures about 8.33  $\mu\text{m}$  in length (plate 5B).

### **Sensilla chaetica**

These sensillae were hair like, and the longest sensillum found in the mala of maxillae and ligula. These sensilla are slightly bent, usually fine and curved near its tip, bifurcated end, the shaft lack any sensory structure ,inserted directly into the cuticle and measures about 14  $\mu\text{m}$  in length (plate 5C & Fig.6A).

### **Digitiform organ and adjacent sensilla**

The digitiform organ, which may function as hygro-thermoreceptors (plate 6A and B) is located on the apical segment of the larval maxillary palps. It

consists of long distally tapering setae in a long oval recess of the palpal cuticle with 15µm in length (plate 6A&B). Adjacent to the digitiform organ on the maxillary palps two sensillum types are present below the digitiform organ. Sensillum is characteristics by small, pit, cuticular depression (plate 6A) and sensillum represent a group of several folded cuticle below the digitiform organ (plate 6A).

### **Campaniform sensilla**

Two campaniform sensilla are present on the apical surface of the antennae are visible on the 2<sup>nd</sup> larval instar of *S. granarius*. The average diameter of them are 13.8µm length and 5 µm width and 4µm length and 4 µm width, respectively (plate 6C).

## **DISCUSSION**

The ultrastructural findings of the current study revealed highly developed sensory structures in pre-adult stages; 2<sup>nd</sup>, 4<sup>th</sup> larval instars and prepupal stages of *S. granarius*, olfactory and thermoreceptors are present in the sensillae of the antennae, maxillary and labial palps.

The distribution and the different types of the antennal sensillae on the different pre-adult stages of *S. granarius* were similar to those of the sum of other coleopteran. The antennae have one segment and two types of sensillae. The antennae have the sensillae basiconica with the two types of pores; many of sensillae twig basiconica are on the apices of the antennae of *S. granarius* these results agree with that reported by (Zacharuk 1962; Scott and Zacharuk 1971; Behan and Ryan 1978; Bloom *et al.*, 1982). The higher number of sensillae basiconica were found on *Languria mozardi* (Coleoptera: Languriidae) (22) (Baker and Ellsbury 1989) and *Tribolium* (Coleoptera: Tenebrionidae) (21) (Behan and Ryan 1978). The types of sensillae on the apices of the maxillary and labial palps of *S. granarius* were similar to those of other coleopterans. The number of sensillae were 12 and 8, respectively, but for *Hypera postica* (Coleoptera: Curculionidae) 12 and 11 (Bland 1983) and for *Leptinotarsa*

*decemlineata* (Coleoptera: Chrysomelidae) were 16 and 10 (Farazmand and Chaika 2008). The higher number were reported for *Melolontha melolontha* (Coleoptera: Scarabaeidae) were 22 and 16 (Eilers *et al.* 2012). The low number of mouth parts sensillae were found in *S. granarius* as compared with *M. melolontha* which show the limited requirements of *S. granarius* for sensillae because all pre-adult stages live inside the kernels.

Sensillae twig basiconica are commonly referred to act as gustatory organs (Schneider 1964; Zacharuk 1980; Zacharuk and shields 1991).

Sensilla twig basiconica1 sensillum on the antennae of *S. granarius* is relatively large and is conical in shape. The sensillum surface is slightly sculptured. Sensilla twig basiconica1 were distributed on the antennae of Lepidoptera (Faucheux 1995; Shields 1996; Roessingh *et al.* 2007; Goldsmith *et al.* 2014 ; Xu *et al.* 2015) and on the antennae , maxillary palps and labial palps of the larvae of Coleoptera ( Bland 1983; Baker and Ellsbury 1989; Giglio *et al.* 2003; 2013; Ortlhoff *et al.* 2014; Xu *et al.* 2015). Bloom *et al.*, (1982); Eilers *et al.*, (2012) reported that S.t.b.1 may be a mechanoreceptor and a gustatory chemoreceptor.

Sensilla twig basiconica2 were found in many insects and were reported in *T. molitor* (Coleoptera: Tenebrionidae) by Bloom *et al.*, (1982). These sensillae were called sensillae basiconica by Goldsmith *et al.* (2014) and supposed that the sensillae were tactile chemosensilla which responded to sucrose, NaCl, amino acids and other chemicals. In the current study the sensillae twig basiconica was located on the apices of the antennae, maxillary palps and labial palps of the 2<sup>nd</sup> ,4<sup>th</sup> larval instars and prepupal stages of *S. granarius*, so there were an easy contact with its surrounding of the kernels and play an important role in its behavior and feeding habits of different stages inside the kernels. The Sensilla twig basiconica5 is found on the antennae of the 4<sup>th</sup> larval instar. This sensillum is short with socket. Eilers *et al.*, 2012 described the same structure of the sensillae and reported that the most olfactory function



that present in larvae of *Melolontha melolontha* come from the spread of these structure on the antennae.

Sensillae basiconica which was observed in the current study on the antennae of 2<sup>nd</sup>, 4<sup>th</sup> larval instars and on the prepupal stages of *S. granarius* was convenient with previous findings for larval dipteran in which two types of pores were reported by Cobb (1999). It was reported that sensillae basiconica act as an olfactory and gustatory receptors (Chu and Axtell 1971; Baker and Ellsbury 1989; Cobb 1999; Baker *et al.* 2000). The presence of pores on sensillae basiconica of *S. granarius* reflects the ability of antennae to perceive different chemical stimuli. These results agree with that reported by Speirs *et al.*, (1986) for *S. oryzae*. Eilers *et al.*, (2012) reported that the abundance of peg like sensillae (sensillae basiconica) is a contact chemo-and mechano-sensillum occurs in the antennae, maxillary and labial palps of the larvae of *Melolontha melolontha*. The sensillae without pores except a pore on their tip of the sensillae, some of these sensillae may play a role in exchange of gases, the entrance of gases to the sensory cell within it through multiporous on their wall. Sensillae like a peg which have pores on their wall, these pores may be closed by a secretion (Altner and prillinger, 1980). Electrophysiological studies cleared that many of these sensillae may function as thermo-hygroreceptors ( Altner *et al.*, 1977; 1978; 1981; Zacharuk *et al.*, 1977 ).

Another type of sensillum is the digitiform sensillum is located on the lateral surface of the apical segment of the maxillary palps of different pre-adult stage of *S. granarius*. It consists of a long, distally slightly tapering seta which lays flat in a longish oval isolation of palpal cuticle. Sensillae digitiformia were reported on the 2<sup>nd</sup>, 4<sup>th</sup> larval instar and on the pre-pupal stage of the maxillary palps of *S. granarius*. Sensillae digitiformia in Coleoptera responded to vibratory stimulation and monitored tunneling activity through contact with tunnel walls (Zacharuck *et al.* 1977). This type of sensillum on the maxillary palp of the wireworm *Ctenicera destructor* has been shown to maintain the

contact and vibratory stimuli (Speirs *et al.*, 1986). Sensillae digitiformia were observed in other insects and were estimated to be chemoreceptors, mechanoreceptors and thermo-hygroreceptors and had electrophysiological responses to CO<sub>2</sub> ( Keil 1996; Farazmaud and Chaika 2008; Eilers *et al.* 2012; Ortloff *et al.*, 2014). The same structure was reported by Speirs *et al.*, (1986) for *S. oryzae* and Eilers *et al.*, (2012) for larvae of *Melolontha melolontha*. Eilers *et al.*, (2012) reported that the maxillary and labial palps of *Melolontha melolontha* is the only structure that estimated the change in air humidity and reported that the structure contain highly lamellated dendrites which characterized for thermohygroreceptors, and this sensillum is responsible for hygro-thermoreceptive structure.

Sensillae chaetica were reported as hair like and the longest sensillum found in the mala of maxillae and ligula of the 2<sup>nd</sup>, 4<sup>th</sup> larval instar and on the pre-pupal stages of *S. granarius*. Many researchers reported that sensillae chaetica were play an important role in monitoring food textile coordinated the power output of the muscles and the positions of the labium, labrum and the mandibles and may act as mechanoreceptors ( Zacharuk and shields 1991; Dey *et al.* 2009, 2011; Goldsmith *et al.* 2014). Xu *et al.*, (2015) reported that sensillae chaetica play an important role in the movements of the maxillary and labial palps for larval *Anoplophora glabripennis* as well as detect the structure of the tunnel. Long curved hairs (setae) are sparsely located on the surfaces of the epicranium, frons, gena, Labrum, mandibles, maxillae and labium, these seta may be have mechanoreceptors function (Speirs *et al.*, 1986).

### CONCLUSION

The findings of the present study clearly showed that *S. granarius* larvae possess well developed chemosensory receptors. The feeding of *S. granarius* larvae avoids chewing through the bran coats to the outside of the kernel, so making its protective cell. It would be interesting to form knowledge which one of these sensors are involved in this response and which one is a

response to the chemical or physical nature of this part of the kernel. It is clear that the findings of this study that there are many receptors on the antennae and mouthparts of the granary weevil larvae and prepupal stages that take part in its survival and adapting in its confined environment. These receptors must provide a good material for much exciting and enlightening research to more knowing about their embryonic and developmental structure and function in the life cycle of this most dangerous pest stored product.

### List of figures

**Plate 1:** Scanning electron microscope (SEM) micrographs of *Sitophilus granarius* showing immature stages.(A) egg, (B) 2<sup>nd</sup> larval instar, (C) 4<sup>th</sup> larval instar, (D) prepupal stages, (E) pupal stages.

**Plate 2:** Scanning electron microscope (SEM) micrographs of *Sitophilus granarius* showing the larval head from a view exposing the mouth parts, antennae, and ocelli. Long curved hairs (setae) are sparsely located on the surfaces of the epicranium, frons, gena, Labrum, mandibles, maxillae and labium.

**Plate 3:** Scanning electron microscope (SEM) micrographs of *Sitophilus granarius* showing the 4<sup>th</sup> larval instar. (A) ligula & labial palp, (B) antenna, (C) maxillary palp & mala. The prepupal stage (D) labium ( ligula & labial palp, (E) mala , maxillary palp, digitiform sensilla.

**Plate 4:** Scanning electron microscope (SEM) micrographs of the mouth parts appendages and antennae pre-adult stage of *Sitophilus granarius* showing,(A) S.t.b.1 (B) S.t.b.2 and S.t.b.3 (C) S.t.b.4 (D) The S.t.b.5 found on the antennae of 4<sup>th</sup> larval instar (E) The S.t.b.6 found on the labial palp.

**Plate 5:** Scanning electron microscope (SEM) micrographs of the antenna and maxilla showing sensilla basiconica and sensilla chaetica. (A) Sensilla basiconica type1 , (B) Sensilla basiconica type 2 , (C) Sensilla chaetica , hair like, the longest sensillum found in the mala of maxillae and ligula, fine, curved near its tip, bifurcated end.

**Plate 6:** Scanning electron microscope (SEM) micrographs of the maxilla and antenna showing sensilla digitiformia and campaniform sensilla.(A&B) Digitiform organ located on the apical segment of the larval maxillary palps, Also showing small, pit cuticular depression (arrowhead) and several folded cuticle below the digitiform organ (asterisk). (C) campaniform sensilla are present on the apical surface of the antennae of the 2<sup>nd</sup> larval instar of *S.granarius*.

### List of abbreviations

S.t.b.1= Sensilla twig basiconica1  
S.t.b.2= Sensilla twig basiconica2  
S.t.b.3= Sensilla twig basiconica3  
S.t.b.4= Sensilla twig basiconica4  
S.t.b.5= Sensilla twig basiconica5  
S.t.b.6= Sensilla twig basiconica6

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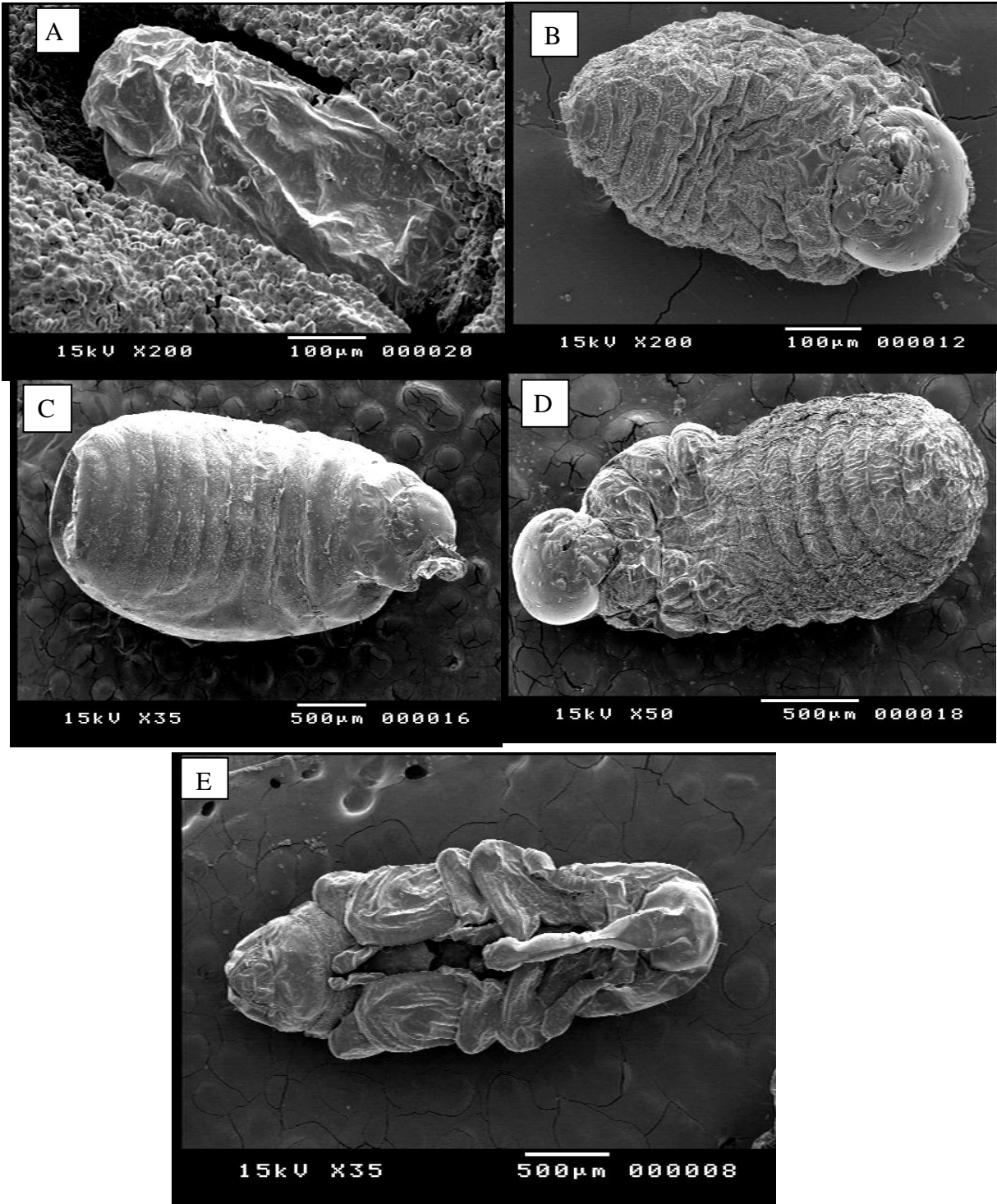


Plate 1

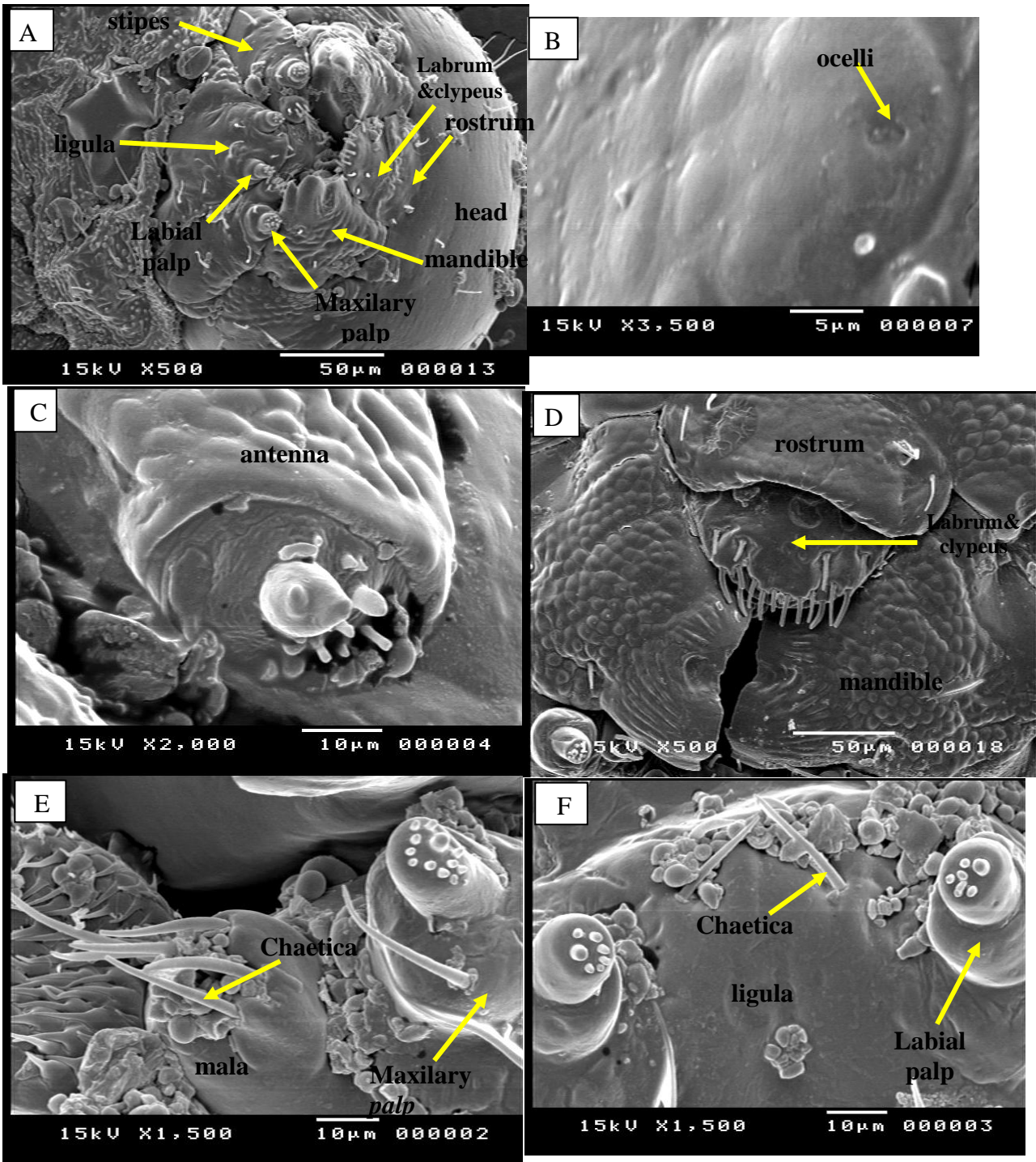


Plate 2

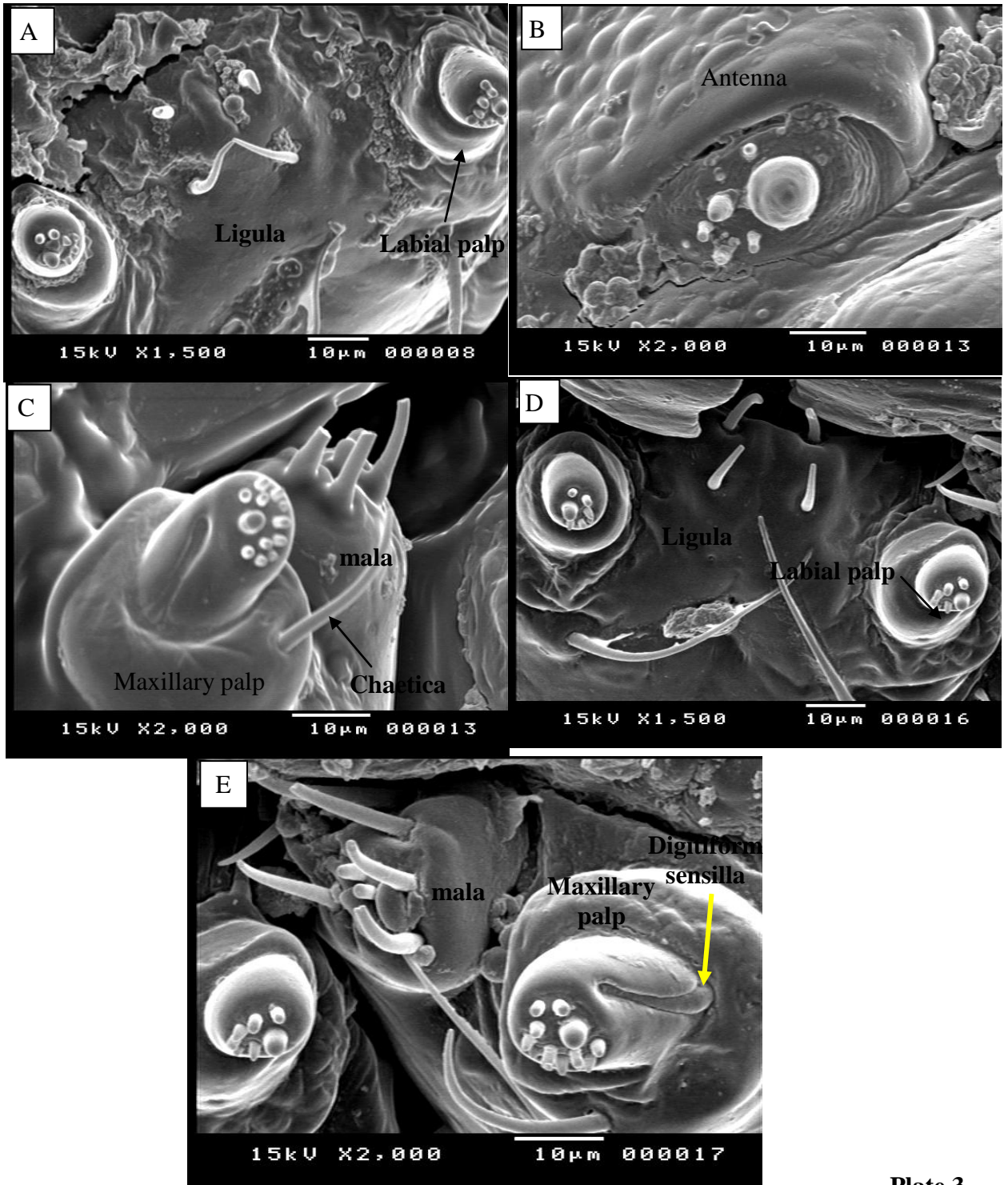


Plate 3

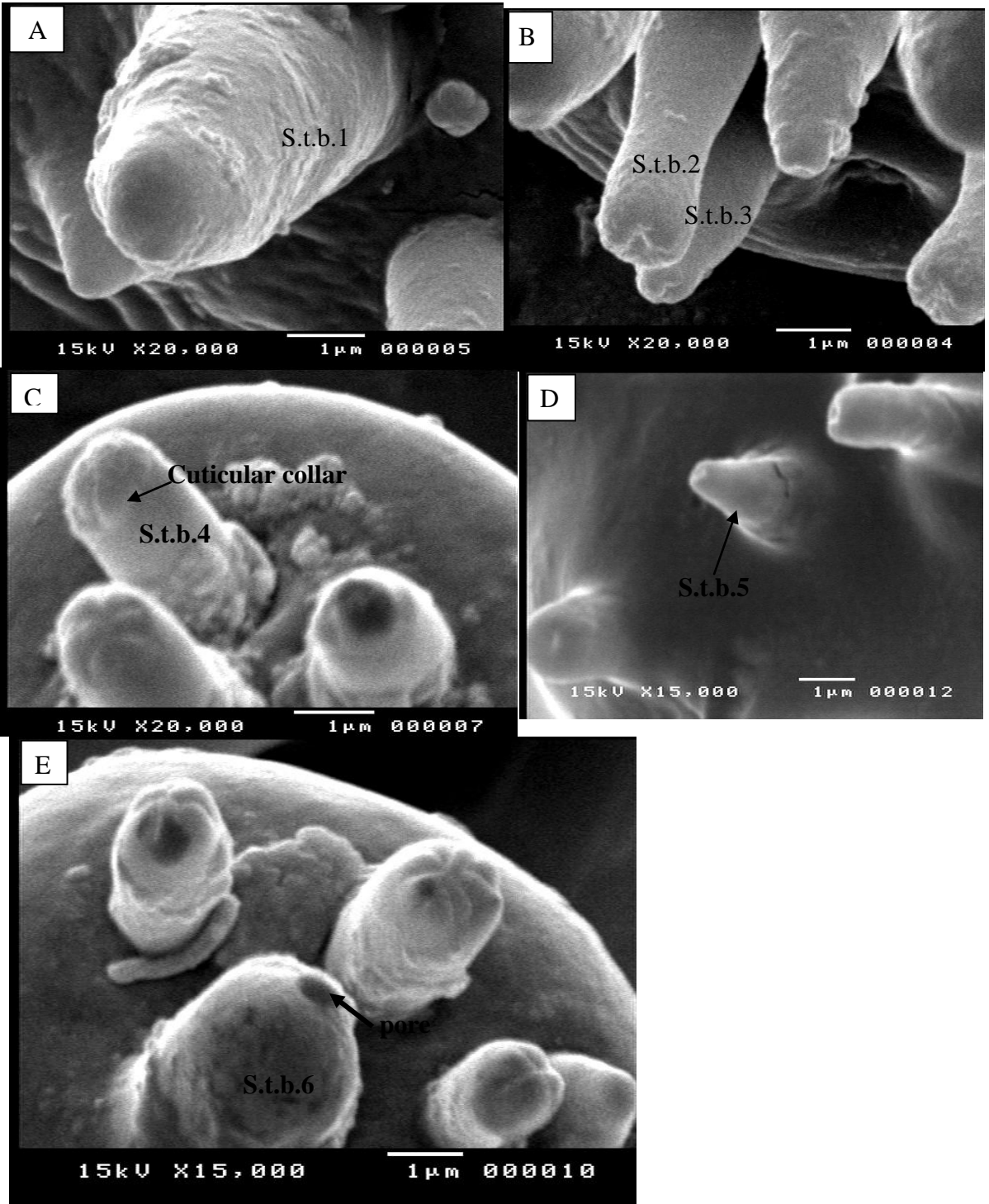


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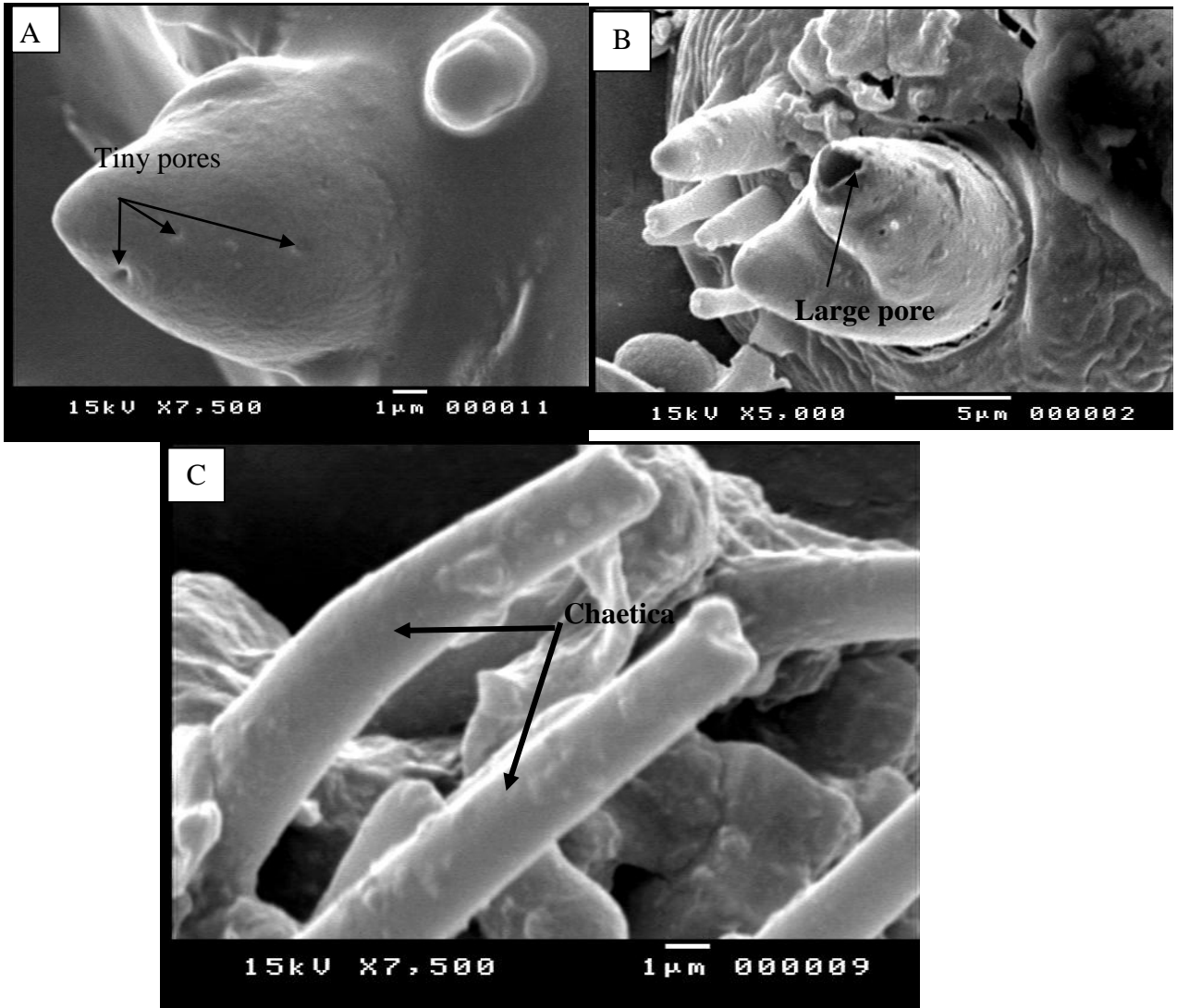


Plate 5

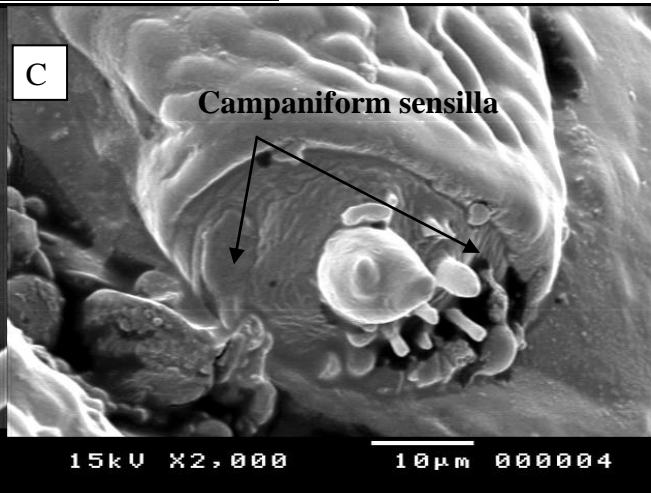
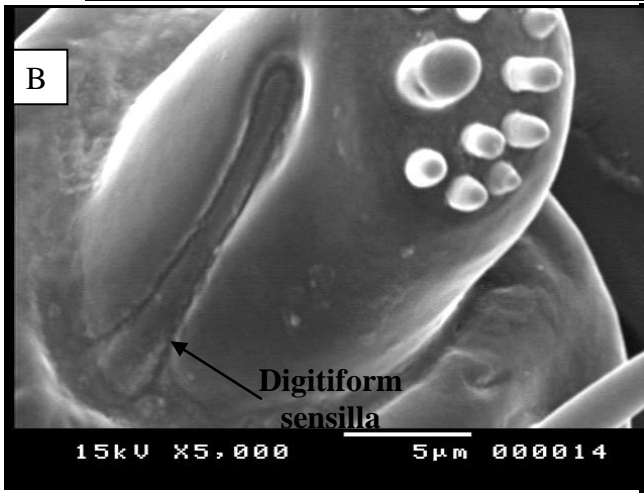
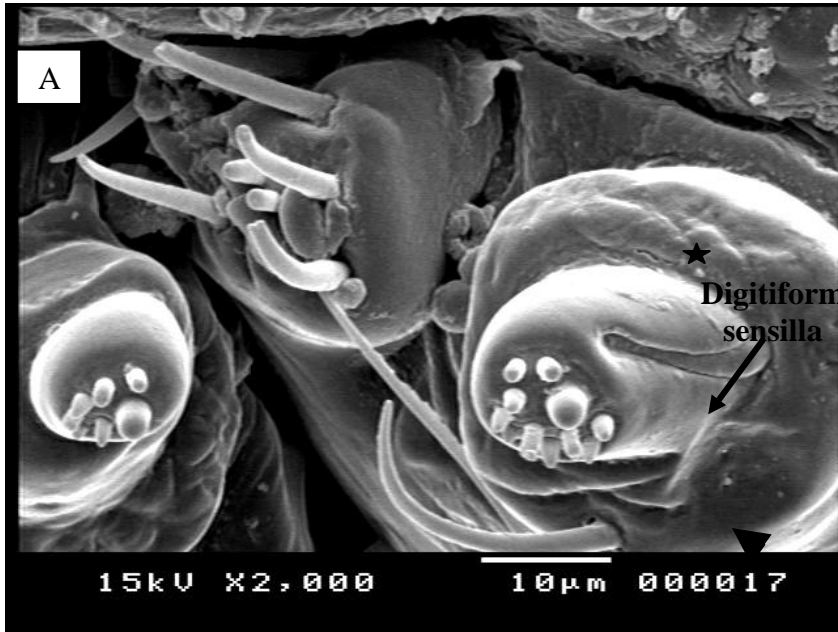


Plate 6

دراسات مورفولوجية بواسطة الميكروسكوب الالكتروني الماسح للشعيرات المنتشرة في  
أجزاء الفم و قرون الاستشعار للأطوار الغير ناضجة لسوسة حبوب القمح *Sitophilus*  
(Coleoptera- curculionidae) *granarius*

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تعتبر سوسة الحبوب المخزونة *Sitophilus granarius* من أهم آفات المنتجات المخزنة في العالم ، بسبب الأضرار التي تحدثها وتعتبر السبب الرئيسي لخسائر ما بعد الحصاد في الإنتاج العالمي بسبب الحشرة الناضجة واليرقات التي تغزو وتتغذى على الحبوب المخزنة. تم فحص مورفولوجيا وبنية أجزاء الفم وقرون الاستشعار للأطوار الغير ناضجة لحشرة *Sitophilus granarius* بواسطة الميكروسكوب الالكتروني الماسح. ان قرون الاستشعار و أجزاء الفم ليرقات سوسة القمح و الطور ما قبل العذراء يحتوى على عدد متنوع من الشعيرات.

قرون الاستشعار تحتوى على شعيرة كبيرة تحتوى على فتحات على جوانب هذه الشعيرة والتي تعرف باسم *sensillae basiconica* . و هذه الثقوب ربما تقوم بوظيفة سمعية. إن الشعيرات تبدو بأشكال مورفولوجية مختلفة. دائما الثقوب او الاغلب تكون وظيفتها مستقبلات شمية او كيميائية.

الطور اليرقى الثانى و الرابع و طور ما قبل العذراء يحتوى كل منهم على زوج من الفكوك القوية لكل منهم زوج من الشعيرات الطويلة. في المنظر الجانبي من الجزء البعيد فى الملماس الفكى توجد *digitiform sensillum* ويعتبر مستقبل ضوئى حرارى و يوجد فى السطح الجانبي من العقلة الأخيرة من الملماس الفكى اليرقى.

و هناك نوع آخر من الشعيرات يسمى *Chaetica sensillae* و شعيرات تشبه الوند و شعيرات قرصية وهى *Campaniform sensilla* وهم يوجدوا في السطح العلوي من قرون الاستشعار في الطور اليرقى الثانى و الرابع و طور ما قبل العذراء.