High resolution ultrastructural investigation of insect sensory organs using field emission scanning electron microscopy

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Field emission scanning electron microscopy (FESEM) is an extremely useful viewing technique for examining ultrastructural features pertaining to insect sensory organs (sensilla) at high magnifications. In comparison to conventional scanning electron microscopy (SEM), FESEM operates superiorly at lower accelerating voltages, offering greater resolution capabilities at higher magnifications and providing greater enhancement of surface cuticular structures (e.g., pores), often unattainable using conventional SEM. Overall, this technique provides a means for unambiguously classifying sensillum types (e.g., those used in the detection of olfactory/smell, gustatory/taste, mechanosensory/touch, and thermo-hygroscopic/temperature-humidity cues) based on external, morphological, cuticular features, such as the absence or presence of cuticular pores. The ability to resolve such external, ultrastructural features at higher magnifications (> 50,000 x) allows inferences to be made about the putative functions of these sensilla.

Keywords insect; sensilla; field emission scanning electron microscopy; ultrastructure; olfactory; gustatory; mechanosensory; ultrastructure; chemosensory; scanning electron microscopy

1. Introduction

Insects bear numerous sensory organs or sensilla used in the sensory perception for smell, taste, sound, touch, vision, proprioception, and geo-, thermo-, and hygroscopeption. These specialized cuticular structures vary in size and shape. These type I sensilla are typically innervated by one or more bipolar sensory neurons. These neurons bear dendrites which are wrapped by at least two accessory or sheath cells. Stimuli are first detected by these neurons. This sensory information is then transduced into an electrical signal, resulting in the generation of a nerve signals comprised of action potentials.

Attempting to unambiguously characterize and classify insect sensilla based on their external, morphological, cuticular appearances using conventional scanning electron microscopy (SEM) can be often very challenging, if not impossible. This greatly depends on the type of sensilla present and the specific morphological structures that need to be resolved (e.g., cuticular pores). This difficulty can be attributed generally to the inability of the scanning electron microscope to provide good resolution of these specific structures at higher magnifications (> 50,000 x). The aim of this paper is to present the benefits and results of using field emission scanning electron microscopy (FESEM) for this purpose, as an alternative approach.

2. Insect sensilla

2.1 Sensillum types

Traditionally, sensillum types have been classified on the basis of the morphology of their cuticular parts, as well as location on the insect [1, 2].

i) Sensilla trichoidea- These are hairs which vary greatly in length and are freely moveable. They are typically innervated by one to several neurons and can be solely mechano-, contact chemo-, thermosensitive, or olfactory in function.

ii) Sensilla chaetica- These hairs are similar to the sensilla trichodea, but have thicker cuticular shafts and are not freely moveable. They are typically innervated by one or more neurons and can be mechanoo- or contact chemosensitive in function.

iii) Sensilla basiconica- These are short hairs (pegs) or cones that are innervated by one to several neurons. They can be solely mechanoo-, contact chemo-, olfactory, thermo- and hygrosensitive in function.

iv) Sensilla coeloconica- These are basiconic pegs or cones that are positioned in shallow pits and innervated by two to several neurons. They are typically chemo-, thermo-, or hygrosensitive in function.
v) Sensilla ampullacea- These pegs are similar to coeloconic sensilla in structure and function, but are positioned in deep pits with narrow openings and innervated by two to several neurons.

vi) Sensilla squamiformia- These are flat, scale-like sensilla that are innervated by one or more neurons. They are typically mechano- or chemosensitive in function.

vii) Sensilla campaniformia- These are dome or bell-shaped sensilla positioned at or below the cuticular surface and are typically innervated by one neuron. They are typically solely mechanosensitive.

viii) Sensilla placodea- These are flat, plate-like sensilla positioned at, above, or below the cuticular surface and are typically innervated by several to many neurons. They are olfactory in function.

iv) Sensilla scolopophora- These are subcuticular, but still maintain an attachment to the cuticle. They are typically innervated by one to three neurons and are proprioceptive in function.

x) Sensilla styloconica- These are cones or pegs that sit on a cylindrical projection or style of insensitive cuticle and are typically innervated by one or a few neurons. They are mechano- and/or chemosensitive in function.

2.2 Sensillum categories

The three main categories of sensilla are those that bear neurons that respond to: 1) mechano- or hygro- and thermosensory stimuli (also called aporous or no-pore sensilla); 2) gustatory or taste stimuli alone (also called uniporous sensilla bearing a single, apical, or terminal pore at the tip of the sensillum) or in combination with mechanosensory stimuli (having a bimodal function), and 3) olfactory stimuli alone (also called multiporous sensilla bearing many pores distributed over the entire sensillum).

3. Field emission scanning electron microscopy (FESEM)

FESEM is an extremely powerful form of microscopy for visualizing and characterizing insect sensilla, based on their external, morphological, cuticular features (i.e., external fine structure). Overall, FESEM provides a reliable means by which to unambiguously classify sensillum types.

FESEM relies on the generation and liberation of electrons from either a thermal field- (or Schottky) or cold field emission source for image formation. The emission source discussed in this paper is that of cold field and the images were taken on a Hitachi S-4500 field emission scanning electron microscope. In contrast to conventional SEM, where a tungsten filament (thermionic source) is heated thereby producing a stream of electrons, in cold field emission sources, a thin, sharp, single crystal, tungsten wire is not heated and remains at ambient temperature. Electrons are thought to originate from a point source on the filament which may be less than 5 nm in diameter and drawn by an electrical field [3]. This extremely fine source of the field emission system allows the attainment of higher resolution [3]. The field emission gun acts as a cathode in front of a primary and secondary anode [3, 5]. A potential difference (extraction voltage of 3-6 kV) is applied between the primary anode and the tip, which results in an electric field concentrated at the tip and produces an electron emission or emission voltage (V_i). Electrons emitted from the tip pass through the first anode and are accelerated by the second anode voltage (V_a) or accelerating voltage of the gun, which is determined by the potential difference between the tip and second grounded anode (electron extraction voltage) [3, 4, 5]. The higher the accelerating voltage, the faster the electrons travel down the column, resulting in a more penetrating beam. These primary electrons are focused and deflected by electron magnetic coils (lenses) to produce a narrow electron probing electron beam diameter (beam current per unit area per solid angle) [5, 6], given the very small source size as it travels down the column and results in
more intense brightness (brightness increases linearly with accelerating voltage), greater improved spatial resolution capabilities (1.5 nm; three to six times better that of conventional SEM), reduction in chromatic aberration, improved depth of field, and greater enhancement of surface detail at higher magnifications (at low voltages, fine features are more easily observed) [4, 5, 6]. In addition, the field emission scanning electron microscope operates at lower accelerating voltages (0.5 to 30 kV), resulting in less electrostatically distorted images with increased image contrast and provides high quality images with minimal sample charging and damage, sometimes minimizing the need to apply conductive specimen coating [4, 5, 6]. The main benefit of using a lower accelerating voltage when observing high magnification views of specimens is that there will be less beam penetration into to the specimen thus obscuring surface detail and less of a likelihood of charging and damage to uncoated, non-conductive, and beam sensitive samples [5, 6]. An additional factor to consider is that if the accelerating voltage is decreased too much, there will also be a decrease in probe current resulting in poor image contrast and low (poor) signal-to-noise ratio [3, 4, 5, 6].

4. Sample preparation

4.1 Cleaning

To remove any unwanted surface debris from the specimens, such dust or other contaminants, selected pieces of insect tissue (e.g., antennae) are removed and placed in 1% aqueous Triton X-100 detergent and sonicated in several changes of Millonig’s phosphate buffer (pH 6.9-7.2) containing 0.54% glucose [7] for 30 s to 1 min. It is necessary to try to use a rinsing solution that closely matches (i.e., pH, temperature, osmotic strength) the same or similar ionic milieu as the specimens. It is also necessary to remove the contaminants prior to fixation, since they will, otherwise, permanently adhere and will be virtually impossible to remove [7]. Two alternative cleaning procedures include using i) a method developed for stonefly larvae [8] and ii) using an alternative cleaning solution than 1% Triton (e.g., 1 drop of “Prell”, commercial hair shampoo, in 50 ml distilled water) [9]. To 10 ml of this cleaning solution, 2.5 ml of ammonium hydroxide is added. Specimens are then sonicated for 10 s and rinsed in two changes of distilled water [9].

4.2 Fixation

After the specimens are cleaned, they are then fixed for 3 h in Carnoy’s fixative solution [10] and rinsed in two changes of absolute ethanol for 10 min each. Alternatively, specimens can be fixed for 24 h in chilled 5% glutaraldehyde (10 ml 25% glutaraldehyde) and 40 ml Millonig’s phosphate buffer containing 0.54% glucose (83 ml 2.26% sodium phosphate monobasic, NaH$_2$PO$_4$ H$_2$O, and 17 ml 2.52% sodium hydroxide, NaOH) (pH 6.9-7.2) for 24 h [7]. Following fixation in glutaraldehyde, the specimens are then rinsed several times in phosphate buffer.

Glutaraldehyde is chosen as the primary fixative since it reacts very rapidly with proteins and because it is a dialdehyde, it stabilizes structures by cross-linking before extraction by the buffer can occur. Since glutaraldehyde is considered to be osmotically inactive, the properties required of a vehicle (buffer) for a fixative are its ability to maintain: i) a constant pH during fixation; 2) suitable osmolarity when mixed with the fixing agent so cells and organelles neither swell nor shrink during fixation, and 3) suitable ionic constitution so that materials are neither extracted nor precipitated during fixation [7].

To minimize specimen charging and to improve conductivity when viewing specimens using FESEM, the tissues are treated with the osmium-thiocarbohydrazide-osmium (OTO) treatment [11] after fixation (e.g., in either Carnoy’s or glutaraldehyde, see above). This procedure is carried out by postfixing the specimens in 1% aqueous osmium tetroxide (OsO$_4$) for 1 h, washing for 45 min in a continuous flow of distilled water, incubating at room temperature in saturated thiocarbohydrazide for 30 min, and washing for 45 min in a continuous flow of distilled water [12]). The OTO treatment is then repeated and the specimens are fixed again and washed in distilled water. The thorough distilled water wash between OsO$_4$ and thiocarbohydrazide applications serves to prevent contamination and the formation of precipitate on the specimens [11]. In order to reduce shrinkage of the specimens and following the OTO treatment, the specimens are block-stained in 2% aqueous uranyl acetate and rinsed for 1 min in distilled water [9].

Post fixation with OsO$_4$ imparts a modest conductive property to fixed tissue [13]. In addition, OsO$_4$ reacts with lipoidal components of tissues that are not fixed by aldehydes and also acts as a stain [7], principally for phospholipid membranes, unsaturated lipids, and certain reactive groups of proteins [14]. The main disadvantage of osmium tetroxide is that it penetrates and reacts with tissues so slowly that considerable changes in structure can occur before fixation is complete [7]. As a second fixative, however, slow penetration is not considered to be a disadvantage since the structures have already been largely stabilized by primary aldehyde fixation [7].

4.3 Rinsing and dehydration

Following fixation or block staining, the specimens are rinsed for 1 min in distilled water to remove excess fixative. Subsequently, the specimens are dehydrated in an ascending ethanol or acetone series starting at 30% (i.e., 30%, 70%,...
85%, 95%, 100%, 100%) in preparation for viewing. This process allows the replacement of water with organic solvents with lower surface tensions [7, 9].

4.4 Drying
Following dehydration, it is important to thoroughly “dry” the specimens to prevent sample destruction, since the field emission scanning electron microscope operates under high vacuum. A procedure called critical point drying is typically used to prevent structural damage to the specimens. In short, critical point drying involves the replacement of water, naturally found in biological specimens, with a dehydrant (e.g., organic solvents, such as ethanol, acetone) and the replacement of the latter solvents with a pressurized transitional fluid, such as liquid CO₂ [6, 13]. After dehydrating the specimen, it is placed in a cooled vessel filled with the dehydrant and sealed. Following several changes of the transitional fluid to ensure that the dehydrant is completely displaced, the temperature of the transitional fluid is heated slowly. After its transition from liquid to the gaseous phase or critical point (point where the density of the liquid phase equals that of the vapor phase), the carbon dioxide is removed while in a supercritical state so that there is no gas-liquid interface present, which would, otherwise, damage the specimen [6, 13]. The specimens are then mounted on SEM stubs. Alternatively, specimens can be air dried (simple dessication) from alcohol, isoamyl acetate, or hexamethyldisilizane, however this method is not recommended since it typically results in undesirable effects, such as excessive flattening, shrinkage, twisting, and collapsing of the specimens [6, 13].

4.5 Coating and mounting
After the specimens have been critical point dried, they are mounted on stub holders in an orientation that will ensure optimal viewing using FESEM. Double-sided, conductive sticky tape is used to adhere the specimens to the stub. Since biological samples tend to be nonconductive, they must be made electrically conductive and need to be grounded to optimize sample coating thickness to permit the production of good images, while obscuring as little of the structure as possible [4, 6, 13]. Coating a sample with even a thin film (e.g., 1 nm) improves and increases the amount of signal produced [4]. The aim is to prevent charging, since they are good insulators, have low atomic number and density which make them inefficient at scattering the electron beam to produce secondary electron or backscatter secondary electron signals [4, 5]. To prevent charging, specimens are thinly sputter-coated with an ultrathin coating of electrically conductive material (i.e., heavy metals including gold and gold-palladium) to increase their density and conductivity due to electron irradiation required during imaging) on the specimens [4]. Dried biological specimens are more susceptible to charging, since they are good insulators, have low atomic number and density which make them inefficient at scattering the electron beam to produce secondary electron or backscatter secondary electron signals [4, 5]. Metal deposition (4-7 nm) is carried out by either using low vacuum sputter coating or by high vacuum evaporation and will result in a high yield of secondary electrons [4, 6, 13]. Failure to coat the specimens will typically result in interference with secondary electron collection, specimen instability, an inability to photograph, and viewing artefacts (e.g., bright blur with no defined edges) [4]. Having said this, however, it is possible to view initially an uncoated specimen to determine if it will, in fact, charge.

5. Viewing insect sensilla using FESEM
The paired antennae of adult insects are segmented appendages. They are each typically divided into three main divisions: scape (basal stalk), pedicel (second segment), and the remainder of the antenna, called the flagellum (often filamentous and multisegmented), which is comprised of flagellomeres [15]. Various types of sensilla occur on the antennae, as well as other body parts (e.g., mouthparts, legs) and function as chemoreceptors, mechanoreceptors, and thermo- and/or hygoreceptors. The antennae of insects are typically sexually dimorphic, with those of males being more elaborate than those of females (e.g., male antennae being larger and more bushy to increase the surface area for detecting molecules of female sex pheromone).

In the tobacco hornworm moth, Manduca sexta (Lepidoptera: Sphingidae), the flagellum of females [12, 16] and males [17] is divided into approximately 80 or more flagellomeres or annuli (Fig. 1 a-d). Male and female annuli bear numerous sensilla. The antennal flagellum in females (Figs. 1a, c) is narrower and more oval in shape than in males (Figs. 1b, d) and the surface area of an individual male annulus is approximately two times that of the female annulus. In males, long, male-specific, trichoid (hair-like) sensilla (Figs. 1b, d) are arranged as arches on the antero-lateral upper and lower margins of each annulus. In addition, other hair-like, shorter sensilla are found on rest of annulus. In contrast, females do not bear these long trichoid sensilla, but shorter ones on the anterior and lateral surfaces (Figs. 1a, c).

Each annulus of M. sexta females bear on average 2216 sensilla [16], comprised of multiporous and aporous sensilla. More than half of the total number of sensilla present on a single annulus are multiporous trichoid sensilla. No uniporous sensilla are present. Both trichoid sensillum types share very similar morphological, cuticular appearances, since they resemble each other with respect to size (i.e., height) and shape (Figs. 1a, c, 2a). Having said this, these shared features do not allow for easy sensillar characterization using conventional SEM, but can be achieved using FESEM. The difficulty lies in the fact that it is typically necessary to resolve structures, such as ridge or pore patterns,
at relatively high magnifications (> 50,000 X), in order to distinguish these sensilla. It is also important to bear in mind to coat the specimens minimally (if coating is required) in order to creation of artefacts.

**Figs. 1a-d.** Field emission scanning electron micrographs of female (Figs. 1a, c) and male (Figs. 1b, d) antennal flagellar annuli of *Manduca sexta*. Figs. 1a and 1b show multiple annuli and the arrow denotes a single annulus. These individual annuli are shown at a higher magnification in Figs. 1c and 1d. Scale bars = 500 µm. Fig. 1c shows a higher magnification of the anterior view of a single annulus from a female flagellum covered with an abundance of numerous, short, hair-like, trichoid sensilla. Scale bar = 500 µm. Fig. d shows a higher magnification of the long, male-specific, pheromonal, trichoid sensilla (arrow) arranged as arches on the anterolateral upper and lower margins of each annulus. The annulus also bears an abundance of other, shorter, olfactory sensilla. Scale bar = 500 µm.

5.1 Multiporous trichoid type A and B sensilla

There are two types of multiporous trichoid sensilla, types A and B on the annulus of female *M. sexta*. Both have the resemblance of long, hair-like sensilla. The type A trichoid sensillum averages 34 µm in length, has a diameter of 4 µm near its base, and tapers to 1 µm at its tip (Figs. 2a, b, c) [12]. The type B trichoid sensillum averages 26 µm in length, has a diameter of 2 µm near its base, and tapers to 0.4 µm at its tip (Fig. 2d) [12]. A conspicuous feature common to both types of sensilla is the presence of cuticular ridges and pores distributed along the entire length of the cuticular shaft. In trichoid type A sensilla, the cuticular shaft bears circumferential ridges that form a circular, helical pattern, with the cuticular pores lying within cuticular depressions in single rows along the midline of each ridge (Figs. 2b, c).

**Figs. 2a-d.** Field emission scanning electron micrographs of female multiporous trichoid sensilla of *Manduca sexta*. Fig. 1a shows two different types of multiporous sensilla: trichoid and basiconic sensilla. An arrow points to the basiconic sensillum. At this magnification, it is impossible to differentiate between these sensilla based on external, morphological, cuticular characteristics. Scale bar = 4 µm. Fig. 1b shows a portion of the cuticular shaft from a multiporous trichoid type A sensillum, which bears circumferential ridges (arrowhead) that form a circular pattern, in addition to pores (arrow) within cuticular depressions. Scale bar = 1 µm. Fig. c shows a higher magnification of the cuticular shaft from a trichoid type A sensillum showing the pores (arrow) arranged in single rows along the midline of each ridge (arrowhead). Scale bar = 1 µm. Fig. d shows a higher magnification of the middle...
portion of the cuticular shaft from a trichoid type B sensillum showing that the cuticular shaft bears diagonal cuticular ridges (arrowhead). Pores (arrow) are arranged in single rows along the distal margin of each ridge. Scale bar = 1 µm.

The trichoid type B sensilla also bear similar cuticular ridges and pores, however, the ridges are arranged in a diagonal fashion, with the pores being arranged in single rows along the distal margin of each ridge (Fig. 2d). The presence of many pores distributed on the cuticular shafts of both trichoid type A and B sensilla indicate that these sensilla most likely detect olfactory stimuli (capable of detecting smell molecules). Interestingly, this function was ascertained by way of neurophysiological experiments showing that trichoid type A sensilla of female *M. sexta* responded to a number of odorants [18].

5.2 Multiporous basiconic type A and B sensilla

The female annulus of female *M. sexta* bears two types of multiporous basiconic sensilla, types A and B (Figs. 3a-f) on the annulus of female *M. sexta*. The basiconic type A sensillum has the appearance of a tall peg. It averages 22 µm in length, has a diameter of 2 µm in diameter near its base, and tapers to 0.65 µm diameter at its tip (Figs. 3a-c) [12]. The type B basiconic sensillum averages 15 µm in length, has a diameter of 1.6 µm near its base, and tapers to 1.1 µm diameter at its tip (Figs. 3d-f) [12]. A conspicuous feature common to both types of sensilla is the presence of a multitude of pores distributed along the entire length of the cuticular shaft (Figs. 3a-f). In the type A basiconic sensillum, the pores are distributed in oblique linear rows along the long axis of the sensillum (Figs. 3a-c). This feature is also maintained in the type B basiconic sensillum, with the exception that the pores are not only distributed in oblique rows, but also along the base of deep furrows (Figs. 3d-f). In addition, the majority of these furrows not only travel along the long axis of the sensillum, but some also travel obliquely, intersecting with adjacent ones (Figs. 3d-f). There are more pores present in type B than A sensilla (i.e., pores/µm²) [12]. The presence of a multitude of pores distributed on the cuticular shafts of both basiconic type A and B sensilla is consistent with these sensilla most likely having an olfactory function.

![Figs. 3a-f](image)

**Figs. 3a-f.** Field emission scanning electron micrographs of female multiporous basiconic sensilla of *Manduca sexta*. Fig. 3a shows the middle of the cuticular shaft from a basiconic type A sensillum. The pores (arrow) travel in oblique linear rows along the long axis of the sensillum. Scale bar = 1 µm. Fig. 3b shows a higher magnification of the cuticular shaft of another type A basiconic sensillum showing many pores (arrow). Scale bar = 0.5 µm. Fig. 3c shows the interior view from the middle portion of the broken cuticular shaft from a type A basiconic sensillum. This view shows that the pores (arrow) extend through the full thickness of the shaft and are also visible on the exterior wall of the sensillum. Scale bar = 0.5 µm. Fig. 3d shows a lower magnification view of a basiconic type B sensillum. Scale bar = 2 µm. Fig. 3e shows a higher magnification view of the cuticular shaft taken from near the middle of a type B basiconic sensillum. Similar to basiconic type A sensilla, the pores (arrow) are distributed in oblique rows along the entire length of the cuticular shaft, but along the base of deep furrows. Some of these furrows also travel obliquely and intersect with adjacent ones. Scale bar = 1 µm. Fig. 3f shows a higher magnification view of the distal end of the cuticular shaft from a basiconic type B sensillum showing that the pores (arrow) extend to the tip of the sensillum. Scale bar = 0.5 µm.

5.3 Multiporous and aporous coeloconic type A and B sensilla and styliform complex sensilla

While the trichoid and basiconic sensilla of female *M. sexta* are visible as long hair-like or peg-like sensilla, respectively, some sensilla, such as the aporous type A and B coeloconic sensilla, are very small, lie in deep pits, and are not easily visible from the annular surface. The coeloconic type A sensillum averages 2 µm in diameter at the base (Figs. 4a, b) [16]. These sensilla are typically surrounded by numerous “teeth” or cuticular spines which form a fringe around the central peg (Fig. 4b). The apical portion of this sensillum bears “fingers”, as well as longitudinal grooves which extend from the base to the tip of the sensillum (Fig. 4b). Minute cuticular pores, found at the base (valley) of each
longitudinal groove, are also present. The presence of many cuticular pores, although less conspicuous as compared with the trichoid and basiconic sensillum types, indicate that these sensilla most likely detect olfactory stimuli.

The coeloconic type B sensillum is similar in appearance and size to the coeloconic type A sensillum (these sensilla both lie in deep pits and are not easily visible from the surface of the annulus), with the exception that this sensillum type does not possess cuticular pores or longitudinal grooves (Fig. 4c) [16]. These sensilla are, therefore, aporous. The lack of pores, as well as the shape of this sensillum type, indicates that it may be sensitive to thermo-hygro-sensory stimuli (capable of detecting fluctuations in temperature and humidity).

The styliform sensillum is a large, aporous sensillum that averages 38-40 µm in length and 14 µm in width (Fig. 4d) [16]. It is the largest sensillum type present on the female annulus and only one is present per annulus. This sensillum is positioned in the middle of the annulus, near its upper margin and it stands erect. This lack of pores, as well as the shape of this sensillum type, indicates that it may bear a thermo-hygro-sensory function.

6. Conclusion

The results of this study clearly demonstrate the merits of using FESEM to unambiguously classify insect sensilla based on their external, morphological, cuticular appearances. The ability to obtain greater resolution capabilities at higher magnifications using FESEM allows greater enhancement of surface cuticular structures. This is necessary especially when many sensillum types are very similar in size and shape (e.g., trichoid and basiconic sensilla) and also bear similar cuticular features (e.g., pores and ridges in trichoid type A and trichoid type B sensilla and pores in the basiconic type A and basiconic type B sensilla). It should be noted that initial visualization of these sensilla with conventional SEM resulted in an inability to distinguish between: i) trichoid type A and trichoid B sensilla; ii) basiconic type A and basiconic type B sensilla, or iii) between many of the trichoid and basiconic sensilla. This problem occurred because the necessary magnifications and resolution capabilities needed to be able to distinguish between these sensilla using such features as e.g., pore and/or ridge patterns was unattainable. The same problems also arose when trying to distinguish between coeloconic types A and coeloconic type B sensilla. For example, it was difficult to resolve the “fingers” present in coeloconic type A sensilla using conventional SEM, which made it impossible to distinguish these sensilla from coeloconic type B sensilla, which lacked these “fingers”. The added benefit of being able to carry out FESEM using low accelerating voltages, also results in less penetration of the sample by the electron beam, allowing for a more accurate and true representation of external, cuticular features. In addition, the capability of applying only a minimal amount of coating (if at all) to prevent charging also ensures that cuticular features, such as pores, ridges, etc. will be preserved.

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