Identification of Antennal Hygroreceptive Sensillum and Other Sensilla of the Firefly, Luciola cruciata

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Identification of Antennal Hygroreceptive Sensillum and Other Sensilla of the Firefly, *Luciola cruciata*

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ABSTRACT—We have identified and characterized the hygroreceptive sensilla on the antenna of the adult male firefly, *Luciola cruciata*, by coupling extracellular electrophysiological recordings from single sensilla with observation of morphology of the sensilla using a field emission scanning electron microscope (FE-SEM). Seven morphologically different types of sensilla were present on the antenna: pored chaetic, poreless chaetic, basiconic, trichoid, capitular and campaniform sensilla and a new type of sensillum. To determine which is the hygroreceptive sensillum, responses to humidity changes were electrophysiologically recorded from each type of these sensilla. Impulses from moist and dry receptor cells could be obtained from only the capitular sensillum along with impulses from a cold receptor cell. The results clearly showed that the capitular sensillum is hygro- and thermoreceptive. The capitular sensillum had a cuticular apparatus that extends about 8 μm above the antennal surface and is externally composed of three parts: an inner stem, an outer wall and a basal dome. This cuticular apparatus was not perforated in any region. About 30 capitular sensilla per antenna were distributed only on the lateral surface of flagellar segments. A new type of sensillum, '*sensillum gemmiformium*' was found. The cuticular apparatus of the gemmiform sensillum consists of a main spheroidal body and one to five protrusions. We discuss the common external features of the hygro- and thermoreceptive sensilla, the role of hygroreception in the behavior of fireflies and possible functions of the other types of sensilla.

INTRODUCTION

The firefly, *Luciola cruciata*, which is the typical species of the fireflies in Japan, lives in or near rivers throughout its life cycle. The larvae of this insect are aquatic, living in clear streams. Its six instars live for more than nine months, feeding on black snails *Semisulcospira* sp. The pupae stay in the ground of the riverbank until emergence in June, a rainy season in Japan. Adult insects do not feed during their short lives of only three weeks or less. They are active in mating behavior, using flashing communication. Most flying adults are males, and the males seek responsive females with flashes in meadows or woods along the river. Both males and females seldom leave their home river range. This life cycle strongly suggests that the fireflies can detect humidity differences in the environment and that they are equipped with hygroreceptive organs that function in habitat selection.

In most insects so far examined hygroreception occurs by means of specialized antennal sensilla. The structure of an unambiguously identified hygroreceptive sensillum was first described in a cockroach, *Periplaneta americana* [21]. This sensillum has a characteristic cuticular apparatus: a mushroom-shaped inner stem surrounded by an outer guard wall, and hence it is named a sensillum capitulum. The cuticular apparatus is entirely non-perforated except for a clogged moulting pore at the terminal of the inner stem. Similar sensilla were electrophysiologically confirmed to be hygroreceptive and have been morphologically examined in species of insects belonging to Blattariae, Orthoptera, Phasmida and Hymenoptera as reviewed by Altner and Prillinger [1] and by Steinbrecht [11]. These sensilla have diverse external appearances, and have been given various names: capitular sensilla [18, 21], coelocapitular sensilla [19], coeloconic sensilla [6, 8] and no-pore sensilla [2, 3]. All of these hygroreceptive sensilla have small poreless cuticular structures that are apparently different from those of the sensilla of the other modalities. In addition, most of these sensilla have three types of sensory neurons: a moist cell, a dry cell, and a thermoreceptor cell [2, 4, 18, 19]. Hence, these sensilla are usually referred to as hygro- and thermoreceptive sensilla.

For coleopteran insects to which fireflies belong, no hygroreceptive sensilla have been identified. Therefore, in this study, we identify the hygroreceptive sensilla of the firefly, *Luciola cruciata*, and we compare the external morphology of the hygroreceptive sensilla of fireflies with those of other insects. In addition, we describe and discuss briefly the characteristics of the other sensilla, especially a novel sensillum, named a '*sensillum gemmiformium*'.

MATERIALS AND METHODS

Adult male fireflies, *Luciola cruciata*, used in this study were generously provided by the Ogi Town Firefly Preservation Society, Saga, Japan.

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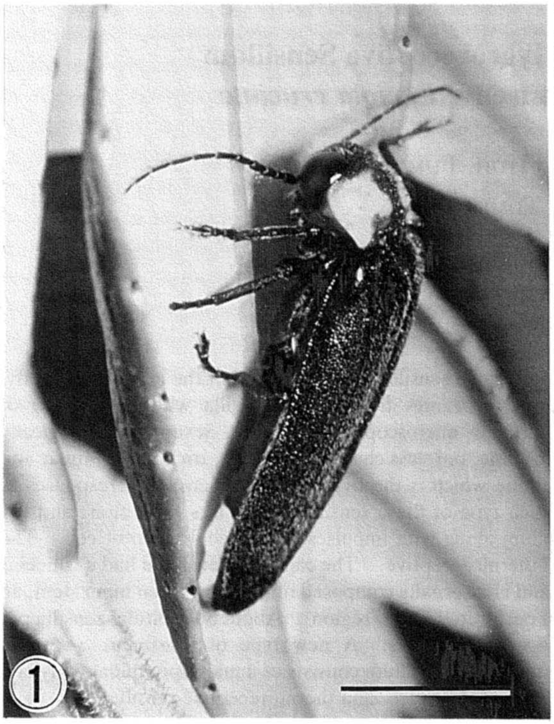
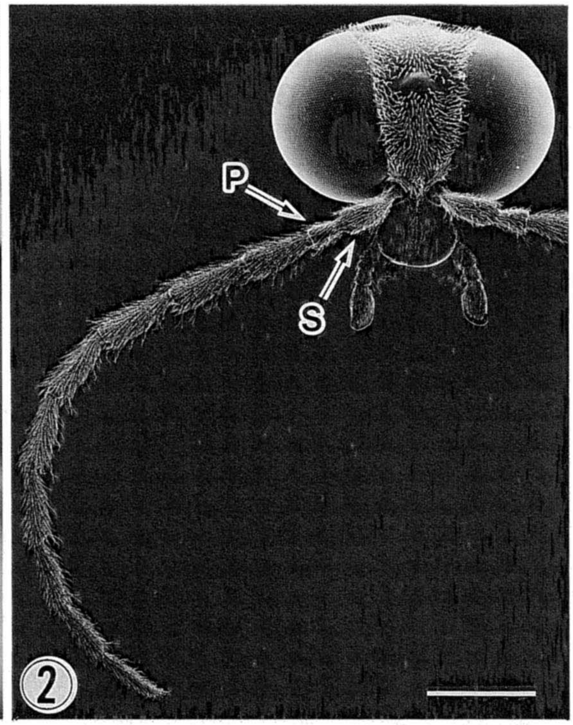




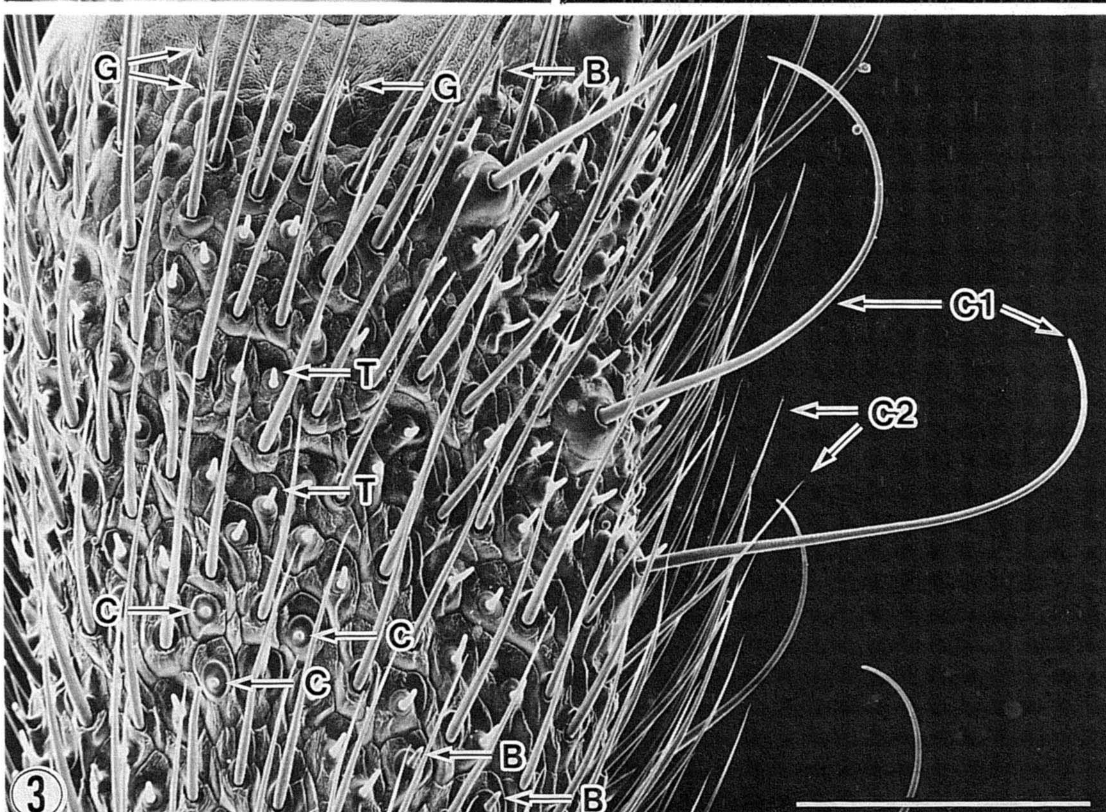


FIG. 1. Lateral view of an adult male firefly, *Luciola cruciata*, illustrating a pair of antennae between two compound eyes. The luminous organs occur on the 5th and 6th abdominal segments. bar=5 mm.

FIG. 2. Low power scanning electron micrograph illustrating a frontal view of the head and the right antenna of a male firefly. The antenna comprises a scape (S), pedicel (P), and nine flagella segments. bar=1 mm.

FIG. 3. Lateral surface of the 1st flagellar segment. Six of seven types of sensilla are visible: pored chaetic (C1), poreless chaetic (C2), basiconic (B), trichoid (T), capitular (C) and gemmiform (G) sensilla. bar=100 μm.

Electrophysiological Recordings Electrophysiological recording methods were basically the same as described in a previous paper [20]. Briefly, the animal was first anesthetized by cooling and then fixed to an acrylic plate. The head of the animal was positioned in a small pool which was filled with cockroach saline solution [16]. An indifferent electrode was placed in this pool. The active electrode was an electrolytically sharpened tungsten wire which was inserted into the base of the cuticular apparatus just deep enough to make electrical contact. Electrical events were amplified, displayed on a cathode ray oscilloscope, and recorded digitally onto magnetic tapes. The conditioning and stimulus air was prepared by mixing dry and fully moistened air.

Morphological Observations The hygro- and thermoreceptive sensilla identified electrophysiologically were marked by advancing the active electrode into the sensilla, thus enlarging the hole or cleft at the recording site on the antennal cuticle. The region around the sensilla was sketched in order to facilitate relocation. Then a small piece of flagellum which contained the identified sensillum was cut out with a razor blade and immediately immersed in 70% acetone. It was dehydrated in a graded acetone series, air dried, and coated with platinum-palladium using a Hitachi E-1030 ion sputter. Observations were made with a Hitachi S-4100 field emission scanning electron microscope (FE-SEM).

RESULTS

Antennal structure

The adult firefly, *Luciola cruciata*, has a pair of antennae at the infero-frontal region between two well-developed compound eyes on the head capsule. Each antenna is about 6 mm long and about 0.3 mm in diameter. The antenna comprises a scape, a pedicel, and nine flagellar segments from proximal to distal, and curves ventrally (Figs. 1 and 2). The scape is 600 μm long and is tapered proximally. Only chaetic sensilla occur on it. The pedicel is 350 μm long, has a shape similar to the scape, and is covered by chaetic and campaniform sensilla. The flagellum is tapered distally, each segment of which has uniform length of 500 to 600 μm, and is covered by many types of sensilla.

We have classified the antennal sensilla into seven types on the basis of their external appearances and location. The seven types are as follows: pored chaetic, poreless chaetic, basiconic, trichoid, capitular, gemmiform and campaniform sensilla (Fig. 3).

Electrophysiological identification of hygroreceptive sensillum

To determine which sensillar type is hygroreceptive, responses to humidity changes were electrophysiologically recorded from each type. When the responses to humidity change from 0% to 100% relative humidity or vice versa were obviously significant, we determined the recorded sensilla as moist or dry hygroreceptive. To determine whether the hygroreceptive sensilla contain also a thermoreceptive cell, responses to temperature decreases about 1 or 2°C were recorded. When significant increases of impulse frequencies were observed and the impulses were discriminated from those of the hygroreceptor cells, the sensilla were determined to contain a thermoreceptor cell. Figure 4 is a typical example showing that the electrical activity from the capitular sensillum is composed of three types of impulses, representing the activities of a moist, a dry and a cold receptor cell. Among 12 capitular sensilla examined the responses of all three receptor cells were simultaneously recorded in 3 cases, but responses of the moist and the dry receptor cell were simultaneously recorded in 5 cases and only the moist or dry receptor cells in 4 cases. These results suggest that most or all capitular sensilla contain two types of hygroreceptor cells and one type of thermoreceptor cell. As for the other types of sensilla we could record undergoing impulse activities from some types of sensilla but no significant responses to humidity changes. Thus we conclude that these types of sensilla are not hygroreceptive. Figures 5 and 6 show capitular sensilla from which the responses to humidity changes were recorded and afterward processed for FE-SEM observation.

Capitular sensillum

The capitular sensillum possesses a cuticular apparatus that is about 8 μm high from the antennal surface, and externally consists of three parts: an inner stem, an outer wall and a basal dome. The outer wall is conical and cylindrical, measuring about 4 μm high and 3.2 $\pm$ 0.6 μm in basal diameter (mean $\pm$ SE, n=16). The outer wall is smooth-surfaced and usually covers the proximal two thirds of the

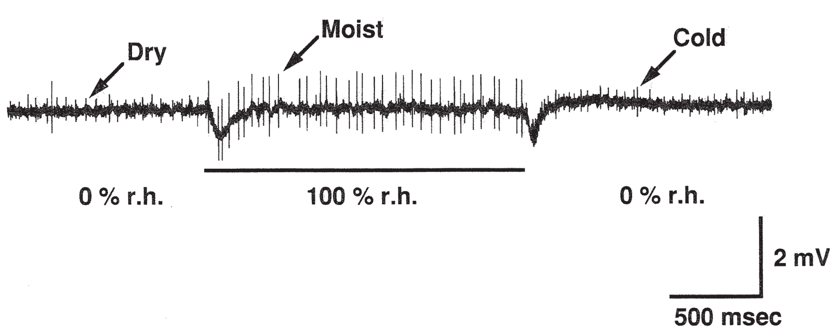
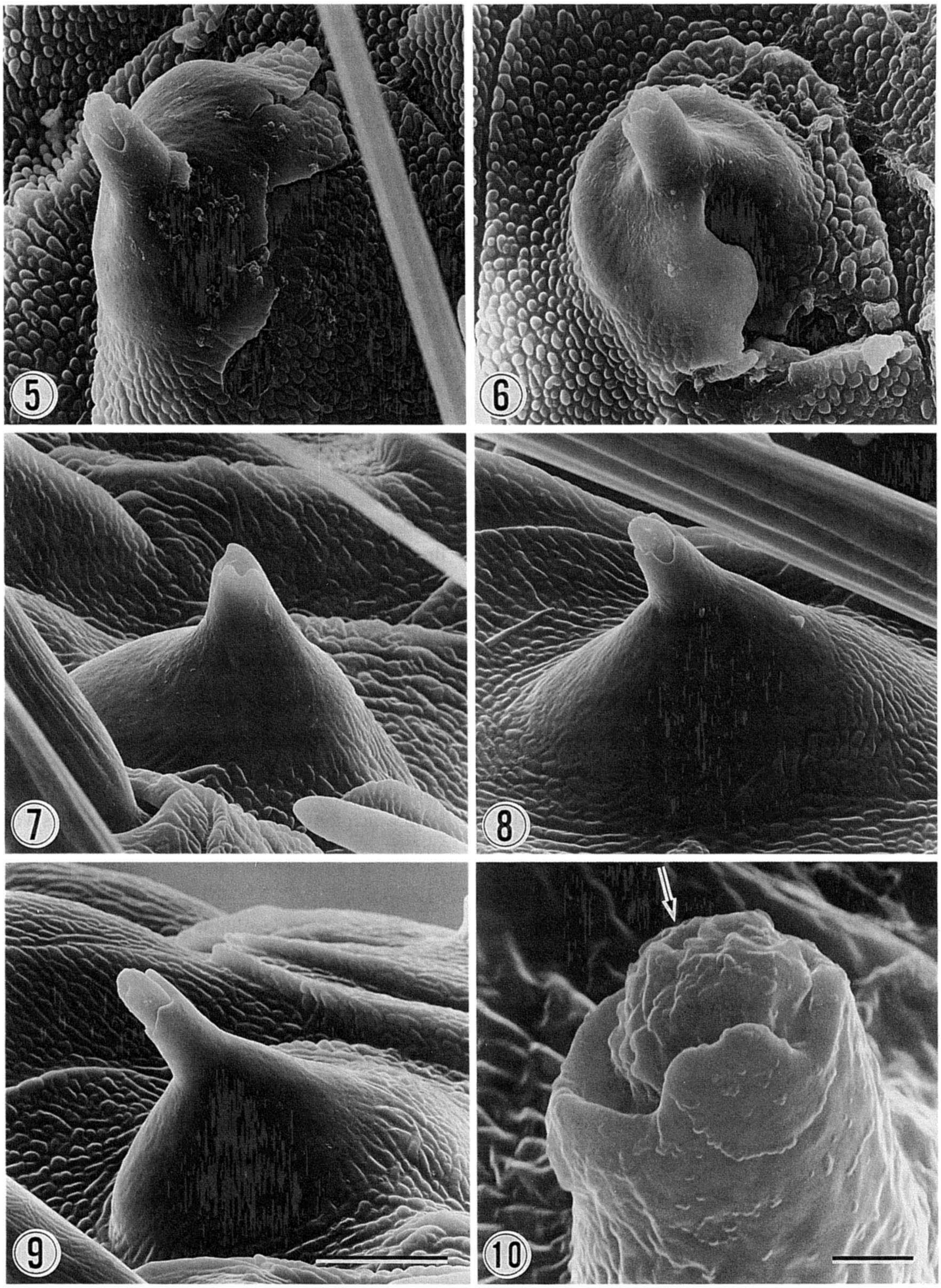


Fig. 4. Electrophysiological responses of a capitular sensillum to humidity changes representing the activities of a moist, a dry and a cold receptor cell. Deflections of the baseline may be reflected by receptor potentials. Temperature is constant at 24°C. *Under line*, 100% relative humidity; *blank*, 0% relative humidity.



FIGS. 5 and 6. Scanning electron micrographs of capitular sensilla from which responses to humidity changes were recorded and afterward processed for FE-SEM observation. The hollow or cleft at the basal dome shows the recording site.
FIG. 7. Capitular sensillum with outer wall covering most parts of the inner stem.
FIG. 8. Capitular sensillum exposing about one third of the inner stem.
FIG. 9. Capitular sensillum exposing about a half of the inner stem. FIGS. 5–9, bar=5 μm.
FIG. 10. Higher magnification of the capitular sensillum. No pores are observed on the outer wall and the inner stem. The head is somewhat rugged, and has a hollow in the apex (arrow). bar=500 nm.

inner stem, continuing onto the basal dome. The wall, however, varies in height from sensillum to sensillum (Figs. 7 to 9). Most parts of the inner stem are covered in some sensilla and are exposed in others. The distal rim of the outer wall is usually irregular. The basal dome is also smooth-surfaced and is 4 μm high and 9 to 12 μm in diameter. The hem of the basal dome is continuous with the sculptured surface wall of the flagellum, which exhibits an imbricate pattern. Neither the outer wall nor the basal dome is perforated.

The head of the inner stem is roundish and has a diameter of 1.4 ± 0.1 μm which is less than the orifice of the outer wall that is 2.1 ± 0.2 μm in diameter. In a high magnification, the head is rugged and lacks pores (Fig. 10), although a single tiny hollow usually occurs at the apex (Figs. 8 to 10). In the sensilla with long naked inner stems, the inner stems were confirmed to never be perforated at their lateral surfaces.

The capitular sensilla are distributed only on the lateral surfaces of the flagellar segments. They are always surrounded by the chaetic sensilla. This arrangement seems to protect the capitular sensilla from harsh contact with solid components of the environment (Fig. 3). The number of the capitular sensilla is larger in the first, second and last flagellar segments than in the other segments. The total number per antenna is 27 ± 3 (n=5) (Fig. 11).

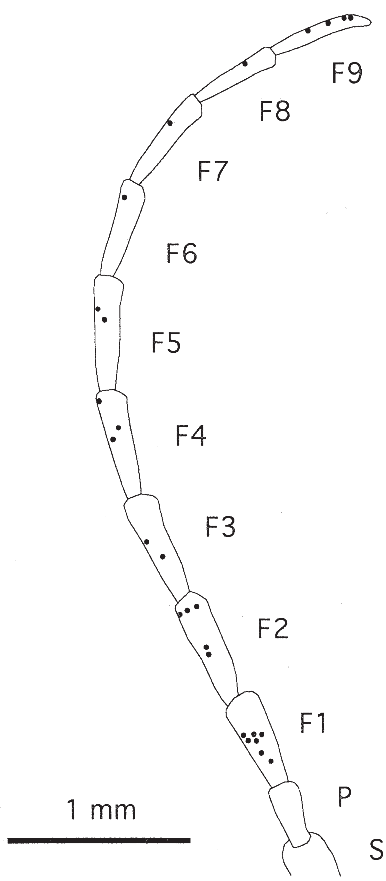


Fig. 11. Lateral view of an antenna showing distribution of the capitular sensilla. S: scape, P: pedicel, F1-F9: 1–9th flagellar segments

Flagellar sensilla other than the capitular sensillum

Chaetic sensillum. Chaetic sensilla are morphologically classified into two types, a pored chaetic sensillum and a poreless chaetic sensillum. The shaft of the cuticular apparatus is 75 to 150 μm long and about 5 μm in diameter at the base in both types of sensilla. It is obliquely ridged on the surface and is articulated to the flagellar surface with a circular socket at the base.

The pored chaetic sensillum is characterized by a single blunt tip of 0.5 μm in diameter with a single terminal pore of 100 nm in diameter at the apex of the shaft, which usually bends in various directions (Fig. 12). It is distributed more densely on the distal portion of each flagellar segment than on the proximal portion. There are about 1.5×10^2 sensilla per antenna.

The shaft of the cuticular apparatus of the poreless chaetic sensillum distally tapers to a single sharp tip without any pores and uniformly slants toward the antennal tip (Fig. 3). This sensillum is distributed everywhere on the flagellar segments without any regular patterns. The total number of sensilla per antenna is about 8×10^3.

Basiconic sensillum. Each basiconic sensillum has a conical cuticular apparatus consisting of a grooved shaft about 10 μm long and a basal dome 8 to 10 μm in diameter. About 15 longitudinal grooves are found on the distal one fourth of the shaft. These grooves converge at the tip (Fig. 13). This sensillum is distributed on all flagellar segments, numbering about 30 per antenna.

Trichoid sensillum. Trichoid sensillum has a smooth-surfaced and blunt-tipped shaft about 15 μm long and a basal dome 6 to 8 μm in diameter (Fig. 14). The wall of the shaft is perforated by numerous minute pores of about 10 nm in diameter (Fig. 15). The trichoid sensillum is distributed on all flagellar segments, the total number of which is about 3×10^3 per antenna.

Gemmiform sensillum. This type of sensillum has a small-sized cuticular apparatus 1.5 to 3 μm in diameter and is equipped with a socket (Figs. 16 and 17). The sensillum is distributed in a limited area close to the distal margins of every other flagellar segment: 1st, 3rd and 5th ones (Fig. 3). They occur on the dorsal side rather than the ventral side. On the basis of its bud-like appearances, we named this type of sensillum '*sensillum gemmiformium*'. The cuticular apparatus of the gemmiform sensillum consists of a main spheroidal body 2 to 3 μm long set in a socket and has protrusions from its body. The protrusions vary in number from one to five and in length from less than 0.5 μm up to more than 5 μm. They have various shapes resembling fingers or petals (Figs 16 and 17). The long finger-like protrusions are often flattened at their terminals, adhering to the flagellar surface (Fig. 16). The number of gemmiform sensilla is about 20 per antenna, more than half concentrated on the 1st flagellar segment.

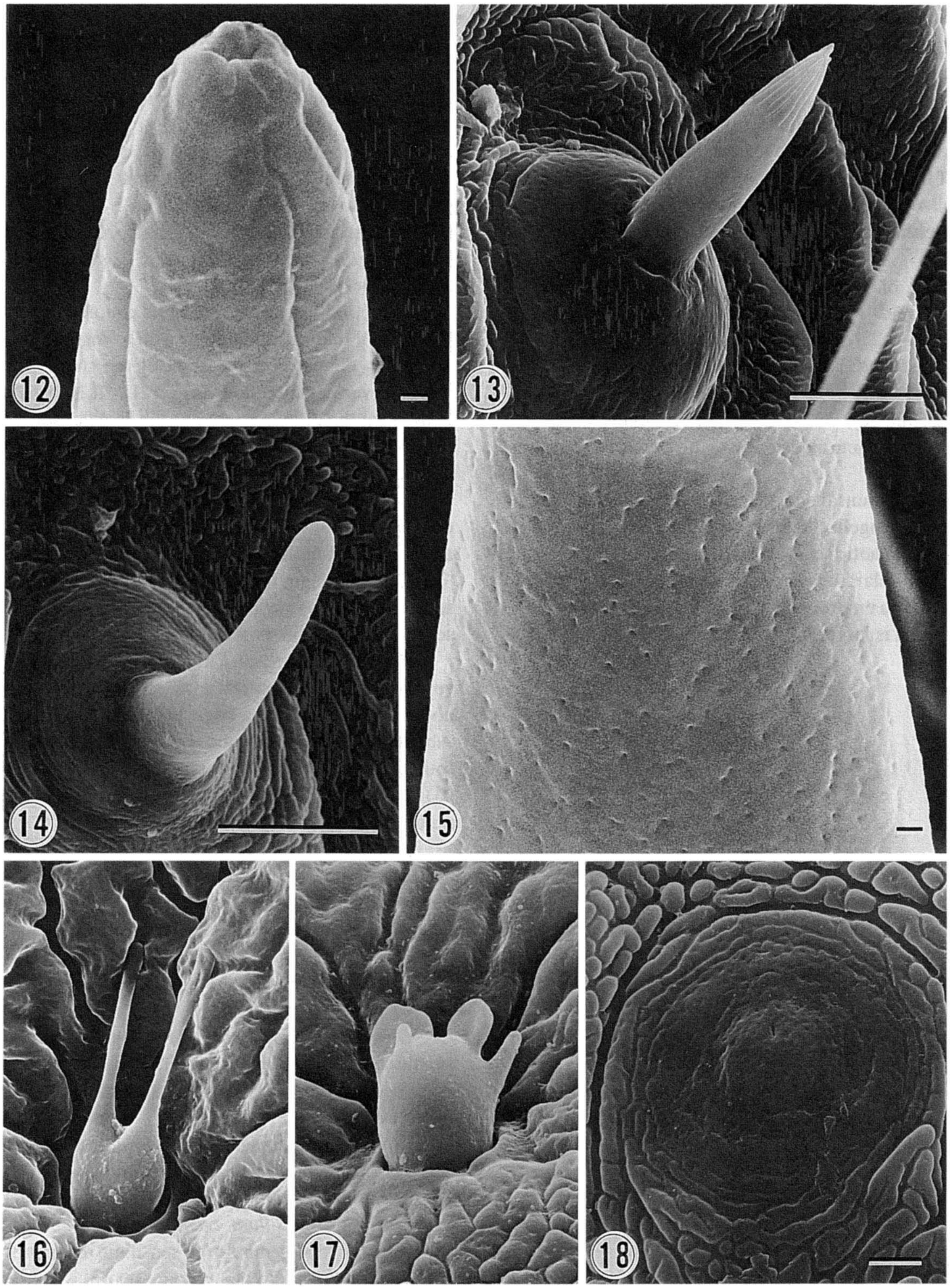


FIG. 12. High power scanning electron micrograph of the distal part of a pored chaetic sensillum. The single terminal pore of 100 nm in diameter is observed at the tip. bar = 100 nm.

FIG. 13. Basiconic sensillum. Apical portion of the cuticular apparatus has longitudinal grooves. bar = 5 μm.

FIG. 14. Trichoid sensillum. Its cuticular apparatus is blunt-tipped and appears smooth-surfaced in this magnification. bar = 5 μm.

FIG. 15. Higher magnification of the side wall of the trichoid sensillum. Numerous minute pores of about 10 nm in diameter are observed. bar = 100 nm.

FIG. 16. Gemmiform sensillum. The cuticular apparatus of this sensillum consists of a main spheroidal body and finger-like protrusions. The tips of protrusions adhere to the antennal surface.

FIG. 17. Gemmiform sensillum. The protrusions are petal-shaped in this example.

FIG. 18. Campaniform sensillum on a flagellar segment. It has an oval dome-shaped cuticular apparatus with a small depression in the center. FIGS. 16–18, bar = 1 μm.

Campaniform sensillum. Campaniform sensillum has an oval dome-shaped cuticular apparatus with a long axis about 3 μm and a short axis about 2 μm (Fig. 18). A small depression is found at the center of the dome. About 10 campaniform sensilla are found on an antenna, about half located on the pedicel.

DISCUSSION

Characteristics of hygro- and thermoreceptive sensillum of firefly

The hygro- and thermoreceptive organ of the firefly is undoubtedly the capitular sensillum based on our electrophysiological recordings (Fig. 4). This is the first identification of the hygro- and thermoreceptive sensilla in coleopteran insects, though the putative hygro- and thermoreceptive sensillum has been reported in *Aleochara* [5].

The resolving power of the FE-SEM used in this study is high enough to visualize the olfactory pores of less than 10 nm as shown in the micrograph of the trichoid sensilla (Fig. 15). No similar pores, however, were found on the cuticular apparatus of any capitular sensilla of the firefly. Therefore, we conclude that the capitular sensillum has no pores.

The head of the inner stem has a rugged surface. This feature is shared with the equivalent structures of hygro- and thermoreceptive sensilla of the cockroach [14], the honey bee [19] and the cricket [6]. Thus, the head of the firefly capitular sensillum may be composed of a specific hygroscopic material which is related to a primary process for hygroreception, as postulated by Yokohari [17]. The head of the firefly capitular sensillum is nearly the same in diameter as the equivalent structures of the honey bee and cricket, while it is about three times as large as that of the cockroach. We, however, do not know what these differences mean.

Tiny apical hollows at the terminal of the inner stem were found in most capitular sensilla of the firefly. Similar structures were commonly observed in the centers of the surfaces of the mechanoreceptive campaniform sensilla of this insect. These structures are known to be vestiges of moulting pores, which are penetration loci of outer dendritic processes at morphogenesis, in both capitular sensilla (unpublished data) and campaniform sensilla [9] in hemimetabolous insects. Although the firefly is holometabolous, the apical hollows seem to be also vestiges of moulting pores by the following reasons. The firefly pupae come out of final instars through moulting, and already have adult-like contours with 11 antennal segments. Emergence, moulting from pupae into adults, is similar to moulting in hemimetabolous insects. This possibly means that the some antennal sensilla are not brand-new but experienced moulting once. Therefore the apical hollows may be vestiges of moulting pores.

The capitular sensilla of the firefly had considerable variation in the structure of their outer walls. These variations were seen not only in different antennae but also in a single antenna. Furthermore, similar variations occurred in specimens prepared in a different dehydrating solution, ethanol. Consequently, the variations in the outer wall are not due to artificial effects, but mirror actual shapes of the sensilla themselves. It remains, however, unknown whether the variations of the outer walls are related to any functional differences in hygro- and/or thermoreception, because we could not find any recognizable differences among sensilla in the present electrophysiological analyses.

The firefly capitular sensillum is markedly different from other sensilla confirmed electrophysiologically to be hygro- and thermoreceptive [2, 4, 6, 8, 21, 22] in that it possesses a wide basal dome. Although the precise function of the basal dome remains unclear, it may contribute to the reduction of mechanical effects propagating from the flagellar surface to the inner stem.

Possible functions of other flagellar sensilla

We have found a new type of sensillum, the gemmiform sensillum. The distribution of gemmiform sensilla is about the same as that of marginal sensilla of the cockroach, limited to the distal margins in alternating segments of the flagella [13, 14]. Although the external features of the gemmiform sensilla are quite different from those of the marginal sensilla, they are both frequently attached to the flagellar wall with the distal parts of the cuticular apparatus. These facts suggest that both types of sensilla may be mechanoreceptive, detecting the distortion of the segmental surface walls caused by antennal movement. The pored chaetic sensillum possesses a single terminal pore and a flexible socket. Hence, this sensillum may be contact chemo- and mechanoreceptive. The poreless chaetic sensillum, on the other hand, has a poreless sharp tip, suggesting that it is a mechanoreceptive organ or a kind of non-sensory structure. The trichoid sensillum has many minute pores on the side wall of the cuticular apparatus, and the basiconic sensillum has the grooved cuticular apparatus. As compared with the external appearance of the functionally identified sensilla of other insects, both trichoid and basiconic sensilla are probably olfactory organs [1, 10]. The campaniform sensillum may detect stress on the surface wall of the antenna [7, 12].

Hygroreception and habitat selection of firefly

Hygrotactic behavior has been demonstrated in several insects including coleopteran insects, *Sitophilus* [15]. The flying and flashing behavior of the adult firefly, *Luciola cruciata*, is strongly influenced by light conditions, temperature, humidity and flashing of other individuals. In preliminary experiments, we estimated the influence of humidity on flashing and flight of males. The results revealed that the males increased the frequency of flashing by two to five times as well as flight activity, when they were transferred from a moist chamber (72% relative humidity) to a dry one (42% relative humidity) at 24°C (unpublished data). The increase of flight activity in a dry environment is perhaps related to attempts to avoid such a condition. Whatever the case, the hygroreceptive ability in this insect plays an important role in its selection of natural habitats.

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