ULTRASTRUCTURE OF THE ANTENNAL CHEMORECEPTORS OF *CULICOIDES NUBECULOSUS* (DIPTERA, CERATOPOGONIDAE)

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Summary :
Although the length and shape of the antennae are different in males and females, the ultrastructural aspects of the sensilla do not differ. The flagellum of both antennae bears two kinds of sensilla : 1) numerous sharp-tipped and blunt-tipped sensilla trichodea. These sensilla have very fine pores in their multiporous walls; they are innervated by neurones which have bifurcating dendrites ascending the hair shafts. Both types of sensilla are abundant on the whole antennal surface and they fit the description of olfactory receptors. 2) Near these sensilla, shorter and less numerous sensilla basiconica and coeloconica occur, which obviously differ in their external aspects, but they present more ultrastructural analogies with a reduced number of dendrites and pores. Both sensilla basiconica and coeloconica are probably thermo or hygroreceptors and/or olfactory receptors.

KEY WORDS : *Culicoides nubeculosus*. sensilla tricodea, basiconica, coeloconica. receptor.

Résumé : ÉTUDE ULTRASTRUCTURALE DES CHÉMORÉCEPTEURS DES ANTENNES CHEZ *CULICOIDES NUBECULOSUS* (DIPTERES, CÉRATOPOGONIDÉS)
Si la taille et la forme de l'antenne diffèrent chez le mâle et la femelle, l'aspect ultrastructural des trois types de soies est identique dans les deux sexes. Il existe deux types morphologiques de soies tricoïdes (longues et courtes) présentant des pores nombreux et très fins qui communiquent avec le liquide sensillaire où baignent de nombreux dendrites émanant de structures ciliaires. Ces soies sont très abondantes sur toute la surface antennaire et décrites comme des récepteurs olfactifs dans les deux sexes. À coté de ces dernières, on trouve des sensilles plus courtes et plus rares, dites respectivement basiconiques et coeloconiques, qui diffèrent sensiblement par leur aspect externe, mais présentent de grandes analogies ultrastructurales, avec un nombre réduit de dendrites et de pores. Ces deux soies sont considérées comme des thermo- ou hygrorécepteurs et aussi comme des récepteurs olfactifs.

MOTS CLES : Culicoides nubeculosus. *soies tricoïdes, basiconiques, coeloconiques. récepteur.*

INTRODUCTION

The olfactory senses of most insects are located on the antennae which possess the appropriate receptors. Each antenna is considered to be an "odor filter" : its efficiency is determined by different factors such as its shape and probably the topographic distribution of the receptors.

Previous experiments on *Culicoides nubeculosus* (involving the masking of the peripheric organs on the male) showed that the antennae bear pheromone receptors (Messaddeq, 1986). Their distribution and the external morphology of the antennal sensilla have been studied by scanning electron microscopy (Messaddeq *et al.*, 1989). A permeability test with silver nitrate revealed that the antennae bear three types of chemoreceptors, namely sensilla basiconica, coeloconica and trichodea (Messaddeq *et al.*, 1990).

Sensilla of *C. furens* females were examined previously by scanning and transmission electron microscopy (Chu-Wang *et al.*, 1975). In 1989, Felipper-Bauer *et al.* showed the morphology of the antennal sensilla of *C. paraensis* females. The latest study was carried out in 1992 by Blackwell *et al.* They made comparative study of the sensory structures on the antennae of males and females of two species : *C. impunctatus* and *C. nubeculosus*. This is a comprehensive study of the chemoreceptive structures on the antennae of *C. nubeculosus* males and females. We studied ultrastructural features using transmission electron microscopy.

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MATERIALS AND METHODS

INSECTS

Culicoides nubeculosus came from a colony maintained since 1973 in the parasitology laboratory insectarium in Strasbourg.

SCANNING ELECTRON MICROSCOPY

Thirty adult males and females were collected from the colony, anaesthetized with cold air, then placed on specimen mounts (aluminium cylinder-type) and coated with the conducting carbon glue (Leit-C). The specimens were sputtered-coated with Palladium-

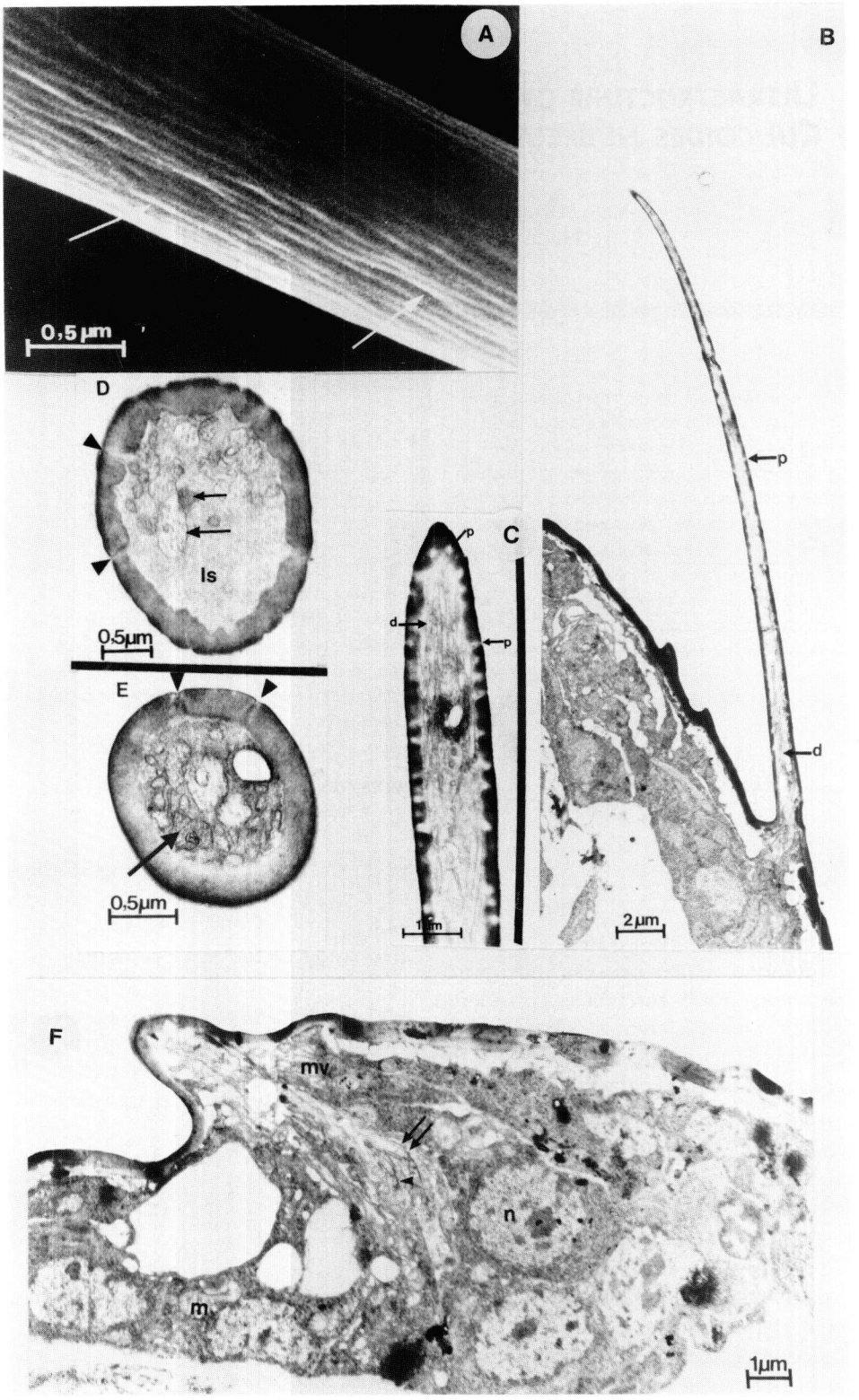

Fig. 1. – Male antennae of *C. nubeculosus*, sensilla trichodea.

A : Blunt-tipped sensilla trichodea. The sensilla walls are slightly hollowed by longitudinal grooves, in which there are small depressions seen on the entire length of the sensilla (arrows). No pores are evident in this picture. B : Sharp-tipped sensilla trichodea on flagellar subsegment 13. Longitudinal section, showing chambers of pores (p) in wall and dendrites (d) in the lumen. Note neurons and enveloping cells at the base. C : Longitudinal section at the tip of a blunt-tipped sensillum trichodeum, with the dendritic ramifications (d) in the sensillary liquid. Each pore (p) expands into a bell-shaped chamber. Note the absence of "pore tubules" in the pore system. D and E : Cross sections of blunt-tipped sensillum, showing the dendritic expansions in the sensillary liquid (l.s), with varied diameters (arrows), containing 1 to 15 microtubules. Fine pores (arrow head) are always situated in the depth of the superficial grooves of the cuticle. F : 13th antennal subsegment. Note a group of enveloping cells, the nucleus"n"of one of these cells and the cytoplasmic veils (mv)at the base of the sensillum. To the left, sensory neurons contain mitochondria (m). The cells bear the ciliary structures, showing 2 groups of neurons innervating the shaft (double arrows), the rootless in the depth (arrow heads).

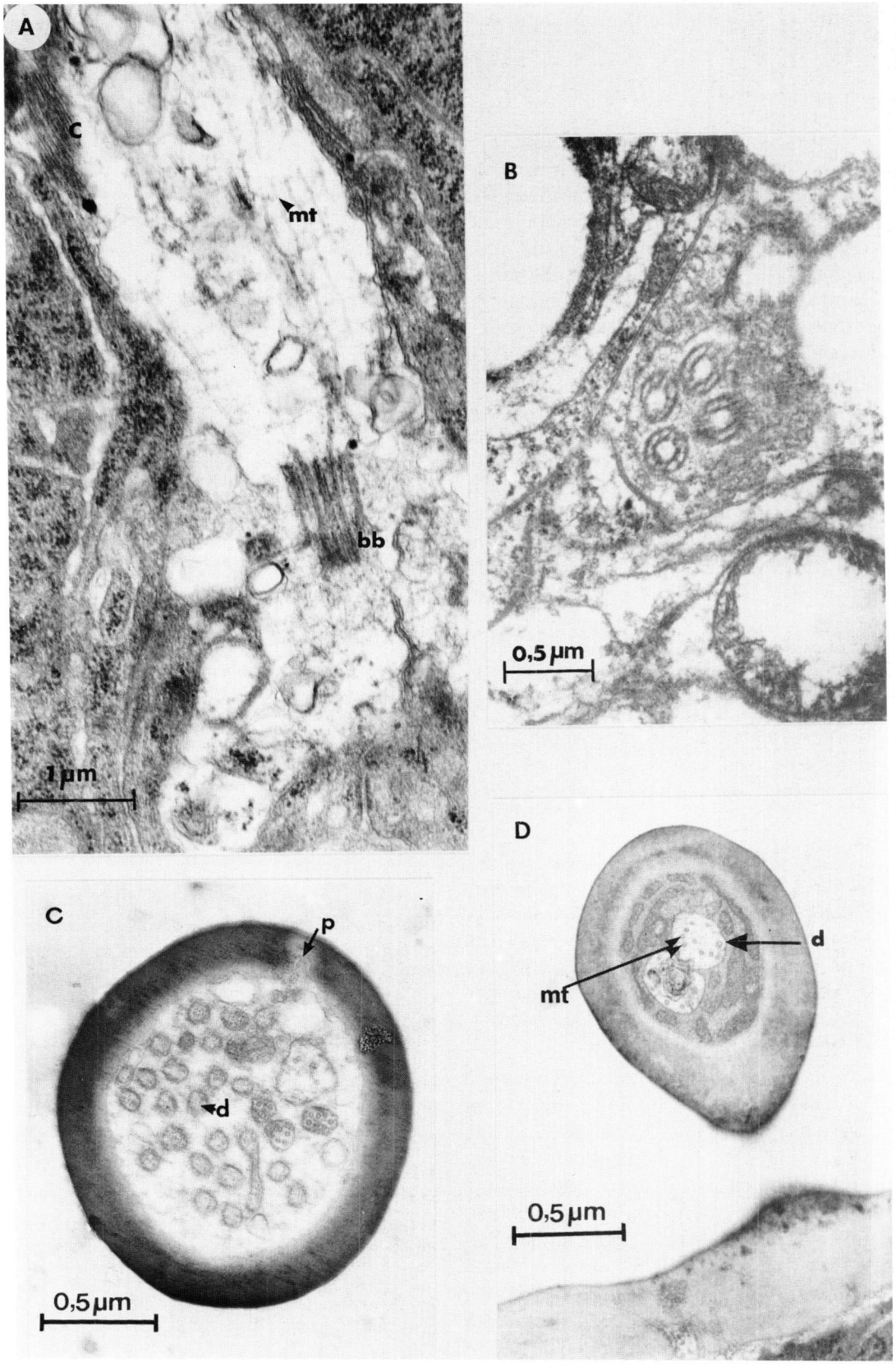

Fig.2. – Male antennae of *C. nubeculosus*, sensilla trichodea.
A : Longitudinal section of dendrites through ciliary region (c), bb = basal body , mt= microtubules. B : Cross section showing 4 dendrites in the ciliary region, which contains "9x2 +0" microtubules. C : A blunt-tipped sensillum trichodeum. Cross section showing a shaft of large diameter with 29 dendrites (d). p=pore. D : A sharp-tipped sensillum trichodeum. Cross section of a shaft showing two large dendritic branches (compare with C). d=dendrite , mt=microtubule.

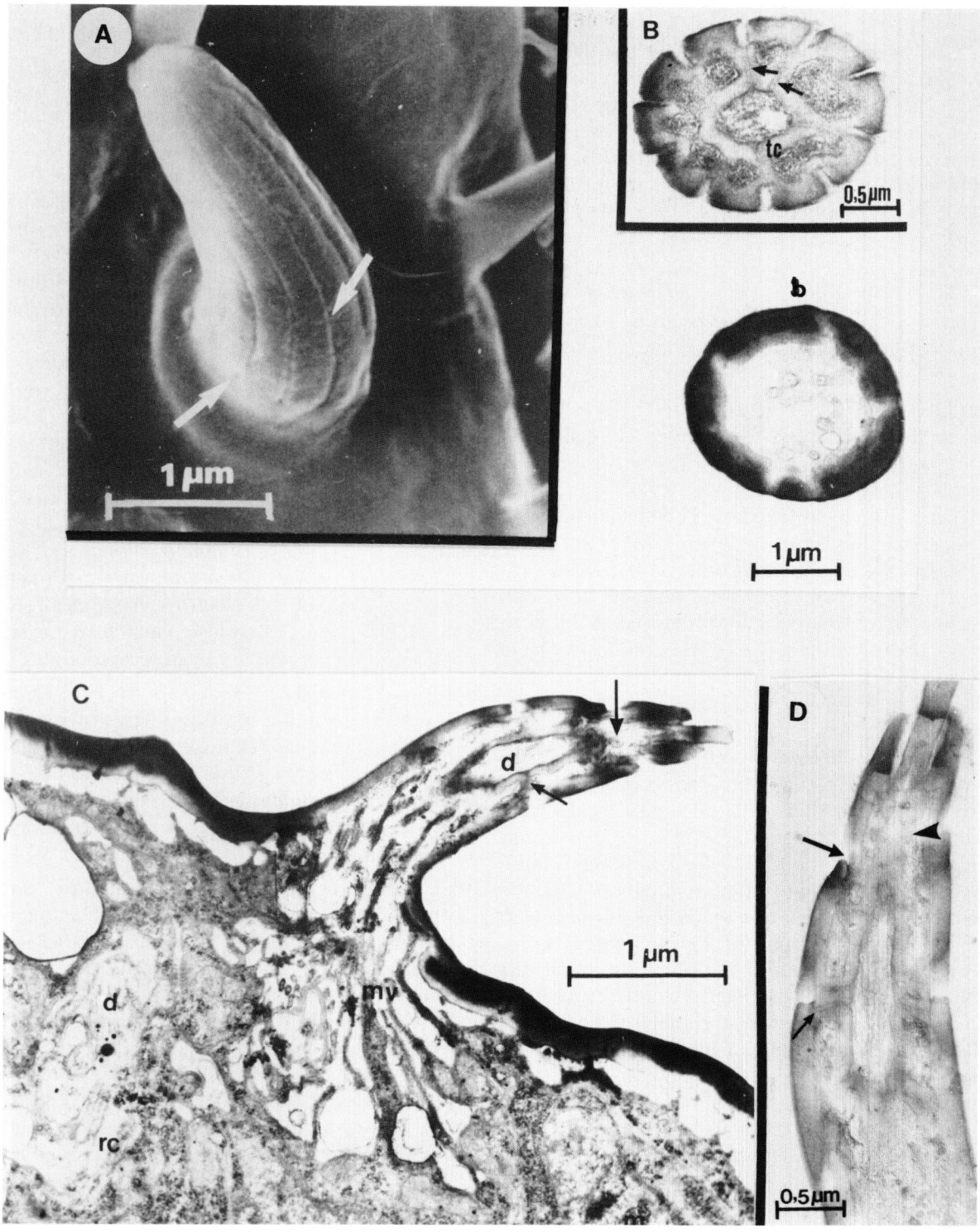

Fig. 3. – Male antennae of *C. nubeculosus*, sensilla basiconica.

A :The body of the sensillum is marked by longitudinal grooves, in which fine pores appear to increase distally toward the tip (arrows). B : 13th antennary subsegment. Cross section of two sensilla near the base. Sensillum basiconicum (above), tc = cuticular tube, pores are noted by arrows ; sensillum trichodeum (below). C : Base of the sensillum basiconicum showing a group of enveloping cells whose microvilli (mv) enter into the sensillum. In the center (in the cuticular tube), there are dendritic expansions (d) which arise in the ciliary region (rc). D : Oblique section of the distal part of the peg, showing the relationship between the axis of sensillum (containing the dendrites) and the cuticular surface. The depth of the longitudinal grooves (large arrow) bear the external opening of pores (fine arrow) which make a passage of 0.32 µm in length to the central channel. Many pores are cut across (arrow head).

DISCUSSION

The present work confirms that the ultrastructural organisation of the chemoreceptive sensilla in *C. nubeculosus* is extremely similar to that of other species of Diptera. Our observations confirm the array of sense organs for *Culicoides* previously identified by electron microscopy (Chu-Wang *et al.*, 1975 ; Filipper-Bauer *et al.*, 1989; Blackwell *et al.*, 1992).

Sensilla trichodea

The sensillar surface in *C. nubeculosus* has pores which open before a short passage into a bell-shaped chamber containing a liquid (Fig. lB).

Messaddeq *et al.* (1989) did not show the presence of pores on the surface, and these are probably open in the longitudinal grooves which cover the sensillar surface.

In 1975, Chu-Wang *et al.* showed that the blunt-tipped sensilla trichodea in *C. furens* have fewer pores basally than the sharp-tipped ones. These sensilla trichodea possess many more pores on their shaft wall, more dendritic expansions into their axes and more neurons than the sharp-tipped trichodea. We have confirmed these observations on *C. nubeculosus.* However, we have observed neither a close relation between pores and dendrites, nor pore tubules in this species. In mosquitoes, the pore tubules were readily observed by Slifer (1970), Chu and Axtell (1971) and by Steinbrecht and Muller (1971). Similar results were obtained in other mosquitoes (Slifer and Sekhon, 1962; Mc Iver, 1970; Kellog, 1970). According to these authors, the presence of numerous dendrites in the lumen, which is in contact with small pores, seems to argue for an olfactory function for both types of sensilla trichodea. Blackwell *et al.* (1992) also think that the single-walled sensilla trichodea are the primary olfactory sensilla, accounting for about 45% and 76% of the total sensory input in males and females of both *C. impunctatus* and *C. nubeculosus.* Results of a permeability test using silver nitrate (Messaddeq *et al.*, 1990) confirm this, but only electrophysiological studies can determine the exact role of these sensilla.

Basiconica and coeloconica sensilla

These types of sensilla have a similar ultrastructure. They are double-walled and multiporous, but have a different appearance and distribution. In transmission micrographs, the difference observed between these types of sensilla is the number of sensory neurons : three to four for sensilla basiconica and five for sensilla coeloconica (Chu-Wang *et al.*, 1975). According to these authors, the ciliary region of the dendrites in *C. furens* (in both types of sensilla) contains a typical pattern of "9+ 0" single microtubules rather than double microtubules as usually found in other insect sensory organs (Chu and Axtell, 1971). In *C. nubeculosus,* we have not obtained the cross sections in this region which are needed to confirm or refute this observation.

Transmission electron microscope studies of both types of sensilla confirm the existence of pores in sensilla basiconica previously suspected by scanning electron microscopy (Messaddeq *et al.*, 1989). Pores in sensilla basiconica are located along the distal 3/4 of the shaft (Fig. 3A). However, they are exclusively distributed on the distal half of sensilla coeloconica, which have deeper grooves that mask the external openings of the pores in scanning micrographs (Fig. 4B). The permeability of both types of sensilla has been confirmed by a test with silver nitrate. Only the regions bearing perforations are impregnated by $AgNO_3$ (Messaddeq *et al.*, 1990). Sensilla basiconica are considered to be hygroreceptors. Ismail (1962), working with *Anopheles maculipennis,* and Kellogg (1970), working with *Aedes aegypti,* confirmed this receptive function by electrophysiological studies. Chu-Wang *et al.*, (1975) also think that the sensilla basiconica of *C. furens* are hygroreceptors.

Mercer and McIver (1973) and Lewis (1971,1972) described the sensilla coeloconica in species of *Simulium* and *Stomoxys,* respectively, which they called sensilla styloconica, that are similar to the sensilla coeloconica in *Culicoides.* According to Slifer (1970), these sensilla may serve as hygroreceptors or olfactory receptors.

We consider the three types of sensilla in *C. nubeculosus* as olfactory receptors because their ultrastructure is like olfactory types of sensilla in other Diptera. The possible role of the one or the other of these sensilla as pheromone receptors is unknown.The pore diameter (10 to 20 nm) of the different sensilla is compatible with the passage of pheromonal molecules. Nevertheless, considering the small number of sensilla basiconica and coeloconica, as well as their small contact surface with air, we suppose that both types of sensilla can detect the atmospheric composition, for example CO_2 or H_2O (Schneider and Steinbrecht, 1968). Therefore, the multiporous sensilla trichodea, which are more abundant and longer than the sensilla we mentioned before, appear better adapted for the detection of diffuse substances, as noted by Steward and Atwood (1963) for *Aedes aegypti.* It seems that the sensilla trichodea are probably pheromonal receptors.

The male antennae of *C. nubeculosus* plays a moderate role in the perception of the pheromones which

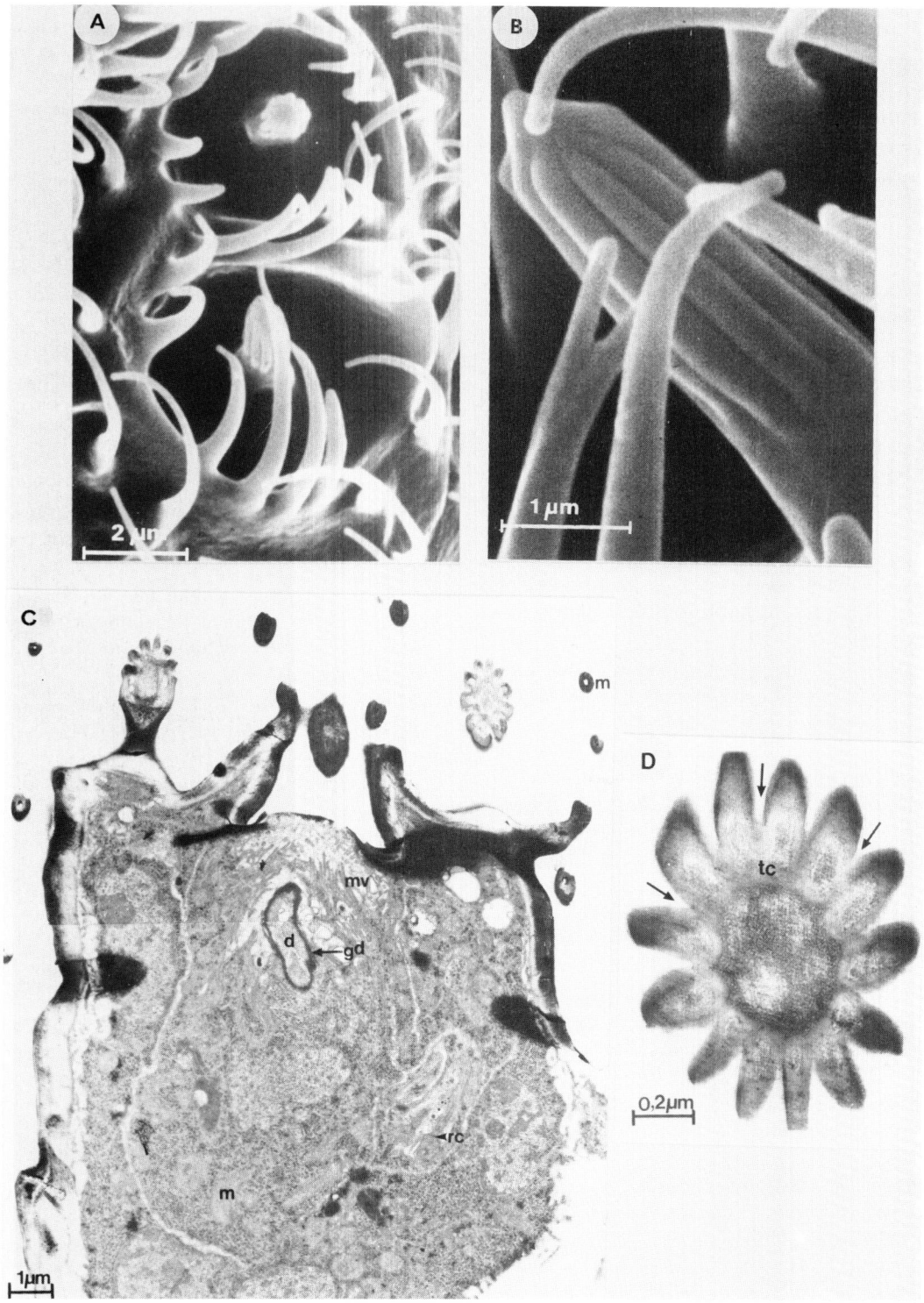

Fig. 4. – Male and female antennae of *C. nubeculosus*, sensilla coeloconica.

A : Antenna of female, two sensilla coeloconica on the 8th subsegment. Peripheral microtrichia are curved to the center. The pit is deeper than the ones bearing sensilla basiconica. B : Antenna of female. Note the deeply marked grooves in the wall, which are deeper than those of sensilla basiconica (Fig.3A). The proximal half of the peg is smooth-walled and distally it abruptly changes into a grooved shaft. C : Antennae of male, 10th antennal subsegment. Oblique cross section of 3 antennal sensory pits . These sensilla are encircled by a number of microtrichia (mc) . Right, the sensillum coeloconicum is cut through the distal part; the one at left is cut through the distal and proximal part . The proximal half of the shaft is smooth-walled and the distal half is highly grooved . In the center, dendrites (d) are surrounded by a cuticular tube (gh) from below the base up to the tip of the shaft . In the longitudinal section, one can see the ciliary structures (rc = ciliary rootlet) approaching the right sensillum. In the center, note a number of microvilli (mv) and enveloping cells with mitochondria (m). D : Cross section of distal half of shaft showing deep grooves occurring in the wall. Arrows indicate channels. tc = cuticular tube.

are secreted by females from the same species (Messaddeq 1986, 1990). Ultrastructural studies of the sensory organs on the antennae show that they are not sexually dimorphic, even if the number and distribution of the sensilla differ in males and females. Three types of sensilla show numerous pores (sensilla basiconica, coeloconica and trichodea), and they are probably chemoreceptors. Physiologically, more numerous sensilla, which possess a great number of pores with a small diameter and containing many neural terminations, i.e. sensilla trichodea, are better candidates as receptors of sexual pheromones.

Electrophysiological studies are needed to determine which receptors may be specifically sensitive to pheromones.

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