# SCANNING ELECTRON MICROSCOPY OF THE ANTENNAL SENSILLA IN FEMALE CULICOIDES PARAENSIS (DIPTERA: CERATOPOGONIDAE)

M. L. FELIPPE-BAUER, P. G. BAUER\* & F. C. SILVA FILHO\*\*

Instituto Oswaldo Cruz, Departamento de Entomologia \* Departamento de Virologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil \*\* Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941 Rio de Janeiro, RJ, Brasil

We studied by scanning electron microscopy the number, types, structure and distribution of the antennal sensilla of the medical important ceratopogonid Culicoides paraensis (Goeldi).

There are about 174 sense organs on the antennal flagellum which are classified as sensilla chaetica; sharp-tipped and blunt-tipped (type I and II) sensilla trichodea; sensilla basiconica; sensilla coeloconica; sensilla ampullacea and styloconic-type sensilla.

The role of antennal sensory organs are discussed regarding the host preference of the biting midges.

Key words: Culicoides paraensis - antennal sensilla - scanning electron microscopy

Antennal sensory organs are important for the interaction of insects with their environment. In hematophagous insects they play a role in the seeking and selection of the host.

The antennal sensilla of medical important dipterans like anopheline and culicine female mosquitoes are intensively investigated (Ismail, 1962, 1964; Slifer & Sekhon, 1962; McIver, 1970, 1982). In biting midges, the sensilla coeloconica are used for taxonomic studies. Recently Wirth & Navai (1978) reviewed the types of the antennal sensilla of Culicoides. A scanning and transmission electron microscopical study of the different types of sensory organs on the antenna of female Culicoides furens (Poey) was made by Chu-Wang et al. (1975).

The investigation of the sensilla of further species of *Culicoides* is necessary, to reveal their importance in taxonomic analysis and to better understand their role in the behavior of these insects.

The present work is a scanning electron microscopical investigation of number, types,

structure and distribution of the antennal sensilla of *Culicoides paraensis* (Goeldi), the biting midge which has been incriminated as vector of the Oropouche virus in the Amazon region (Freitas et al., 1980; Pinheiro et al., 1981, 1982; Roberts et al., 1981).

The terminology of the antennal sensory organs corresponds to the one used by Wirth & Navai (1978). General aspects of ultrastructure and function of the sensilla may be found in the studies of Chu-Wang et al. (1975) and McIver (1982).

### MATERIALS AND METHODS

Specimens of female Culicoides paraensis from Pará (Rodovia Belém-Brasília, km 25), Brazil, were collected biting on man. The specimens were fixed in 70% ethanol and sent to our laboratory by Dr A. C. Linhares, of the Evandro Chagas Institute, Belém.

For light microscopy 20 selected specimens were mounted on slide in Berlèse's medium. For scanning electron microscopy 10 specimens were dehydrated through graded series of ethanols, dried by critical point method, coated with gold in a vacuum evaporator and examined with a JEOL 25 SII scanning electron microscope.

Received March 13, 1989. Accepted April 19, 1989. The measurements of the length of the antennal segments and the antennal ratio (combined lengths of segments 11-15 divided by the combined lengths of the segments 3-10) were made with an ocular micrometer. The lengths of the sensilla were measured on the electron micrographs. The data are presented as mean values and, in parenthesis, as minimum and maximum values.

#### RESULTS

The antennae of *Culicoides paraensis*, as in most ceratopogonids, consist of 13 flagellar segments connected with a cup-shaped pedicel and attached to the head by a flattened ring-shaped scape.

The length of the flagellum of female C. paraensis is about 0.57 (0.53-0.62) mm. The 13 flagellar segments correspond to the antennal segments 3-15 which occur in the proportion of 16-13-15-17-16-16-16-17-17-18-19-18-28. The antennal ratio corresponds to 0.79 (0.75-0.85). The antennal segments 3 and 4 are ovoid; the following ones are elongated, nearly cylindrical. The diameters of the segments decrease distally, Segments 3 and 4 present the greatest diameter with 0.031 mm and 0.025 mm respectively; segments 5-10 show similar diameters with an average of 0.020 mm, and segments 11-15 are slightly smaller with 0.018 mm. The whole antenna is covered by dense microtrichia. The scape present 5 to 6 articulated thick-walled sensilla (sensilla chaetica), 36  $\mu$ m long. On the pedicel, 3 to 6 of these sensilla are found with a length from 17 to 23  $\mu$ m.

The antennal flagellum contains about 174 sense organs. The sensilla are classified according to their external morphology in sensilla chaetica; sharp-tipped and blunt-tipped (type I and II) sensilla trichodea; sensilla basiconica; sensilla coeloconica; sensilla ampullacea and styloconic-type sensilla.

Sensilla chaetica (Table; Figs 2, 3, 5)

The sensilla chaetica are long articulated bristles, arising from a socket, and presenting thick walls and sharp points. These sensilla, also called "verticils", appear as dark antennal hairs when observed by light microscopy.

In C. paraensis they are arranged in the basal portion of the proximal 8 flagellar segments in whorls of 4 to 5, with a length of about 38 to 58  $\mu$ m. On the 15th antennal segment there is a single, smaller one, 19  $\mu$ m long, located at the distal tip (Fig. 3). The surface of sensilla chaetica presents longitudinal grooves as revealed by scanning electron microscopy (Fig. 5).

Sensilla trichodea (Table; Figs 3-4, 6)

The sensilla trichodea are thin-walled, tapering and not articulated setae, which arise from clear areas on the cuticle. They are lightly pigmented, appearing more or less transparent on light microscopy, and have been referred to as "hyaline sensory filaments".

In *C. paraensis* they are found on all flagellar segments. Two morphological types can be distinguished: blunt-tipped and sharp- or pointed-tipped sensilla.

The blunt-tipped trichodea occur on the 8 first flagellar segments, averaging 31/antenna. They can further be divided, according to their length in 2 types, here described as type I and II. The type I blunt-tipped trichodea (Fig. 4) are located in the basal portion of the segments, 2 on each segment, and average 50 to 66  $\mu$ m long. The type II blunt trichodea (Fig. 4) are situated on the distal portion of the segments, with 2 on antennal segments 3-9 and only one on segment 10. They are smaller than the type I, measuring 14 to 26  $\mu$ m long.

The sharp-tipped trichodea (Figs 3, 6) appear most tapered and darker than the blunt-tipped ones on light microscopy. They represent the most numerous sensilla, averaging 62/antenna. They occur on the 5 distal flagel-lomeres, with a length from 40 to  $50~\mu\text{m}$ . There are approximately 10 sensilla on antennal segments 11-13, and 12 on segment 14, generally arranged in two whorls. On segment 15 there are 20 diffusely distributed sensilla.

Sensilla basiconica (Table; Figs 3, 6)

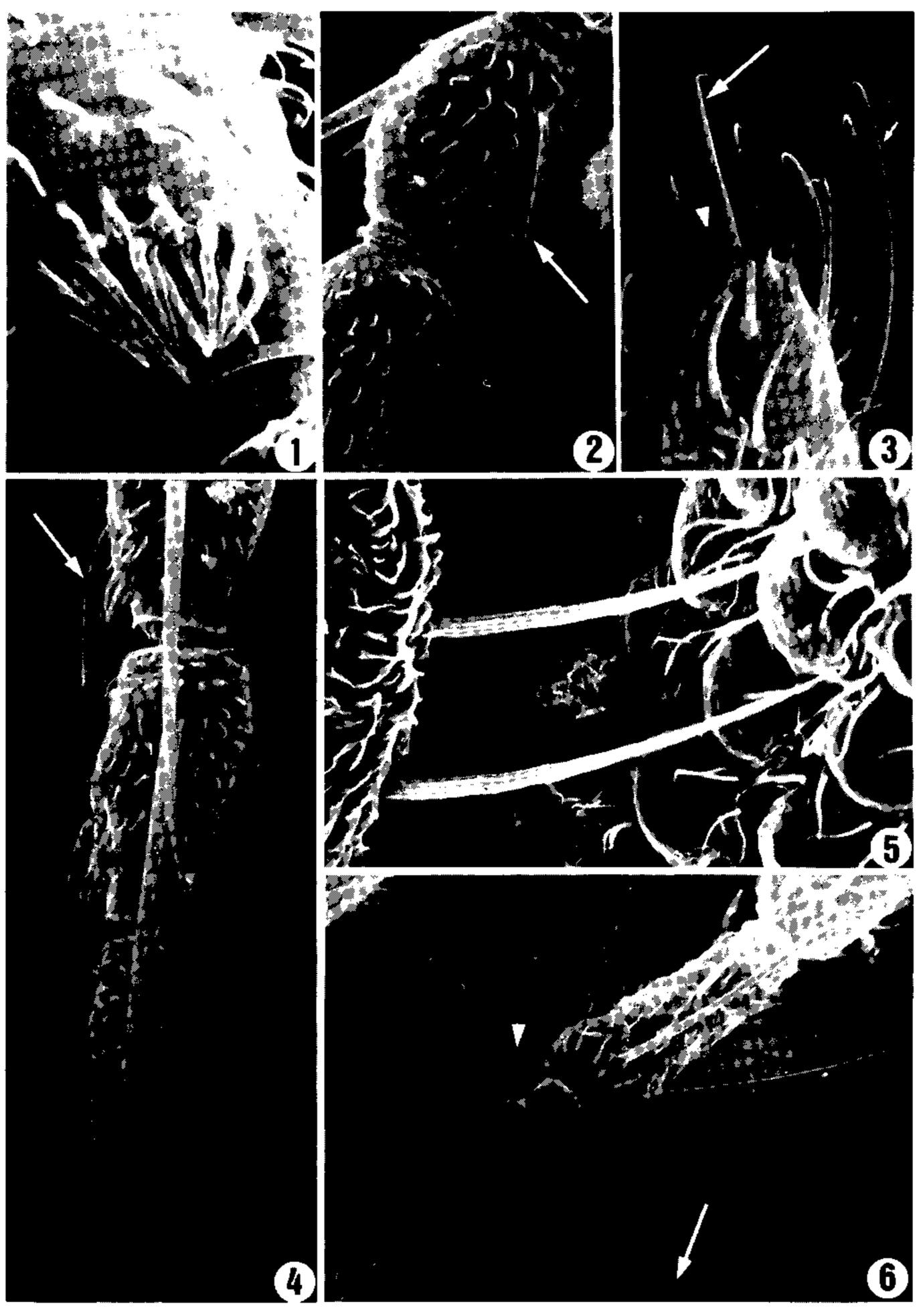
The sensilla basiconica are thin-walled, pegshaped structures, with slightly curved tips, which have been referred to as "sensory pegs or cones" and "soies digitiformes".

TABLE

Number and types of sensilla on the antennal segments of female Culicoides paraensis (Goeldi)

Antennal segments	Sensilla								
	chaetica	trichodea				- · · · - · · · · · · · · · · · · · · ·			
		blunt-tipped		- sharp-tipped	basiconica	coeloconica	ampullacea	styloconic-type	Total
		I	II	**************************************					
3	5 (4-5)	2	2	0	0	2	1	0	12
. 4	5 (5-6)	2	2	0	0	0	1	0	10
5	5 (5-6)	2	2	0	. 0	0	0	0	9
6	5 (4-6)	2	2	0	0	0	0	0	9
7	4 (4-5)	2	2	0	0	0	0	0	8
8	4 (4-5)	2	2	0	0	2	0	0	10
9	4 (4-5)	2	2	0	0	2	0	0	10
10	4 (4-6)	2	1	0	0	2 (2-3)	0	0	9
11	0	0	0	10 (9-10)	5 (4-7)	0	0	0	15
12	0	0	0	10 (10-12)	5 (4-6)	0	0	0	15
13	0	0	0	10 (10-12)	6 (4-7)	0	0	0	16
14	0	0	0	12 (10-12)	6 (4-7)	0	0	0	18
15	1	0	0	20 (17-20)	8 (5-10)	0	0	4	33
al	37	16	15	62	30	8	2	4	174

The sensilla numbers are based on 20 examined antennae and are presented as mean (minimum-maximum) values.



Scanning electron microscopy of antennal sensory organs of *Culicoides paraensis* (Goeldi). Fig. 1: sensilla coeloconica with 2 contiguous pits on 8th antennal segment, X 3680, Fig. 2: 4th segment showing sensilla chaetica (arrow) and the narrow opening of the sensilla ampullacea (arrowhead), X 1450, Fig. 3: apice of 15th segment with a group of styloconic-type sensilla (black arrowhead), sensilla basiconica (white arrowhead), sensilla chaetica (big arrow) and sharp-tipped sensilla trichodea (small arrow). X 1650, Fig. 4: 8-10th segment showing type I (arrowhead) and type II (arrow) blunt-tipped sensilla trichodea and sensilla coeloconica (asterisk). X 1440, Fig. 5: two sensilla chaetica on the scape revealing typical longitudinal grooves. X 1750, Fig. 6: 12-13th segment showing the sharp-tipped sensilla trichodea (arrow) and sensilla basiconica (arrowhead), X 1060.

In C. paraensis they are arranged in the distal portion of antennal segments 11-15, in whorls of 4, with a length from 6 to 8  $\mu$ m. Further sensilla basiconica can be found sparsely distributed between the sharp-tipped trichodea of these segments. There are approximately 30 basiconica per antenna with an increase in number distally.

## Sensilla coeloconica (Table; Figs 1, 4)

The sensilla coeloconica are thin-walled peg organs sunken into depressions of the antennal wall. These sensilla are also known as "olfactory pits" and "sensory tufs".

In C. paraensis they are present on the distal portion of antennal segments 3,8-10, with 2 contiguous pits per segment (Fig. 1), and average 8/antenna. Each pit contains a single central peg (sensillum coeloconicum) encircled by a ring of approximately 10 microtrichia. The sensilla coeloconica are very common in Culicoides and easy to detect by light microscopy due to the microtrichian ring.

# Sensilla ampullacea (Table; Fig. 2)

These sensilla consist of a peg organ located in a deep flask-like pit with a narrow opening. They have anteriorly been referred to by many authors as "sensilla coeloconica" (Campbell & Pelham-Clinton, 1960; Atchley, 1970; Cornet, 1974).

In C. paraensis they are present on the distal portion of the antennal segments 3 and 4, with 1 on each segment, on the opposite side of the sensilla coeloconica.

# Styloconic-type sensilla (Table; Fig. 3)

On the extreme tip of the 15th antennal segment, we could observe a group of four minute cone-shaped pegs, 0.2  $\mu$ m long, which are arising from wider cuticular bases. Their morphological appearence reminds that of sensilla styloconica. They are found near the unique sensilla chaetica of this segment, with which they form a ring on the antennal apice.

#### DISCUSSION

Chu-Wang et al. (1975) mentioned 4 types of antennal sensilla in *Culicoides furens*: s. chaetica, s. trichodea, s. basiconica and s. coeloconica. Wirth & Navai (1978) mentioned the occurrence of a further sensilla on the antenna of *Culicoides*, the sensilla ampullacea. In our study we present 6 types of sensilla in *Culicoides paraensis*, including the sensilla ampullacea, which is in accordance with Wirth & Navai (1978), and styloconic-type sensilla. A detailed discussion of the last two types of sensilla in *Culicoides* is made by Felippe-Bauer & Bauer (in preparation).

In C. furens, Chu-Wang et al. (1975) could find the blunt-tipped sensilla trichodea on all flagellar segments of the antenna. On the proximal 8 segments they are longer than on the distal 5. In C. paraensis we encountered two types of blunt-tipped trichodea with distinct lengths, both occurring only on 8 proximal flagellar segments, as previously mentioned by Atchley (1970) and Cornet (1974). The segmental distribution of the sensilla sharp-tipped trichodea, chaetica and basiconica on the antennae of C. paraensis is similar to the one described by Chu-Wang et al. (1975) in C. furens.

arrangement of antennal Number and sensilla of *Culicoides*, specially the sensilla coeloconica, have been used for many years in taxonomic studies. Furthermore, Jamback (1965) suggested that the number of sensilla coeloconica may be important in the determination of host preference of Culicoides. He observed that ornithophilic species showed more sensilla coeloconica than mammophilic species. Braverman & Hulley (1979), analyzing 12 species of *Culicoides* from southern Africa, found that the species with preference for mammals showed sensilla coeloconica on 6 or less antennal segments, while ornithophilic species presented them on 12 or 13.

Females of *C. paraensis* have 8 sensilla coeloconica on 4 antennal segments (3,8-10), corresponding to number and distribution of mammophilic species (Jamback, 1965; Braverman & Hulley, 1979). However, *C. paraensis* have been cited as feeding on man (Sherlock & Guitton, 1964; Hair & Turner, 1968; Gazeau & Messermith, 1970; Herzog et al., 1988), cattle (Hayes et al., 1984; Mullen et al., 1985), goats and rab-

bits, but also on galliform birds (Humphrys & Turner, 1973; Tanner & Turner, 1974).

Humphrys & Turner (1973) and Tanner & Turner (1974) showed a predominance of *C. paraensis* feeding on galliform birds compared with rabbits and goats, but we think that the small number of captured specimens is not significant to indicate a preference for birds. Therefore, host feeding activity studies in regions where *C. paraensis* is predominant, should be made, to get better data about its biting habits.

More detailed investigations about the antennal sensory organs as well as studies of the behavior of *Culicoides* are necessary to reveal the relationship between each other.

#### **RESUMO**

Microscopia eletrônica de varredura das sensilas antenais em fêmeas de Culicoides paraensis (Diptera: Ceratopogonidae) — Estudos sobre o número, tipo, estrutura e distribuição das sensilas antenais do ceratopogonídeo de importância médica, Culicoides paraensis (Goeldi), são realizados com microscopia eletrônica de varredura.

Encontram-se aproximadamente 174 órgãos sensoriais no flagelo, os quais são classificados em sensila caética; sensila trichoidea, de ápice afilado e de ápice curvo (tipo I e II); sensila basicônica; sensila ampulácea e sensila do tipo estilocônica.

É discutido o papel dos órgãos sensoriais da antena na relação Culicoides/hospedeiro.

Palavras-chave: Culicoides paraensis — sensilas antenais — microscopia eletrônica de varredura

### ACKNOWLEDGEMENTS

To Dr A. C. Linhares, Evandro Chagas Institute, for sending us the specimens for the study, Dr Wanderley de Souza and collaborators, Biophysical Institute of Federal University of Rio de Janeiro, for the scanning electron microscopy facilities and Dr Leonidas M. Deane for reading the manuscript.

### **REFERENCES**

ATCHLEY, W. R., 1970. A biosystematic study of the subgenus Selfia of Culicoides (Diptera: Ceratopogonidae). Univ. Kans. Sci. Bull., 46: 937-1022. BRAVERMAN, Y. & HULLEY, P. E., 1979. The

- relationship between the numbers and distribution of some antennal and palpal sense organs and host preference in some *Culicoides* (Diptera: Ceratopogonidae) from southern Africa. J. Med. Entomol., 15:419-424.
- CAMPBELL, J. A. & PELHAM-CLINTON, E. C., 1960. A taxonomic review of the British species of Culicoides Latreille (Diptera: Ceratopogonidae). Proc. R. Soc. Edinburgh Sect. B, 67: 181-302.
- CHU-WANG, I-WU; AXTELL, R. C. & KLINE, D. L., 1975. Antennal and palpal sensilla of the sandfly Culicoides furens (Poey) (Diptera: Ceratopogonidae). Int. J. Insect Morphol. & Embryol., 4: 131-149.
- CORNET, M., 1974. Caractères morphologiques utilisés pour l'identification des *Culicoides* (Diptera: Ceratopogonidae). Cah. O. R. S. T. O. M., sér. Ent. méd. et parasitol., 12: 221-229.
- FREITAS, R. B.; PINHEIRO, F. P.; SANTOS, M. A. V.; TRAVASSOS DA ROSA, A. P. A.; TRAVASSOS DA ROSA, J. F. S. & FREITAS, E. N., 1980. Epidemia de vírus Oropouche no leste do Estado do Pará, 1979. Revista da FSESP, 25: 59-72.
- GAZEAU, L. J. & MESSERMITH, D. H., 1970. Rearing and distribution of Maryland *Culicoides* (Diptera: Ceratopogonidae). *Mosq. News*, 30: 34-38.
- HAIR, J. A. & TURNER, E. C., 1968. Preliminary host preference studies on Virginia Culicoides (Diptera: Ceratopogonidae). Mosq. News, 28: 103-107.
- HAYES, M. E.; MULLEN, G. R. & NUSBAM, K. E., 1984. Comparison of *Culicoides* spp. (Diptera: Ceratopogonidae) attracted to cattle in an open pasture and bordering woodland. *Mosq. News, 44:* 368-370.
- HERZOG, M. M.; FELIPPE-BAUER, M. L.; MALA-GUTI, R. & CARVALHO LEITE, T. C., 1988. A contribution to the study of Simulium and Culicoides of Rio de Janeiro: Monthly incidence and biting activity. Mem. Inst. Oswaldo Cruz, 83: 95-99.
- HUMPHREYS, J. G. & TURNER, E. C., 1973. Blood-feeding activity of female *Culicoides* (Diptera: Ceratopogonidae). *J. Med. Ent.*, 10: 79-83.
- ISMAIL, I. A. H., 1962. Sense organs in the antennae of Anopheles maculipennis atroparvus (v. Thiel.), and their possible function in relation to the attraction of female mosquito to man. Acta Trop., 19: 1-58.
- ISMAIL, I. A. H., 1964. Comparative study of sense organs in the antennae of culicine and anopheline female mosquitoes. *Acta Trop.*, 21:155-168.
- JAMBACK, H., 1965. The Culicoides of New York State (Diptera: Ceratopogonidae). N. Y. State Mus. Sci. Bull., 399: 1-154.
- McIVER, S. B., 1970. Comparative study of antennal sense organs of female culicine mosquitoes. Can. Entomol., 102:1258-1267.
- McIVER, S. B., 1982. Sensilla of mosquitoes (Diptera: Culicidae). J. Med. Entomol., 19: 489-535.
- MULLEN, G. R.; HAYES, M. E. & NUSBAUM, K. E., 1985. Potential vector of bluetongue and epizootic hemorrhagic disease viruses of cattle and white-tailed deer in Alabama. p. 201-206. In: Alan R. Liss, Inc. T. L. Barver and M. M. Jochim, Eds. Bluetongue and related Viruses, 746 p.

- PINHEIRO, F. P.; HOCH, A. L.; GOMES, M. L. C. & ROBERTS, D. R., 1981. Oropouche Virus. IV. Laboratory transmission by Culicoides paraensis. Am. J. Trop. Med. Hyg., 30: 172-176.
- PINHEIRO, F. P.; TRAVASSOS DA ROSA, A. P. A.; GOMES, M. L. C.; DE DUC, J. W. & HOCH, A. L., 1982. Transmission of Oropouche Virus from Man to Hamster by midge Culicoides paraensis. Science, 215: 1251-1253.
- ROBERTS, D. R.; HOCH, A. L.; DIXON, K. E. & LLEWELLYN, C. H., 1981. Oropouche Virus. III. Entomological observations from three epidemics in Pará, Brazil, 1975. Am. J. Trop. Med. Hyg., 30: 165-171.
- SHERLOCK, I. A. & GUITTON, N., 1964. Dermato-

- zoonosis by Culicoides' bite (Diptera: Ceratopogonidae) in Salvador, State of Bahia, Brazil. Mem. Inst. Oswaldo Cruz, 62: 145-159.
- SLIFER, E. H. & SEKHON, S. S., 1962. The fine structure of the sense organs on the antennal flagel-lum of the yellow fever mosquito Aedes aegypti (Linnaeus). J. Morphol., 111: 49-67.
- TANNER, G. D. & TURNER, E. C., 1974. Vertical activities and host preferences of several Culicoides species in a southwestern Virginia forest. Mosq. News, 34: 66-70.
- WIRTH, W. W. & NAVAI, S., 1978. Terminology of some antennal sensory organs of *Culicoides* biting midges (Diptera: Ceratopogonidae). *J. Med. Entomol.*, 15:43-49.