

Scanning Electron Microscopy of *Onchocerca volvulus* Microfilariae from Guatemala

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Abstract: Microfilariae of *Onchocerca volvulus* from Guatemala were examined by scanning electron microscope. There are numerous transverse striations on the cuticular surface. The number of annulations varies from 325 to 357. The anterior end forms a round cephalic cap which bears a V-shaped hook. Two small openings are present on the cephalic cap. They are probably the openings of one of the amphidial channels and buccal cavity. The tail tapers gradually and ends in the terminal appendage which lacks the transverse striations. The geographical difference in surface structure of *O. volvulus* microfilariae was discussed.

Key words: *Onchocerca volvulus*, *Microfilaria*, *Guatemala*, *Scanning electron microscopy*.

INTRODUCTION

The clinical manifestations of onchocerciasis have long been known to vary from one geographical area to another. The experimental studies on transmission of onchocerciasis have shown that *Onchocerca volvulus* microfilariae from patients in one area develop well to infective larvae in *Simulium* spp. from their own area, but poorly, or not at all, in *Simulium* from other areas (Duke *et al.*, 1966; De Leon and Duke, 1966; Duke, 1967; Duke, 1970). These findings probably suggest the presence of a number of different strains of *O. volvulus*, each adapted for transmission by a different species or forms of *Simulium* (Duke, 1976). Microscopists, therefore, have searched for the morphological evidence of strain difference between *O. volvulus* isolated from different geographical areas, by means of the histochemistry and electron microscopy (Laurence and Simpson, 1968; Omar, 1978; Omar *et al.*, 1982; Franz, 1980).

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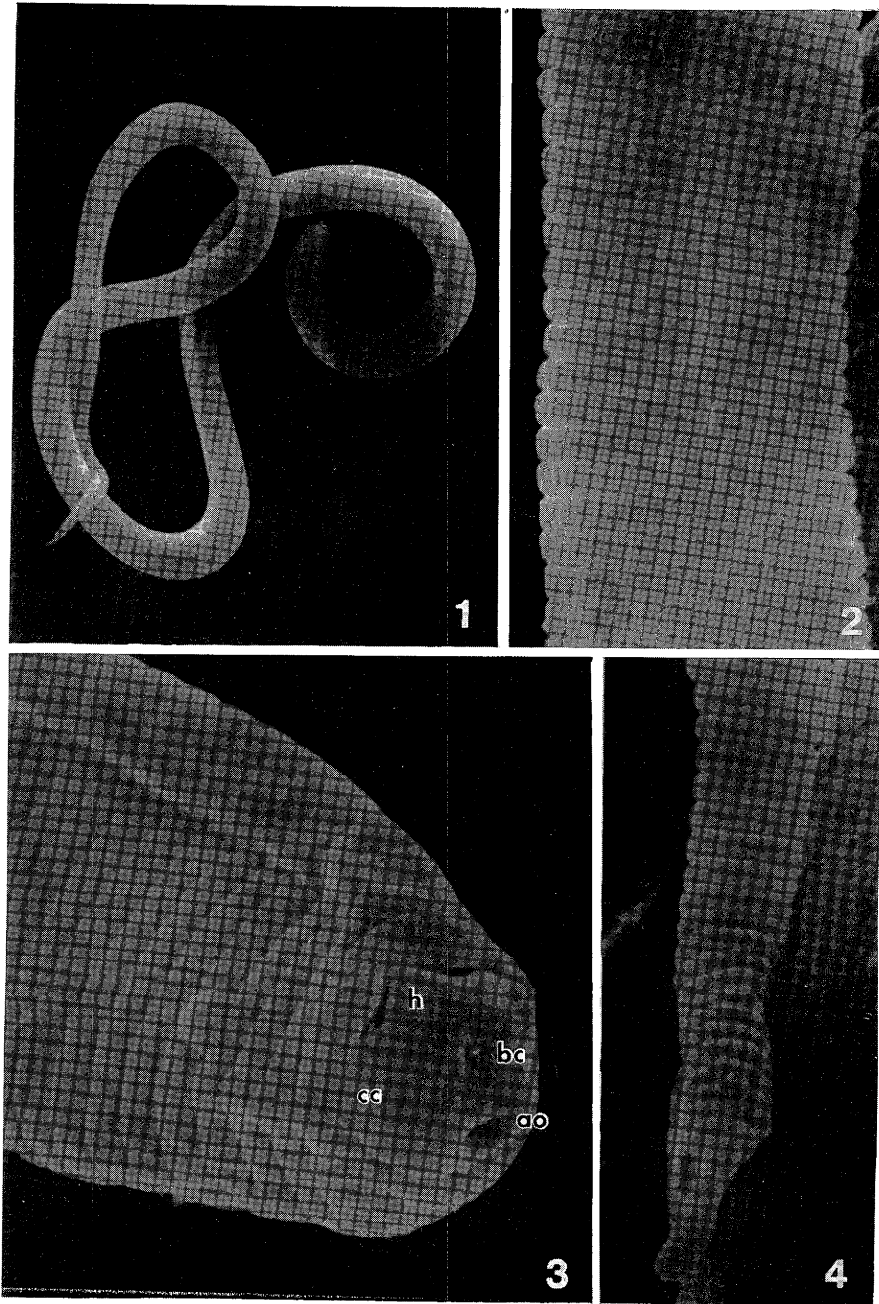
The adult worms of *O. volvulus* isolated from patients in Guatemala were studied by scanning electron microscopy and compared with those isolated from other areas (Franz, 1980). However, Guatemalan *O. volvulus* microfilariae remained to be examined. The present paper deals with the surface architecture of *O. volvulus* microfilariae isolated from Guatemalan patients.

MATERIALS AND METHODS

O. volvulus microfilariae were obtained from human onchocercomas removed surgically from patients living in Municipio de San Vicente Pacaya, Guatemala. Six to eight hours postoperation, the excised onchocercomas were minced and soaked in physiological saline for about 30 min. to allow microfilariae to emerge. Fragments of the onchocercomas and adult worms were removed as completely as possible. After repeated centrifugation at 1,500 rpm for 5 min. and washing with saline several times, microfilariae were fixed in cold, 5% glutaraldehyde with 0.1 M phosphate buffer, pH 7.4. Specimens were dehydrated in a graded series of ethanol, transferred into 100% amyl acetate through the mixture of ethanol and amyl acetate, dried in a liquid CO₂ critical-point dryer, mounted on stubs, and rotary-coated with gold in a vacuum evaporator. The specimens were then examined with the JEOL 100 CX electron microscope.

RESULTS

There are numerous transverse striations on the cuticular surface (Fig. 1). At the midbody of microfilaria, the width of each annulation was 0.6–0.8 μ (Fig. 2). The annulations numbered 325, 329, 334, 342 and 357 in 5 specimens which were positioned for accurate observation. The anterior end of a microfilaria forms a round cephalic cap. The cephalic cap bears a V-shaped hook, 0.5–0.6 μ in length. There are two small openings on the cephalic cap. One of them, 0.4–0.5 μ in diameter, is present at the site opposite to the hook and the other, 0.2 μ in diameter, is at the center of the cap (Fig. 3). The tail tapers gradually and ends in the terminal appendage, 2.0–2.5 μ long, which lacks the transverse striations (Fig. 4). Despite of the careful search, the openings of the excretory and anal pores could not be observed.



Figures 1–4. Scanning electron micrographs of *O. volvulus* microfilariae. 1. Whole body. $\times 1,000$. 2. Cuticular annulation at midbody. $\times 5,000$. 3. Anterior region. A hook and two small openings are present on the cephalic cap. $\times 10,000$. ao: amphidial opening; bc: opening of buccal cavity; cc: cephalic cap; h: hook. 4. Tail. Terminal appendage lacks transverse striations. $\times 10,000$.

DISCUSSION

The application of scanning electron microscopy for the differentiation of filarial worms within the same genus have provided an additional basis for specific diagnosis (Shoho and Uni, 1977; Wong and Brummer, 1978). Scanning electron microscopy, therefore, has been used for the morphological studies on *O. volvulus* isolated from different geographic regions (Martinez-Palomo and Martinez-Baez, 1977; Franz, 1980; Franz and Schuz-Key, 1981).

Franz (1980) reported that there was no geographical difference in surface structure between the adult worms isolated from Liberia, Upper Volta, Tanzania and Guatemala. The present paper described the surface architecture of microfilariae of *O. volvulus* isolated from the patients in Guatemala. Our results are comparable with the surface structure of microfilariae of *O. volvulus* from Mexico (Martinez-Palomo and Martinez-Baez, 1977) and from Liberia (Franz and Schulz-Key, 1981). The surface structure of Guatemalan microfilariae is identical with those of Mexican and Liberian microfilariae. However, the number of annulations of Mexican and Liberian microfilariae was not examined. It might be interesting to compare the number of annulations of microfilariae isolated from different geographic regions.

The surface structures of microfilariae of *Dirofilaria immitis*, *Brugia malayi* and *B. pahangi* have been published elsewhere (Aoki and Katamine, 1975; Aoki *et al.*, 1976). *B. malayi* and *B. pahangi* microfilariae are sheathed. They differ markedly from microfilariae of *O. volvulus* in having larger number of annulations, the spines on the cephalic space, and the striations on the cuticular surface of the terminal appendage of the tail. *D. immitis* microfilariae are unsheathed. The cephalic cap has a hook and two small openings. The tail has the appendage of 4–5 μ long, which lacks the transverse striations. The number of annulations ranges 296–321. It is interesting that *O. volvulus* microfilariae, as mentioned above, show striking resemblance to microfilariae of *D. immitis*, despite of the fact that they inhabit the different tissues; *O. volvulus* microfilariae are present mainly in the skin, while *D. immitis* microfilariae are in the blood stream. *O. volvulus* microfilariae migrate the skin and invade frequently the various organs and tissues other than the eyes. Sakamoto *et al.* (1983) reported that *D. immitis* microfilariae can migrate the skin of a mouse and survive there for 3–4 weeks, when microfilariae were inoculated subcutaneously. These findings may suggest a relation between the cuticular structure and behaviour of microfilariae in the tissues, i.e., the unsheathed microfilariae with a hook on their cephalic cap probably migrate the tissues. It will be interesting to study the surface structures of unsheathed microfilariae of other species; microfilariae of *Dipetalonema streptocerca* which inhabit the skin and those of *D. perstans* which are in the blood stream.

We observed the two small openings on the cephalic cap of *O. volvulus* microfilaria. The opening at the center of the cap probably corresponds to the opening of the buccal

cavity and the other is the opening of one of the amphidial channels, based on the transmission electron microscopy of several species of microfilariae (Kozek, 1968, 1971; McLaren, 1969, 1972; Tongu, 1974; Martinez-palomo and Martinez-Baez, 1977). We failed to observe the openings of anal and excretory pores, which were found on the microfilariae of *D. immitis* (Aoki and Katamine, 1975). The anal pore and excretory pore might be covered by the dense plug as reported by Martinez-Palomo and Martinez-Baez (1977).

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グアテマラの回旋糸状虫仔虫の走査電子顕微鏡像

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グアテマラで得た回旋糸状虫 (*Onchocerca volvulus*) 仔虫の体表微細構造を走査電子顕微鏡で観察した。体表には多くの輪状溝が存在し、虫体は多くの体環状を示す。体環の数は325-357とほぼ一定である。頭端は半球状で、その一端より長さ0.5~0.6 μ の鉤が後方にのびる。また頭端には amphidial channel と buccal cavity の開孔部と考えられる2つの小孔が存在する。尾部では体環は徐々に小さくなり、尾端2.0~2.5 μ は棍棒状を呈し、その表面には輪状溝が存在しない。排泄孔、肛門孔の開孔部は観察されなかった。オンコセルカ仔虫体表構造の虫体の strain による違いについて考察した。