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SHORT REPORT

# FIRST REPORT ON SURFACE ASPECTS OF *Mansonella ozzardi* (SPIRURIDA: ONCHOCERCIDAE) MICROFILARIAE BY SCANNING ELECTRON MICROSCOPY: PRELIMINARY RESULTS

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ABSTRACT

Blood samples from *Mansonella ozzardi* infected volunteers from Vila Antimary (Amazonas State) were processed and a protocol to isolate and prepare microfilariae was carried out in order to perform Scanning Electron Microscopy (SEM) analysis. Data obtained from ultrastructure showed some undescribed structural points of the parasite such as a dimple in the anterior end of the larva and small points –orifice-like– that may be related to amphidial structures or simply pores. Another interesting feature was the tip of the tail which is very similar to that found in the rodent parasite *Dunnifilaria meningica*.

KEY WORDS: *Mansonella ozzardi*; microfilariae; Scanning Electron Microscopy; ultrastructure.

*Mansonella ozzardi* (Spirurida: Onchocercidae) is a filarial nematode found in South and Central America as well as some Caribbean Islands. In the Caribbean islands *Culicoides* spp cause transmission and dissemination of the parasite, while in Brazil it is transmitted during the blood meal of hematophagous diptera from the genus *Simulium* spp (Shelley et al., 1980), which is very widespread in communities in the Alto Amazonas (Solimões river), Negro and Purus rivers (Adami et al., 2014; Nascimento et al., 2009). The exact locality of adult worms in the human body remains a mystery, but during experimental infections in *Erythrocebus patas* monkeys the worms were detected in the subcutaneous tissue (Orihel & Eberhard, 1982).On the other hand, the larval stage of the worm - the microfilariae - is found in the peripheral blood of parasitized individuals - since their behavior is not periodical (Nathan et al., 1978) and is identified based on its morphological characteristics, and

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more recently polymerase chain reaction, PCR (Medeiros et al., 2018). The parasite has high prevalence rates in some areas of the Brazilian Amazon and it is relatively easy to find the microfilariaes in the blood of infected individuals (Adami et al., 2014). Remarkable morphological studies on *M. ozzardi* microfilariae were performed by Raccurt & Kozek (1983a, 1983b), comparing Simuliid and Culicoid transmitted larval stage forms using light and electron microscopy. Subsequently, studies on morphological features in blood samples revealed the presence of atypical forms of the microfilariae in Brazil (Adami et al., 2008) and Peru (Arrospide et al., 2010). Studies on the morphology of *M. ozzardi* are scarce and the surface of its larval stage through Scanning Electron Microscopy (SEM) had never been reported.

The initial procedures were performed in Vila Antimary in Amazonas State ($9^0$4´01´´S $67^0$23´50.1´´O) under field conditions. Thus, blood samples from volunteers - previously selected according to high parasitemia levels - were collected and 8 mL were placed in a 15 mL Falcon® tube with Dextran (1 mL) and Sodium citrate (1 mL). The solution was gently inverted ten times, and kept for 45 minutes at room temperature to precipitate erythrocytes. Microfilariae enriched supernatant was washed with a saline solution twice and the sediment was transferred to Eppendorff® tubes and fixed with 1 mL of a mixture of glacial acetic acid (Sigma Chem. Co, St Louis, USA), 37% formaldehyde (Sigma) and 70% Ethanol (Vetec, Brazil). Microfilariaes were fixed to a support with 1% gelatine (Sigma), washed three times with sodium cacodylate buffer 0.1 M (pH =7.2), and post fixed with two drops of 2% osmium tetroxide . The following step was dehydration in ethanol and drying using the critical point method. They were then set up on metallic supports, covered with gold and observed using the JEOL 5310 Scanning Electron Microscope®. Measurements were performed with the aid of the Semafore Analysis software coupled to the electron microscope.

The present study was approved by the Ethics in Research Committee of Oswaldo Cruz Foundation, under approval certificate nº. 281/05.

The ultrastructure revealed that the cuticle was covered in transversal striations that surround the entire extension of the larva as far as the beginning of the cephalic space. Amplification of the image revealed a depression in the anterior extremity – like a slit with small orifices (Figure 1). The posterior extremity – tail region – showed a slender and pointy termination. The striations are present to the end of the tail, which seems to have a blunt termination with a discrete bifurcation (Figure 2).

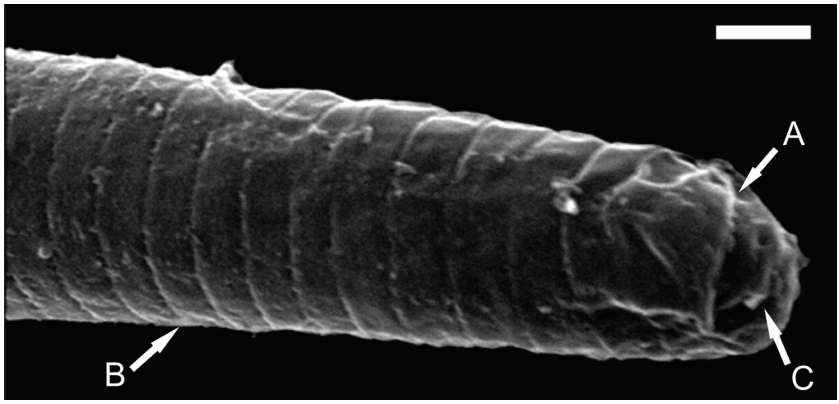


*Figure 1*. Anterior region of *Mansonella ozzardi* microfilariae showing in A: amphydial structure; B: transversal cuticular striation and C: Lip. Bar = 1 µm.

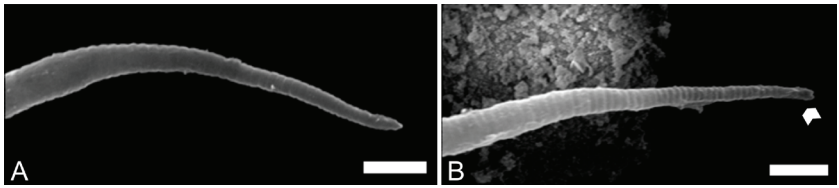


*Figure 2*. A: Posterior extremity showing tail with a slender pointed termination. B: Discrete bifurcation of the tip of the tail (arrow). Bar = 1µm.

Larval stages from other filarial genera such as *Dirofilaria immitis* (Aoki & Katamine, 1975), *Onchocerca volvulus* (Martínez-Palombo & Martinez-Báez, 1977), *Wuchereria bancrofti* (Franz & Zielke, 1980), Loa loa (Kozek & Uriel, 1983) and *Dunnifilaria meningica* (Gutiérrez-Peña, 1989) have already been described through SEM - but surface studies with microfilariae from *M. ozzardi* had never been performed before. Transversal striation seems to be a common characteristic in other filarial genera but the dimple in the anterior end of the larva has been previously described as an oral opening in *W. bancrofti* microfilariae (Franz & Zielke, 1980). Besides, small points observed - such as orifices – may be related to amphidial structures or simply pores, as seen in *Dirofilaria meningica* (Gutiérrez-Pena, 1989). In the posterior extremity the transversal striations cover the entire body of the microfilariae reaching the tail. Interestingly, the tip of the tail and its entire structure are very similar to those found in the rodent parasite *D. meningica* (Gutiérrez-Peña, 1989). However, new SEM will be necessary to clarify these specific points.

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