Morphological Study of Adult Male Worms of *Schistosoma mansoni* Sambon, 1907 by Confocal Laser Scanning Microscopy

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Aiming to detail data obtained through brightfield microscopy (BM) on reproductive, excretory and digestive system, specimens of Schistosoma mansoni *eight weeks old, were recovered from SW mice, stained with Langeron's carmine and analyzed under a confocal laser scanning microscope CLSM 410 (Carl Zeiss). The reproductive system presented a single and lobate testis, with intercommunications between the lobes without efferent duct. Supernumerary testicular lobe was amorphous and isolated from the normal ones. Collecting tubules (excretory ducts), followed by the excretory bladder, opening to the external media through the excretory pore, were observed at the posterior extremity of the body. In the digestive tract, a cecal swelling was noted at the junction that originates the single cecum. It was concluded that through confocal laser scanning microscopy, new interpretations of morphological structures of S.* mansoni *worms could be achieved, modifying adopted and current descriptions. The gonad consists of a single lobed testis, similar to that observed in some trematode species. Moreover, the same specimens can be observed either by BM or CLSM, considering that the latter causes only focal and limited damage in tissue structures.*

Key words: *Schistosoma mansoni* - adult male - morphology - confocal laser scanning microscopy - reproductive system

Since the middle of the past century, morphological features of the genus *Schistosoma* Weinland, 1858 have elucidated taxonomic questions about the main species known to infect man. Indeed, using morphological criteria, Sambon in 1907 proposed the species name *S. mansoni* for the worms producing the lateral-spined ova, basing his proposal on the different size and shape of the eggs and of female worms from those of the typical *S. haematobium* (Bilharz, 1852) Weinland, 1858. Finally the initial controversy about the *S. mansoni* and *S. haematobium* species had a definitive answer when Pirajá da Silva (1908) described a schistosome worm whose measurements were distintc from other species and had a lateral-spined egg.

In Brazil, brightfield microscopy has been used in morphometric studies of *S. mansoni* adult worms recovered from several hosts (Kastner et al. 1975, Dias & Piedrabuena 1980, Machado-Silva et al. 1994) or pertaining to different strains (Magalhães & Carvalho 1973, Paraense & Corrêa 1981, Machado-Silva et al. 1995) aiming to define some quantitative parameters.

The surface topography of adult schistosomes has been exhaustively visualized in a number of scanning electron microscope (SEM) studies (Miller et al. 1972, Hockley 1973, Kuntz et al. 1976, Sakamoto & Ishii 1977, McLaren 1980), which also showed tegumental alterations after schistosomicides treatment (Kohn et al. 1982, Magalhães Filho 1987) or after incubation in various media (Kalapothakis et al. 1988). Recently, we have demonstrated that the far anterior region of the gynaecophoric canal is spiness, the right side of the gynaecophoric canal presents a greater amount of tubercles compared to the other in which the spines prevailed. The excretory pore situated at the distal extremity of the body has a "volcano gate-like" aspect (Machado-Silva et al. 1997). However, scanning electron microscopy deals with the surface of

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the adult schistosome and it not make possible to study its inner organization (McLaren 1980).

Otherwise, the advent of commercial availability of the confocal laser scanning microscope (CLSM) in 1987, provided a superior tool to investigate external and internal helminth morphologic aspects (Lenzi et al. 1996a, b, 1997). Thus some articles came out describing the neuroanatomy of helminths (Johnston et al. 1990, Skuce et al. 1990) and the entire excretory and gut system of different life-cycle stages of *S. mansoni* using monoclonal antibodies (Bogers et al. 1994).

In this paper we show the usefull application of CLSM, employing non-immune staining, for the description of the microanatomy of reproductive, excretory and digestive systems of *S. mansoni* male worms, showing some original contributions that complement the morphological features already described by conventional brightfield and SEM microscopies.

MATERIALS AND METHODS

Male adult worms - The source of adult worms and the isolation procedures to collect them were described elsewhere (Machado-Silva et al. 1994).

Processing of specimens and morphological analysis - Eight weeks old adult worms were recovered from Swiss Webster mice, fixed in AFA (2% acetic acid, 3% formaldeyde and 95% of 70% alcohol), stained with Langeron's carmine (Merck - ART 2233), cleared with beechwood creosote and preserved in Canada balsam. Whole-mounts were analyzed by CLSM (LSM 410, Zeiss), in DIC and/or reflected mode, using 543 He/Ne laser, with LP 570 filter. The images were transferred from the LSM computer to Microsoft Imager™ and Corel Draw 6.0™ for final adjustments of contrast, brightness and gamma-correction and then printed in a Codonics NP 1600™ printer.

RESULTS

The male reproductive system in the ventral area is distinguished by the presence of thick and muscular genital pore (Figs 1, 5) at the proximal end of the gynaecophoric canal. The seminal vesicle is ventrally joined to the genital pore through the ejaculatory duct (Fig. 2), and on the opposite end, is connected with the testis through the deferent duct (Fig. 3). There is a single testis composed by combining lobes, ventrally intercommunicated between them (Figs 3, 4), which drain the sperm directly to the deferent duct, without the intermediacy of an efferent duct (Fig. 3). Supernumerary testis lobe exihibits an amorphous appearance (Fig. 6) and is isolated from the normal lobes, which are enclosed by a thin albuginea-like capsule (Figs 1, 3).

Collecting tubules (excretory ducts) (Fig. 10) join to form the excretory bladder (Fig. 7), opening to the exterior through the excretory pore, located at the posterior extremity of the body (Fig. 8). In the digestive system, a cecal swelling is observed at the junction that originates the single cecum (Fig. 9). A closed digestive system is evident (Fig. 10).

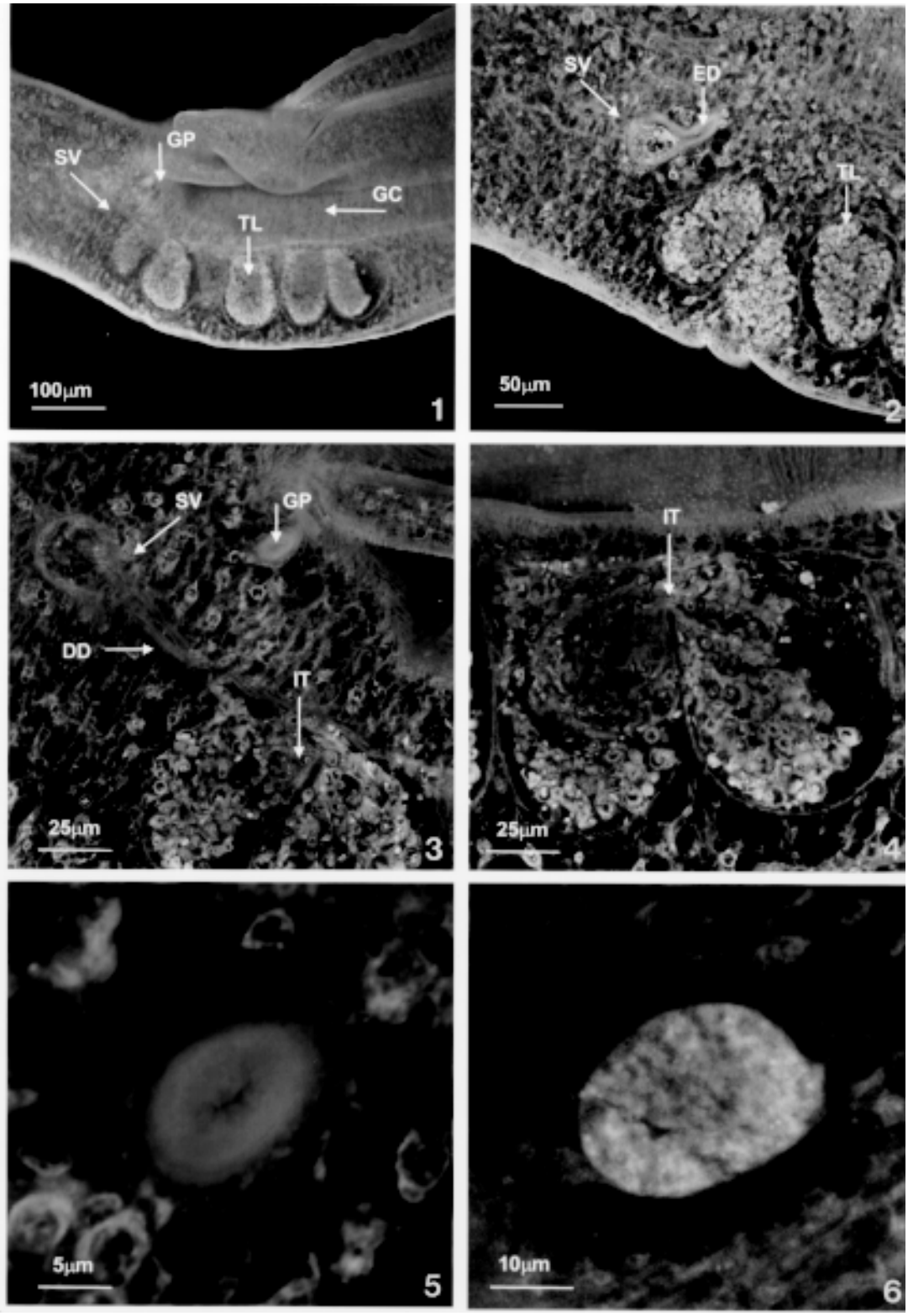
DISCUSSION

In this work we present part of our experience on the use of non-immune staining procedure (Langeron's carmine) which usefully complement specific studies on *S. mansoni*. The CLSM optimizes this staining making possible to optically section relative large whole specimens, and to digitally store and process such images on a computer for reconstructions of galleries (Fig. 7) or 3-D images, representative from many levels of in and outside of the optically sectioned specimen (Lenzi et al. 1997).

At the present, CSLM has confirmed previous descriptions related to the localization of genital pore and seminal vesicle (Figs 1, 2, 3) although, some structures are now having another interpretation, modifying adopted and current concepts. Some digeneans have a single, two or numerous testes which are joined to a efferent duct (Travassos et al. 1969, Kastner et al. 1975, Fried & Graczyk 1997). Otherwise, by CLSM those features were not confirmed, due to the finding of intercommunications between posterior to the anterior gonadal lobes (Figs 3, 4). So, we named them testicular lobes instead of testes.

According to Fried and Graczyk (1997) spermatozoa produced in the testes of some trematodes are transferred to the seminal vesicle by way of the *vas efferentia* (efferent duct) and *vas deferens* (deferent duct). Our data herein indicate that *S. mansoni* is not included in this group. Efferent duct was not evident in the male reproductive system though deferent duct was demonstrated (Fig. 3). Consequently, we suppose that intercommunications between lobes are the route that spermatozoa follow to reach deferent duct.

Male worms of *S. mansoni* with supernumerary testes (testicular lobes) have been mentioned in several papers (Vogel 1947, Najim 1951, Saoud 1966, Coles & Thurston 1970, Soliman et al. 1984, Machado-Silva et al. 1994), however, the origin of this fact is unknown (Machado-Silva et al. 1995). Detailed morphological descriptions of those structures are scarces. The supernumerary testicular lobe had an amorphous aspect (Fig. 6), without connection with the normal set. This observation do not ratify data showing that the texture of the contents of this lobe is similar to that of normal set (Najim 1951). It is also in disagreement with a



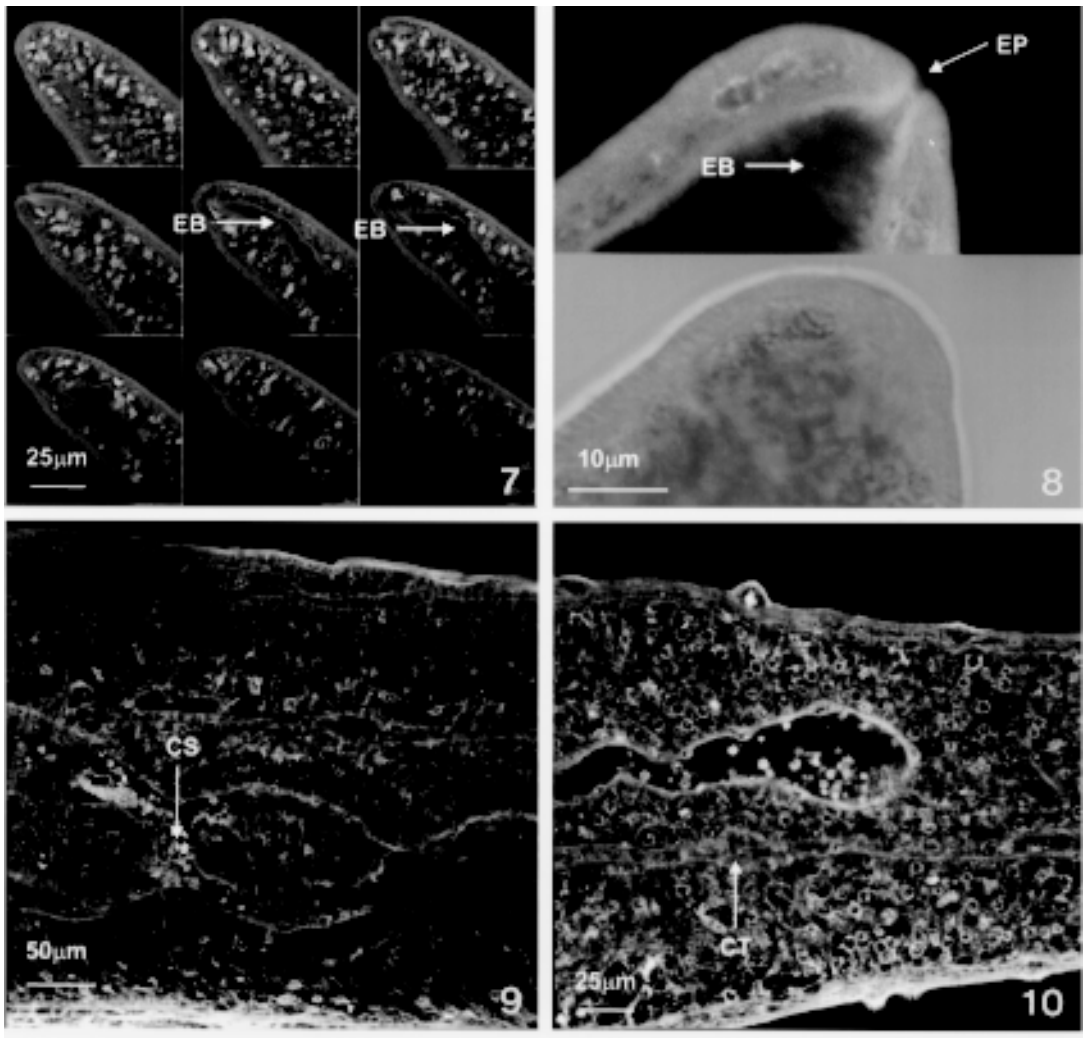
Schistosoma mansoni by confocal laser scanning microscopy. Reproductive system. Figs 1, 2, 3: the general morphology of the reproductive system. Fig. 4: intercommunication between testicular lobes. Fig. 5: genital pore. Fig. 6: supernumerary testicular lobe. DD: deferent duct; ED: ejaculatory duct; GC: gynaecophoric canal; GP: genital pore; IT; intercommunication between testicular lobes; SV: seminal vesicle; TL: testicular lobes.

possible duct connecting the supernumerary lobe with preceeding ones (Vogel 1947).

CLSM also allowed a detailed morphological study of the excretory (osmoregulatory) system, providing complementary informations to previous studies by brightfield microscopy (Kastner et al. 1975), immunohistochemistry (Bogers et al. 1994) and SEM (Machado-Silva et al. 1997). The results so far obtained (Figs 7, 8) demonstrate that in *S. mansoni* this system is alike other digeneans (Fried & Graczyk 1997).

S. mansoni adult male has a closed digestive system (Fig. 10) as other digeneans (Fried & Graczyk 1997) however, a cecal swelling (Fig. 9) was observed at the junction that originates the single cecum. This feature has not been described before.

It can be concluded that through CLSM, new interpretations of morphological structures of *S. mansoni* worms could be achieved, modifying adopted and current descriptions and adding new details to previous studies by brightfield microscopy (BM) (Piraja da Silva 1908, Vogel 1947, Saoud 1966, Magalhães & Carvalho 1973, Kastner et al. 1975, Paraense & Corrêa 1981, Machado-Silva et al. 1994, 1995) and SEM (McLaren 1980, Machado-Silva et al 1997) microscopies. Besides, a same specimen can be observed either by BM or CLSM, considering that the later causes only focal and limited damages in tissue structures.



Schistosoma mansoni by confocal laser scanning microscopy. Excretory and digestive systems. Fig. 7: tomographic sections (gallery) showing the excretory pore and the excretory bladder. Fig. 8: detail of the excretory pore, better visualised by reflected (upper figure) than by transmitted mode (lower figure). Figs 9-10: cecal swelling and end extremity of the digestive system. CS: cecal swelling; CT: collecting tubules; EB: excretory bladder; EP: excretory pore.

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