

# Reproductive System Abnormalities in *Schistosoma mansoni* Adult Worms Isolated from *Nectomys squamipes* (Muridae: Sigmodontinae): Brightfield and Confocal Laser Scanning Microscopy Analysis

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*Schistosoma mansoni* adult worms with genital anomalies isolated from *Nectomys squamipes* (Muridae: Sigmodontinae) were studied by confocal laser scanning microscopy under the reflected mode. One male without testicular lobes (testicular agenesis/anorchism) and two females, one with an atrophied ovary and another with 17 uterine eggs, were identified. The absence of testicular lobes occurred in a worm presenting otherwise normal male adult characteristics: tegument, tubercles and a gynaecophoric canal with spines. In both female specimens the digestive tube showed a vacuolated appearance, and the specimen with supernumerary uterine eggs exhibited a developing miracidium and an egg with a formed shell. The area of the ventral sucker was similar in both specimens however the tegument thickness, ovary and vitelline glands of the specimen with the atrophied ovary were smaller than those of the one with supernumerary eggs. These reported anomalies in the reproductive system call attention to the need to improve our understanding of genetic regulation and the possible role of environmental influences upon trematode development.

Key words: *Schistosoma mansoni* - reproductive system abnormalities - adult worms - trematode - anorchism - confocal laser scanning microscopy

The somatic development and reproductive system of adult worms of *Schistosoma mansoni* suffer intense selective pressure from the vertebrate hosts. Full somatic development of adult worms, characterized by large size, complete formation of reproductive organs and complete tegumental maturation, is reached only in permissive hosts (mice, hamster and water rat) (Cioli et al. 1977, Machado-Silva et al. 1997). However, anomalous specimens can occur sporadically even in favorable hosts. In this context, Najim (1951) found a male adult with two sets of testes, which he considered as supranumerary testes. Machado Filho (1966) recovered a female with six uterine eggs, without however any visible morphological alterations. Dias and Ribeiro (1980) found in *S. mansoni* eggs containing two active miracidia the feces from a patient.

This article describes morphologically the abnormalities of the reproductive system of *S. mansoni* adult worms (two females and one male) isolated from *Nectomys*

*squamipes* (Muridae: Sigmodontinae), which is a permissive host, using brightfield and confocal laser scanning microscopies.

## MATERIALS AND METHODS

The study was based on an examination of anomalous adult *S. mansoni* specimens, obtained from the mesenteric veins of nine-week-old *N. squamipes*, presenting the following characteristics: one male without testicular lobes and two females, one with an atrophied ovary and another with 17 uterine eggs. The animal, from which the male specimen was obtained, was infected with cercariae from *Biomphalaria glabrata* infected with a Brazilian *S. mansoni* strain (CM) isolated from a patient in the municipality of São Lourenço da Mata (Recife, State of Pernambuco). The anomalous females came from another *N. squamipes* infected with cercariae belonging to a strain isolated from *Rattus rattus*, maintained in Brazilian *B. glabrata* and originating from Guadeloupe (Antilles) (GD strain). After conventional perfusion, the worms were fixed in AFA (acetic acid, formaldehyde and alcohol 95%), stained with chloride carmine, cleared in methyl salicylate and assembled on glass slides (Neves et al. 1998). Voucher specimens were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC: 34771a, 34771b, 34772), Rio de Janeiro, Brazil. Digital images were obtained using a brightfield microscope (Olympus, BX 50) linked to image analysis software (Image Pro Plus-version 3.0, Media Cybernetics, USA) and were used for morphometric evaluation of the following measurements:

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area of the suckers and distance between them; area, smaller and larger diameters and perimeter of uterine eggs and egg spine area. From acquired confocal images (LSM-410, Zeiss), using a 543 nm He/Ne laser and a LP 570 filter under reflected mode, the thickness of the tegument under the ventral sucker of the three specimens, the width and length of the ovary and vitelline glands were measured by specific software (version 3.98, Zeiss) pertaining to the equipment.

Normal adult worms were isolated from experimentally infected *N. squamipes* with the same strains under the same conditions, and were used as controls.

### RESULTS

For the male specimen the morphometric values were: 22056  $\mu\text{m}^2$  for the area of the oral sucker, 19831  $\mu\text{m}^2$  for the ventral sucker, 255  $\mu\text{m}$  for the distance between them and 11  $\mu\text{m}$  for the thickness of the tegument. Testicular lobes were not identified, even after virtual tomography under reflected mode by confocal laser scanning microscopy (CLSM). However, the specimen presented other male adult characteristics such as a fully developed tegu-

ment, tubercles with spines and a gynaecophoric canal (Fig. 1). In contrast, the confocal analysis of the corresponding body segment from normal adult males clearly developed testicular lobes and a seminal vesicle (Fig. 2).

In females worms the atrophy of the ovary observed by brightfield microscopy in one specimen was also confirmed by CSLM (Fig. 3), contrasting with the regular ovary of normal females (Fig. 4). The supernumerary uterine eggs seen in another specimen exhibited a miracidium in developmental stage and an egg with a formed shell (Fig. 5). In both female specimens the digestive tube was deformed by large dilatations of the lumen, containing amorphous and vacuolated material (Fig. 6). The comparison between the anomalous females, except for the area of the ventral sucker, showed that the specimen with atrophied ovary showed smaller measurements than the female with supernumerary eggs (Tables I, II).

### DISCUSSION

The actual incidence of dysmorphogenetic defects among *S. mansoni* adult worms is completely unknown. However it has been established that this incidence may

TABLE I

Comparative measurements between anomalous and normal *Schistosoma mansoni* females isolated from *Nectomys squamipes* (GD strain), using brightfield microscopy

	Atrophied ovary	Supranumerary eggs	Normal females <sup>b</sup>
Suckers			
Area oral sucker ( $\mu\text{m}^2$ )	1810.0	2621.0	2190.1
Area ventral sucker ( $\mu\text{m}^2$ )	1911.0	1871.0	2112.4
Distance between suckers ( $\mu\text{m}$ )	89.0	161.0	160.0
Egg <sup>a</sup>			
Area ( $\mu\text{m}^2$ )	-	1923.0	2698.0
Larger diameter ( $\mu\text{m}$ )	-	70.0	97.0
Smaller diameter ( $\mu\text{m}$ )	-	33.7	34.0
Perimeter ( $\mu\text{m}$ )	-	186.3	246.0
Egg spine			
Area ( $\mu\text{m}^2$ )	-	133.3	121.5
Larger diameter ( $\mu\text{m}$ )	-	17.7	17.0
Smaller diameter ( $\mu\text{m}$ )	-	8.7	8.0
Perimeter ( $\mu\text{m}$ )	-	50.3	49.4

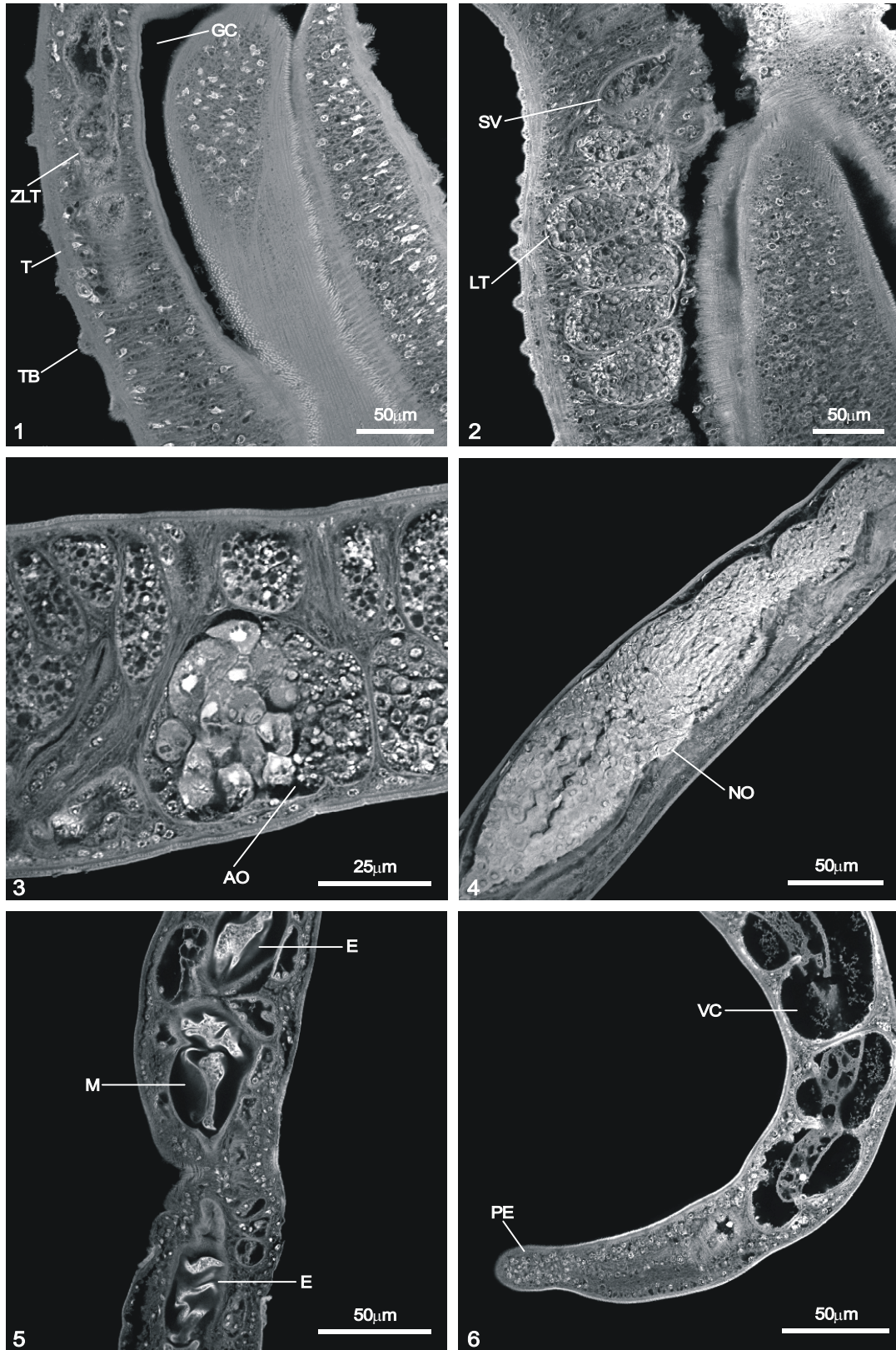
a: the mean of three eggs; - not detected; b: mean (n = 15)

TABLE II

Comparative measurements between anomalous and normal *Schistosoma mansoni* females isolated from *Nectomys squamipes*, using confocal scanning laser microscopy

	Atrophied ovary	Supranumerary eggs	Normal female <sup>b</sup>
Tegument			
Thickness ( $\mu\text{m}$ )	4.9	6.8	4.5
Ovary			
Area ( $\mu\text{m}^2$ )	3313.0	4931.0	25398.0
Length ( $\mu\text{m}$ )	92.9	114.1	306.0
Width ( $\mu\text{m}$ )	48.4	56.9	83.0
Vitelline glands <sup>a</sup>			
Area ( $\mu\text{m}^2$ )	85.4	825.0	483.0
Length ( $\mu\text{m}$ )	14.7	40.6	41.0
Width ( $\mu\text{m}$ )	7.6	21.2	13.0

a: mean of n = 5 (female with atrophied ovary) and n = 8 (female with supernumerary eggs); b: mean (n = 15)



Reproductive system abnormalities in *Schistosoma mansoni* (confocal laser scanning microscopy) - Fig. 1: adult male with anorchism. The mark indicates the zone where the testis use to be. ZLT: zone without testicular lobes; T: tegument; TB: tubercle; GC: gynaeophoric canal. Fig. 2: normal adult male with testis consisting of seven lobes (LT), close to seminal vesicle (SV). Fig. 3: female with atrophied ovary (AO) showing oocytes in different stages of development. Fig. 4: normal adult female with normal ovary (NO). Fig. 5: female with dilated uterus with supranumerary eggs presenting miracidium (M) in maturation process and defined eggshell. Fig. 6: female with dilated intestinal lumen, containing amorphous and vacuolated material (VC). PE: posterior end

vary according to host, environmental/teratogenic factors, including drugs, polygenic disorders, single gene mutations and others (Thorogood 1997).

*S. mansoni* is a dioecious trematode, as a result of evolutive pressure, acquired different and complementary functions where the males became oriented to fecundation (Basch 1990, Platt & Brooks 1997) and female transportation to the site of oviposition, which is facilitated by the complex musculature of the suckers (Mair et al. 2000, Morand & Muller-Graf 2000), while the females invested in the production of eggs (Morand & Muller-Graf 2000). Therefore, any imbalance in this relationship can affect the reproduction of this parasitic flatworm. In female schistosomes, an intimate and permanent association with the male is necessary for reproduction to occur (Kunz 2001).

The finding of atypical adult worms collected from permissive hosts with alterations in the location of the testicular lobes has already been reported (Najim 1951, Machado-Silva et al. 1994, Neves et al. 1998). In the present article, we described a male specimen without testicular lobes (testicular agenesis), not related to immature stage (below 21-days-old). Although sexual maturation without synchronism can occur (Barbosa et al. 1978), in our experiment, the hosts were with nine weeks post infection, time enough for all the specimens of the entire population to reach the adult stage. Furthermore, the tegument of the male with testicular agenesis had tubercles and its thickness was compatible with that of adult stage (Machado-Silva et al. 1994, 1997, 1998, Neves et al. 1998). Finally, it is worthwhile to point out that young specimens (21 days old) already present two testicular lobes (Clegg 1965).

Structural modifications of the female reproductive system can occur during maintenance of the trematode in vitro (Basch 1981) or in unisexual infections (Erasmus 1973). In the present experiment, alterations were evidenced in mixed infections. One of the females of the GD strain presented atrophy in both ovary and vitelline glands. Other components of the reproductive system (oviduct, ootype, uterus) presented normal aspect, resembling those of immature females derived from unisexual infection (Erasmus 1973). After 63 days of infection, it is not expected to find immature females, considering that the immature sexual stages appear only during the morphogenetic period (30-38 days) (Popiel 1986). Conversely, the preservation of the reproductive morphogenesis is determined by the permanent pairing of specimens (LoVerde & Chen 1991, Southgate et al. 1998), which depends on the sex ratio between them. In the present article this ratio was balanced (1:1), which lead us to assume that all specimens were paired, even though it has been demonstrated that males are not monogamous (Pica-Mattocia et al. 2000). According to Kunz (2001), the male signal does not differentiate pluripotent stem cells into germ cells. Instead, it regulates the multiplication and differentiation of germ cells that are already present, located in separate organs and committed to become oocytes or vitellocytes before pairing with a male. Vitelline cells are haploid, suggesting a common origin of the vitelline gland and ovary. In *S. mansoni* cultured in vitro from cercariae to adults, vitelline cells are essentially normal, demon-

strating that specific host influences are not necessary for their development (Basch & Samuelson 1990).

Another female derived from the same strain was also atypical, presenting supernumerary eggs and intestinal dilatation. This situation is rare and confirms a previous report of a specimen with six supernumerary eggs (Machado Filho 1966). Morphological alterations have not been evidenced in the other components of the reproductive system, and the eggs were inside a dilated uterus. This observation, together with the fact that the eggs were mature, with miracidium and configured shell, and the intestinal and uterus lumens were dilated, suggested that the main reason was egg intrauterine retention by unknown obstructive mechanism, rather than egg production and or secretion defects. The intestinal and uterine lumen dilatation may also have been due to impaired or insufficient female musculature. Female reproductive changes could be partially related to intestinal enzymatic deficiencies, since products of hemoglobin digestion by intestinal hemoglobinas are shuttled into the vitellaria, where they serve as egg yolk for the soon-to-develop miracidium (Newport & Agabian 1990).

In our experiment, even considering that the strains are different, they had also been maintained in the laboratory and, moreover, other analyzed specimens did not present alterations similar to those presently described. As the same intermediate and definitive hosts were utilized for both strains, we consider that these strains were under the same selective pressure.

The scientific communication of these types of rare development anomalies or defects, expressing phenotypic variations will undoubtedly help to improve our understanding of the molecular, genetic and cellular mechanisms underlying normal development in parasitic helminthes.

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