DOI: 10.1590/S0037-86822011005000027.

Article/Artigo

Schistosoma mansoni specimens first described by Pirajá da Silva in Brazil (1908) re-examined by confocal laser scanning microscopy

Espécimes de *Schistosoma mansoni* descritos por Pirajá da Silva no Brasil (1908) reexaminados pela técnica de microscopia de varredura a laser confocal

José Roberto Machado-Silva[1], Renata Heisler Neves[2] and Delir Corrêa Gomes[2]

ABSTRACT

Introduction: *Pirajá da Silva* made a seminal contribution to helminthology by demonstrating both schistosome eggs in feces of patients from the State of Bahia and the morphology of *Schistosoma mansoni* adult worms. **Methods:** In this study, a microscopic investigation of the whole-mounts deposited at the Helminthological Collection of the Oswaldo Cruz Institute is presented. Confocal laser scanning microscopy was used as the main investigation technique. **Results:** At the anterior end of the adult male, suckers with well-developed musculature and germinative cells inside the testicular lobes were observed, as well as spines located in the mid region of the male gynecophoric canal. Tegumental tubercles and transversal and longitudinal muscular bundles were observed at the dorsal surface. The female reproductive system presented a uterus lacking eggs and an elongated ovary with germinative cells. The vitellaria were restricted to the extreme posterior end of each female connected to a short vitelline duct. **Conclusions:** The results reported in this study demonstrate that the characteristic studied here are similar to those previously reported, using fresh worms. Moreover, this study also highlights the importance of deposits of specimens in helminthological collections, which further permit revisiting whole-mounts in such institutions.

Keywords: *Schistosoma mansoni. Pirajá da Silva*. Confocal laser scanning microscopy.

RESUMO

Introdução: Pirajá da Silva fez contribuição magnífica à helmintologia ao descrever ovos de *Schistosoma mansoni* nas fezes de um paciente, no Estado da Bahia e a morfologia de vermes adultos. **Métodos:** Neste estudo, apresentamos uma avaliação microscópica das lâminas montadas e depositadas na Coleção Helmintológica do Instituto Oswaldo Cruz. A técnica empregada nesta nova análise foi a microscopia de varredura a laser confocal. **Resultados:** Na parte anterior dos vermes adultos machos, observamos ventosas com musculatura bem desenvolvida e células germinativas dentro dos lobos testiculares. Visualizamos, também, espinhos localizados na região mediana do canal ginecóforo. Na superfície dorsal, encontramos tubérculos e feixes musculares transversais e longitudinais. Em relação ao aparelho reprodutivo feminino, pudemos distinguir um ovo no interior do útero e o ovário alongado com células germinativas. As glândulas vitelínicas estavam restritas à parte posterior das fêmeas conectadas por um ducto vitelínico curto. **Conclusões:** As características morfológicas são similares as estudadas anteriormente por Pirajá da Silva com vermes frescos. Além disso, este estudo demonstra a importância de se depositar espécimes nas coleções helmintológicas abrindo possibilidade de novos estudos com estas lâminas.

Palavras-chaves: *Schistosoma mansoni*. Pirajá da Silva. Microscopia de varredura a laser confocal.

1. Laboratório Romero Lascasas Porto, Departamento de Microbiologia, Imunologia e Parasitologia, Faculdade de Ciências Médicas, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ. 2. Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Rio de Janeiro, RJ.

Address to: Dr. José Roberto Machado-Silva. Lab. Helmintologia Romero Lascasas Porto/DMIP/FCM/Centro Biomédico/UERJ. Rua Prof. Manoel de Abreu 444/5º andar, 20511-070 Rio de Janeiro, RJ, Brasil.
Phone: 55 21 2868-8048
e-mail: machado@uerj.br

Received in 25/10/2010
Accepted in 02/12/2010

INTRODUCTION

The 18th and 19th centuries were very productive periods for the life sciences, during which new staining techniques were developed[1]. Hill first used carmine as a dye for microscopic studies three centuries ago. In the following century, Ehrenberg used carmine to examine microorganisms[2]. In addition, important technical achievements of the microscope were developed in 1830 and the late 1800s, culminating in the improvement of microscope resolution[3]. Taxonomic studies in helminthology were likely feasible, due to both routine light microscopy and available dies. At the same time, this was the principal period for classification in Europe[4].

The first decades of the 20th century brought great advances in emerging Brazilian medical and public health science[5]. Over a 100 years ago, Pirajá da Silva provided a notable contribution to helminthology, describing the presence of schistosome eggs in feces of patients from the State of Bahia[6,7]. He also provided a detailed description of the morphology of *Schistosoma mansoni* adult worms[8].

Since its discovery, *Schistosoma mansoni* has been the subject of intense investigation, describing the features of adult worm based on light microscopy[9-13]. With the advances in microscopy technology, confocal laser scanning microscopy has become an invaluable tool for helminthology, mainly for schistosomiasis mansoni studies[14-20]. The present article revisited the *S. mansoni* specimens first described by Pirajá da Silva in Brazil, using confocal laser scanning microscopy.

METHODS

Whole-mounts stained with carmine and mounted with canadium balsam were deposited at the Helminthological Collection of the Oswaldo Cruz Institute (*Coleção Helmintológica do Instituto Oswaldo Cruz*, CHIOC; no. 780), Rio de Janeiro,

RJ, Brazil (**Figure 1**). The images were captured by a camera lucida (Axioplan Axiophoto) attached to a Zeiss light microscope MC100 SPOT. Two adult specimens of each sex were analyzed by confocal laser scanning microscopy (LSM 510- ZETA Zeiss, laser confocal microscopy, Germany), using reflected mode with a 543nm He/Ne laser and LP 570 filter. The overall morphology of the adult worms (suckers and tegument) and reproductive organs were focused[21].

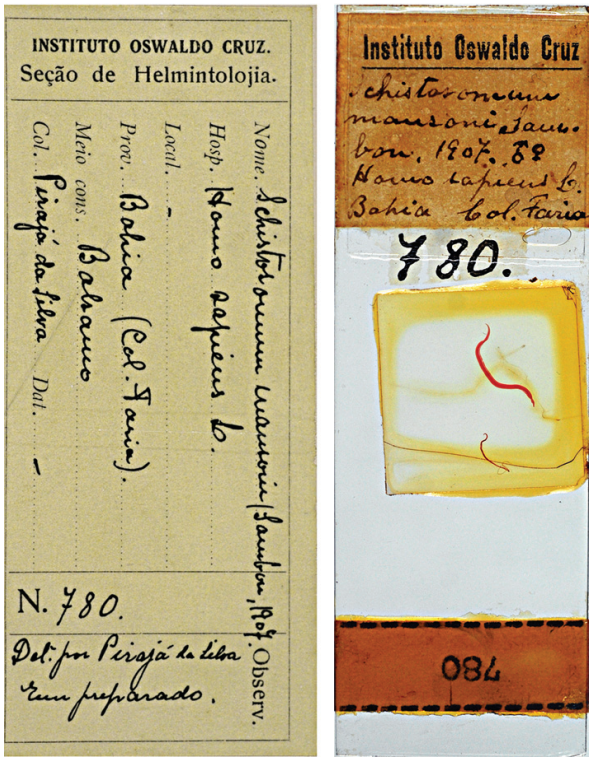

FIGURE 1. Card label and whole-mount slide with male and female adult worms of *Schistosoma mansoni* stained with carmine and mounted with canadium balsam deposited at the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) (number 780).

RESULTS

The confocal images revealed well-developed muscular suckers, seven testicular lobes filled with germinative cells. There were spines in the mid region of the male gynecophoric canal. Tegumental tubercles and transversal and longitudinal muscular bundles were observed at the dorsal surface. Within the female reproductive system, a uterus lacking eggs and an elongated ovary could be distinguished at the anterior part of the body of the worm. The vitelline gland (vitellaria) was restricted to the extreme posterior end of each female joined by a short vitelline duct (**Figure 2**).

DISCUSSION

Nowadays, microscopic examination of stools is a practical method for confirming the presence of worms in the intestine of vertebrate hosts[22]. Even though this observation was not intended to be a diagnostic tool, helminth eggs collected from intestinal contents were first reported 160 years ago[22]. The presence of foreign bodies with helminth eggs was not mentioned nor identified by the authors due to limitations in know-how at that time[22].

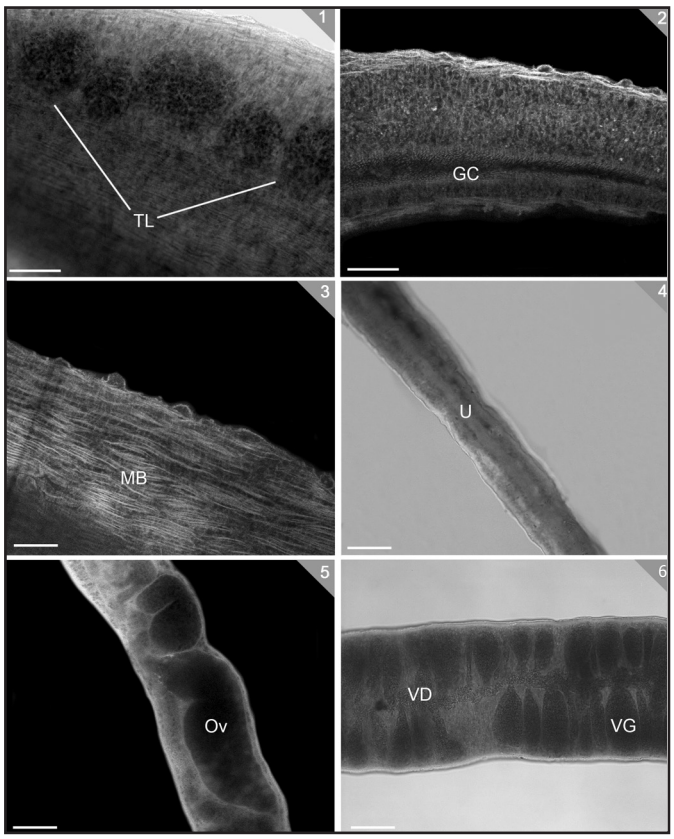

FIGURE 2 - Confocal images of male and female adult worms of *Schistosoma mansoni* stained with carmine and mounted with canadium balsam.

1. TL: testicular lobes, 2. GC: gynecophoric canal, 3. MB: muscular bundle and dorsal surface with tubercles, 4. U: uterus, 5. Ov: ovary, 6. VD: vitelline duct, VG: vitelline gland.

During the course of autopsies on patients with hematuria, Bilharz detected some white worms in the blood of the portal vein, with flat bodies and sexual differentiation, which he immediately recognized as something new[6]. He later described and named them *Distomum haematobium*, which was later reclassified as *Schistosoma haematobium* based on the presence of the gynecophoric canal in the male worm[7]. Moreover, Bilharz reported eggs with a terminal spine[23].

A hundred years ago, Pirajá da Silva detected schistosomes eggs (lateral spine) during microscopic examination of stools from 20 individuals. He also explained that no eggs were detected in the urine[6]. Thus, Pirajá da Silva discovered schistosomiasis in Brazil, which was already suspected to occur due to migration of slaves from Africa to Brazil. At that time, new ideas concerning parasite etiology were in agreement with the development in understanding regarding disease causation[5]. It also represents a period of great advances in the emerging Brazilian medical and public health science[5].

From the zoological standpoint, the discovery was in line with the major period of classification in Europe[4]. Although the discovery of new species is the highest goal that can be achieved among those interested in taxonomy, the contribution of Pirajá da Silva was not recognized by other studies[6]. A plausible explanation is that research from Europe did not value the contributions derived from researchers living in third world countries[6]. It is worthwhile mentioning, however, that Brumpt recognized the discovery and published a copy of a *Schistosoma mansoni* female depicting the uterus with an egg showing a lateral spine[24].

From today's point of view, it is noteworthy that both carmine and light microscopy led to a distinguished history in helminthology. Furthermore, carmine staining renders the samples fluorescent and thus makes it feasible to examine parasites by optical sectioning with the confocal microscope[14,15,19]. In addition, specimens deposited within helminthological collections permit further revisiting studies that may lead to new morphological descriptions[25]. In more recent history, both light microscopy and confocal laser scanning microscopy experienced advances that have been used to develop further studies regarding the morphology of adult worms of *S. mansoni*[9,10,11,21]. In this study, specimens of *S. mansoni* deposited in the Helminthological Collection of the Oswaldo Cruz Institute were re-examined. This approach identified male worms with several testicular lobes, while the females showed an ovary in the anterior end of the body, a uterus and vitelline glands in the two posterior thirds of the body, as published previously[26,27]. More important in this context is the fact that the internal morphological structures remained preserved. The state of preservation of the specimens determined the accuracy of the current descriptions, indicating that other characteristics were visible. Confocal images showed well-developed muscular suckers, germinative cells within the testicular lobes, spines in the mid region of the gynecophoric canal and tegumental tubercles. A vitelline gland (vitellaria) joined by a short vitelline duct was also observed. It is worth mentioning that these characteristics have been described during the examination of freshly collected worms[15,16,19-21].

To the best of our knowledge, this the first time that a historic breakthrough in helminthology has been re-examined using confocal microscopy. Unlike other studies[28], the proposal was not meant to be a taxonomic study on specimens mounted in permanent preparations. Given the results, we suggest that further morphological and molecular studies should be performed on material deposited in helminthological collections.

ACKNOWLEDGMENTS

The authors are grateful to the curator of the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) for his cooperation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

The Instituto Oswaldo Cruz/FIOCRUZ, the Universidade do Estado do Rio de Janeiro, the Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro and the Conselho Nacional de Desenvolvimento Científico e Tecnológico all supported this work.

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