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SCHISTOSOMA MANSONI SAMBON, 1907: COMPARATIVE MORPHOLOGICAL STUDIES OF SOME BRAZILIAN STRAINS

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SUMMARY

The morphology of *Schistosoma mansoni* adult male worms from three strains which have been maintained in albino mice for several generations, was compared to a strain that has been isolated from the natural host *Nectomys squamipes* (Rodentia:Muridae) captured in Sumidouro (Rio de Janeiro State) and have been maintained in the same sylvatic rodent under laboratory conditions. Total length of specimens, distance between suckers, the number of testes and extention of testes grouping were the taxonomic characters analysed. The worms recovered from *N. squamipes* showed expressive differences ($p < 0.01$) compared to the other strains regarding the considered morphological characters. The strains that were maintained in mice presented statistical differences ($p < 0.01$) in several characters. Some adult worms besides the normal position of the testes also showed an atypical arrangement of these glands. It can be concluded that the morphology of adult worms may be used to distinguish *S. mansoni* strains and that morphological changes in adult worms are not induced by successive inoculations of a strain in mice.

KEYWORDS: *Schistosoma mansoni*: Morphometrics; *Nectomys squamipes*: albino mouse.

INTRODUCTION

It is believed that both man and *Schistosoma* species had appeared in Africa and their relationship had since started[3]. *Schistosoma mansoni* is native from regions of western Africa and has reached Brazil through the trade of slaves two centuries ago[22] and this recent introduction was ratified on basis of molecular comparative studies of African and Brazilian strains[4]. The adaptation of the different *Biomphalaria* species, the expansion of the agricultural frontiers with the spreading of the infection which followed the adjustment of the schistosomes to different species of *Biomphalaria* had contributed to the natural selection of biological populations of the parasite. Actually the process is taking place, although now is due to the presence of wild rodents that act as natural hosts of *S. mansoni*[20,23,28].

The diversity of the clinical forms of schistosomiasis seems to be due to different biological populations of *S. mansoni*, although this concept has not been fully accepted[9]. Studies of the parasite/invertebrate host relation-

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TABLE 1
Source of strains and hosts maintained in laboratory

STRAINS	ISOLATION HOST/DATE	LABORATORY VERTEBRATE HOST	MAINTENANCE INVERTEBRATE HOST
BH-Belo Horizonte, MG	*Biomphalaria glabrata*-1985	*Mus musculus* swiss albino	*B. glabrata*
SJ-São José dos Campos, SP	*B.tenagophila* 1963	*Mus musculus* swiss albino	*B. tenagophila*
CMO-Ceará Mirim-RN	*Oryzomys subflavus** 1979	*Mus musculus* swiss albino	*B. glabrata*
SNN-Sumidouro, RJ	*Nectomys squamipes*-1987	*Nectomys squamipes*	*B. glabrata*

* wild rodent: Cricetidae

ships[16,18] added further data on this association and on the biological characteristics of *S. mansoni* in Brazil[5,8,13,18] leading to the conclusion that this helminth showed intra-specific variations and, thus, could not be considered as an uniform species. Besides, these strains were morphologically diagnosed on basis on taxonomic parameters, as the total length of the body and the number of testes in the adult male worm. Comparative morphometric studies of BH (Belo Horizonte, Minas Gerais) and SJ (São José dos Campos, São Paulo) showed that the worms from BH strain are longer and have a greater number of testes[12,19]. Male worms from an isolated strain of sylvatic rodent (*N. squamipes*), are significantly bigger than others derived from a sympatric human strain, both maintained in mice[11].

In order to evaluate host-induced morphological changes and to determine morphological differences in *S. mansoni* adult male worms, a comparative study of the morphology of Brazilian allopatric geographic strains, which were maintained through sucessive inoculations in albino mice with another maintained in *N. squamipes* was undertaken.

MATERIAL AND METHODS

The origin, date of isolation and maintenance conditions of the studies strains are expressed in table 1. BH, SJ and CMO strains from Belo Horizonte (Minas Gerais State), São José dos Campos (São Paulo State) and Ceará-Mirim (Rio Grande do Norte State), respectively were maintained in the Departamento de Malacologia do Instituto Oswaldo Cruz - FIOCRUZ and SNN strain was isolated from an experimentally infected *N. squamipes* and maintained in the Departamento de Patologia & Laboratórios, Faculdade de Ciências Médicas - UERJ. Studied worms resulted from the first inoculation of this strain in laboratory conditions.

Worm isolation:

For each strain (BH, SJ, CMO) ten albino mice, percutaneously infected with 80 cercariae each, were used. Four specimens of *N. squamipes* were infected with 500 cercariae each of the SNN strain. Seven weeks after exposure infecting larvae, all infected mice and *N. squamipes* were sacrificed either by cervical displacement (mice) or in an ether chamber (*N. squamipes*) and perfused[28,29].

Morphological analysis:

Processed as reported elsewhere[11]. For examination through a digital analyser or light microscopy, 50-70 male worms were studied.

Statistical analysis:

The Mann-Whitney test was applied, considering as significative differences values of $p \leq 0.05$[27].

RESULTS

The morphometric data regarding the studied strains are shown in Table 2. SNN strain presented, for the

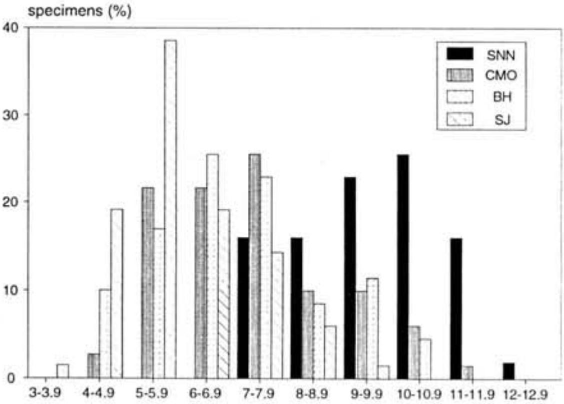

Fig. 1 - Comparative frequency (%) of the analysed morphological characters in the strains: total length (mm).

analysed characters, higher values than the others. With relation to the total length of worm of this strain, all differences were significative ($p<0.01$) in any tested combination (Table 2). Comparative analysis with other strains also showed statistic differences, except in BH x SJ. It was verified that all strains presented a higher frequency of specimens occurring in different ranges (Fig. 1).

When comparing the number of testes the differences were significative ($p<0.01$) in several combinations, except for SNN x BH and CMO x SJ (Table 3). The concentration of a greater number of specimens in a determined range was also coincidental with the number of testes in the worms of the different strains except for CMO (Fig. 2). SNN and BH strains are situated in the same range of 8 testes and SJ in that of 6. Most of the specimens, about 90%, from SNN, BH and SJ strains had 6-8 testes. An atypical localization of the testes was observed in SJ (8.8%), SNN (8.0%) and BH (4.4%) strains, but not in CMO.

The values regarding the distance between the suckers were significantly different ($p<0.01$) in any comparison of the strains, except for BH x SJ (Table 3). Only in the CMO strain a higher frequency of worms occurred in a same range of length (Fig. 3). All the strains presented significative differences ($p<0.01$) concerning the extention occupied by testes (Table 3). Only SJ strain had specimens in all ranges of length (Fig.4).

DISCUSSION

It was verified that adult male worms of Brazilian strains of *S. mansoni* show morphological differences among themselves (Table 2) and infra-specific variations occur in the specimens even when they are age matched (Figs. 1,2,3,4). Conversely, it was shown that the worms do not have their morphological characteristics changed even after sucessive inoculations in albino mice for several generations.

Some questions can be raised for the interpretation of our data:

1- Trematodes that are maintained in different hosts show differences in their growth pattern[34], possibly due to the phenotypic plasticity as occurs in plants[26]:

Two criteria are used to define the susceptibility of a host to the *S. mansoni* infection: the ability of adult worms in reaching the complete somatic development

TABLE 2

Morphometric data (X and SE) on the proposed morphological characters for each strains

STRAINS	CHARACTERS			
	1	2	3	4
	$\overline{X}$ SE	$\overline{X}$ SE	$\overline{X}$ SE	$\overline{X}$ SE
SNN	9.5±1.3	7.6±1.0	0.36±0.07	0.29±0.1
BH	6.9±1.5	7.7±0.9	0.30±0.06	0.23±0.08
CMO	7.2±1.5	6.4±1.4	0.20±0.08	0.18±0.07
SJ	5.9±1.2	6.8±0.8	0.27±0.08	0.22±0.09

1 - Total length (mm)
2 - Testes number
3 - Extention testes grouping (mm)
4 - Distance between suckers (mm)

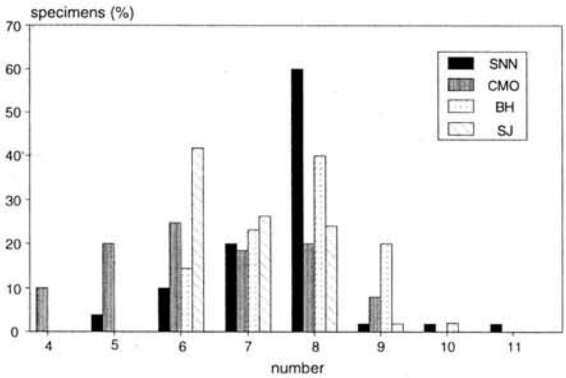

Fig. 2 - Comparative frequency (%) of the analysed morphological characters in the strains: testes number.

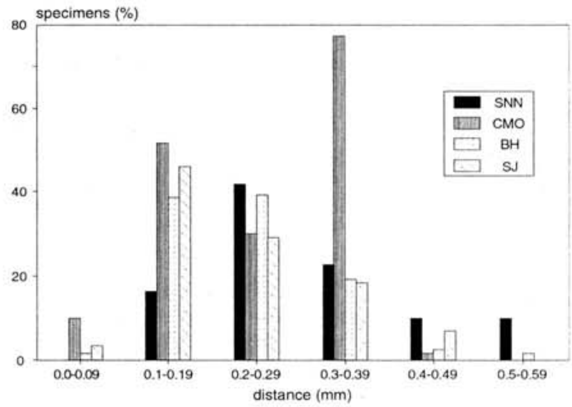

Fig. 3 - Comparative frequency (%) of the analysed morphological characters in the strains: distance between suckers (mm).

and their efficacy to eliminate eggs in feces[31]. These authors infected several mammal species (mice, hamsters, guinea pigs, albino rats, rabbits, among others) and verified that these animals presented different degrees of susceptibility. In rabbits, adult worms are bigger, nevertheless the eggs do not pass with the feces.

Adult worms recovered from a permissive host (mouse) are bigger than those maintained in non-permissive animals (albino rat) where they become atrophied. But this situation is modified when the worms are transferred to a permissive host through surgery[1]. Conversely, male specimens have their length drastically reduced when the strain circulating in a natural host (*N. squamipes, Rattus rattus*) and inoculated in a mouse[11] or in the laboratory albino rat[7]. Host-induced morphological changes are also present in the reproductive system of the adult specimens, as for example the variation in the number of testes in specimens from the same strains depending on the fact they were isolated either from a mouse or a hamster[25], *N. squamipes* or albino mouse[11].

All the studied characters were different in the comparison between SNN and CMO strains (Table 3) suggesting mouse-induced morphological alterations in this last strain because it was isolated from a sylvatic rodent (*O. subflavus*).

TABLE 3
Statistical comparison of strains for each morphological character

STRAINS	CHARACTERS			
	1	2	3	4
SNN x BH	p<0.01	NS	p<0.01	p<0.01
SNN x CMO	p<0.01	p<0.01	p<0.01	p<0.01
SNN x SJ	p<0.01	p<0.01	p<0.01	p<0.01
BH x CMO	NS	p<0.01	p<0.01	p<0.01
BH x SJ	p<0.01	p<0.01	NS	p<0.01
SJ x CMO	p<0.01	NS	p<0.01	p<0.01

1 - Total length
2 - Testes number
3 - Distance between suckers
4 - Extention testes grouping
notes: p<0.01 + significative difference
NS = not significative difference

The morphometric data referring to the worms of the SNN strain (isolated from an experimentally infected *N. squamipes*) confirm that adult worms find perfect conditions for their somatic development in their sylvatic host (Table 2). In this host, although adult worms are smaller than those found in the rabbits[31], there is a great amount of eggs in the feces[28]. Total length of the specimen has proved to be an important taxonomic character acting as a marker in epidemiological surveys in areas where humans and/or wild rodents are supposed to participate in the transmission of schistosomiasis mansoni[23], suggesting that this criterion should be adopted for the characterization of the strains in these area.

Under laboratory conditions, *S. mansoni* strains undergo an intense selection process, because they are maintained in an artificial host (mouse), differing from natural hosts, humans and/or rodents[23,28]. Sucessive inoculations in mice may cause modification in the genetic structure of the strains, expressed by enzymatic alterations[8,12] and morphological changes in the adult worms[32]. However, the present data do not confirm this last theory, as the values from BH and SJ strains agreed with others previously reported[12,19].

2 - The intra-specific variations of *S. mansoni*[15] may be characterized by morphological studies[19]:

The phenotypic characteristics of adult worms from BH, SJ and CMO strains were different, and sometimes significative differences were not related to more than a single taxonomic character (Table 3). Previous observations considering the same taxonomic characters showed that sympatric strains have less distinctive morphological differences among themselves[11] indicating that morphological similarities or differences also depend on the

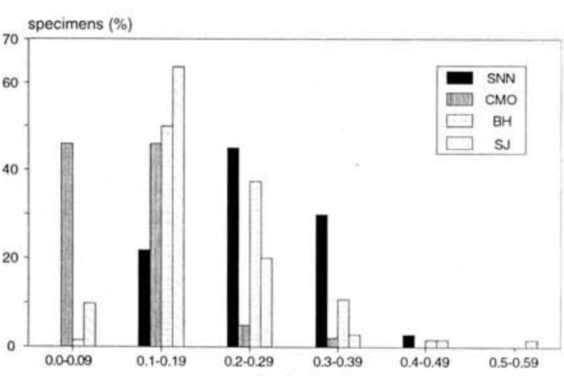

Fig. 4 - Comparative frequency (%) of the analysed morphological characters in the strains: extension of testes grouping (mm).

proximity or diversity of the strains origin. This diversity can be mentioned in order to explain the significative differences among between BH, SJ and CMO strains (Table 3) and those from Sumidouro which were maintained in albino mice[11]. In this last comparison, the differences may be due to a greater spacial geographic distance between them, considering that the increase of diversity is proportional to the distance that separate populations (isolation-by-distance effect)[21].

The study of the spacial distribution of testes (extension of testes grouping) revealed two facts:

1- all the strains showed statistical differences among themselves (Table 3) compared to previous results with other strains[11].

2- testes atypically located, as referred for African and Puerto Rico strains[2,4,24,30] and those isolated from wild vertebrates in Brazil[11,33]. This anomalous localization of testes in Brazilian *S. mansoni* strains maintained routinely in laboratory for several generations, is mentioned for the first time, and the origin of this fact is unknown.

The distance between suckers of the adult worm has not been considered as a valuable criterion for the morphological characterization of strains. Nevertheless, the values obtained for this character (Fig. 4) showed that the strains differed significantly except for BH x SJ (Table 3), in agreement with previous reports[12]. Nevertheless, based on the same criterion it can be demonstrated that there are statistical differences between an isolated strain from a naturally infected *N. squamipes* captured in Sumidoro and other from a sympatric human strain[11]. The results so far obtained allow us to conclude that:

1 - the adult worms recovered from a natural host are bigger than those in mice (fenotipic plasticity).

2 - intra-specific varieties are observed during morphological studies of male adult worms.

3 - sucessive inoculations of a strain in mice for several generations do not induce morphological changes in adult worms and even in strains maintained in laboratory for several generations, adult worms also present testes atypically located.

RESUMO

Schistosoma mansoni Sambon, 1907: estudos comparativos da morfologia de algumas cepas brasileiras.

A morfologia de vermes adultos machos de tres cepas de *Schistosoma mansoni*, mantidas por várias gerações em camundongos albinos, foi comparada com uma cepa isolada do hospedeiro natural *Nectomys squamipes* (Rodentia:Muridae) e mantida, em laboratório, neste mesmo roedor silvestre. Como caracteres taxonômicos foram analisados o comprimento total, o numero de testículos, a distância entre as ventosas e a distância ocupada pelos testículos nos espécimes. Os vermes recuperados de *N. squamipes* apresentaram diferenças significativas ($p<0,01$) em relação às outras cepas para quaisquer caracteres morfológicos estudados. As cepas mantidas em camundongos apresentaram diferença estatística em vários caracteres ($p<0,01$). Alguns vermes adultos além da disposição normal dos testículos, apresentavam também uma localização atípica destas glândulas sexuais. Conclui-se que a morfologia dos vermes adultos pode ser utilizada para caracterizar cepas de *S. mansoni* e que as passagens sucessivas de uma cepa em camundongos não induzem a alterações morfológicas nos vermes adultos.

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