Differences in Brazilian Strains of *Schistosoma mansoni* Evaluated by Means of Morphometric Analysis of Cercariae of Both Sexes

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Morphometrics of Brazilian strains (BH, SJ and CMO) of Schistosoma mansoni *cercariae were obtained with a computerized image analyzer (IMAGE PRO PLUS, MEDIA CYBERNETICS), considering the following characters: body area, tail, furcae, oral and ventral suckers and distance between them. For statistical analysis, the variance test (one-way Anova) was applied and significant differences of* $p < 0.05$ *were considered. All morphometric values in the BH strain were significantly higher (*$p < 0.05$*) than in the others. Lower values were obtained in females of SJ strain for all characters, excepting the body area. Only this character showed to be significantly different in males and females of the three strains. Specimens of both sexes in the BH and SJ strains showed significant differences regarding all characters. It was observed that this morphometric analysis permits the characterization of strains and also the sex identification in* S. mansoni *cercariae. Due to its feasibility, this method can be applied as a tool in laboratories devoid of more complex equipment.*

Key words: *Schistosoma mansoni* - cercariae - strains - morphometrics

Based on the phenotypic characteristics of adult worms of both sexes, it was demonstrated that the *Schistosoma mansoni* Sambon, 1907 strains present morphometric differences (Saoud 1966, Coles & Thurston 1970, Magalhães & Carvalho 1973, Paraense & Corrêa 1981, Soliman et al. 1984, Machado-Silva et al. 1994, 1995, Neves et al. 1998). Moreover, *S. mansoni* strains can be identified by the chetotaxic study of the sensorial argentophilic papillae of cercariae (Bayssade-Dufour 1977, 1979, Cassone et al. 1979, Pino et al. 1988, 1997). Nevertheless, there are few reports on the morphometric analysis of non-sensorial structures in the cercariae aiming at taxonomic approaches (Zanotti-Magalhães et al. 1993). In this paper, data on male and female cercariae of different strains of *S. mansoni* are presented and discussed, since these larval forms show morphometric differences that also permit the identification of strains.

MATERIALS AND METHODS

Strains - All strains (BH – Belo Horizonte, Minas Gerais; SJ – São José dos Campos, São Paulo; CMO – Ceará-Mirim, Rio Grande do Norte) are from Brazil and their maintenance under laboratory conditions was reported elsewhere (Machado-Silva et al. 1995).

Obtainment of single-sex cercariae - Miracidia were isolated from faeces of Swiss Webster (SW) albino mice experimentally infected with *S. mansoni* cercariae from each strain. One hundred and ninety-one specimens *of Biomphalaria glabrata* Say, 1819 and the same number of specimens of *B. tenagophila* Orbigny, 1835, measuring 2-4 mm in diameter, were infected with one miracidium each, of the referred strains. All infections were sympatric. Thirty-five days post-infection, snails were individually exposed to artificial light and those positive for cercariae were isolated. Shed cercariae from each snail were utilized in the infection of albino mice and for staining procedures.

The authors dedicated this paper in honor of the Instituto Oswaldo Cruz, on the occasion of the centenary of its foundation, May 25th 1900.

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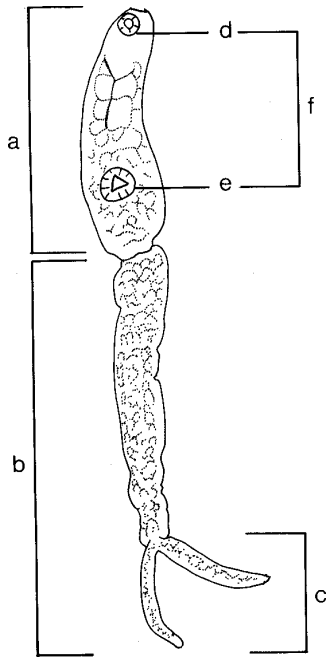
Two 7-day-old SW mice (*Mus musculus* Linnaeus, 1758) were percutaneously infected, with 50 cercariae each, shed by two positive snails/strain, respectively. Remaining cercariae from each snail were preserved for study.

Infected animals were sacrificed 30 days later in an ether chamber. Adult worms (males or females) were recovered from the portal and mesenteric venous system in Petri dishes containing a 0.85% NaCl solution. Sex of worms was confirmed under a stereoscope microscope and specimens were associated to the source of infecting cercariae. Samples of *S. mansoni* cercariae of the BH strain were previously identified regarding the sex and were supplied by the staff of the Department of Malacology, Oswaldo Cruz Institute.

Preparation of cercariae - Cercariae were fixed (10% formaldehyde solution), stained (carmine + ethanol 70º GL), dehydrated (ethanol 70º-100º GL) and clarified (phenol). Each step was performed using conical tubes with the cercarial solution, centrifuged at 1,000 g for 5 min. Whole mounts were obtained with two drops of the sedimented stained and clarified cercariae on slides under coverslip and preserved in a 1:1 Canada balsam and beechwood creosote solution.

Morphometric analysis of cercariae - A computerized image analyzer system (IMAGE PRO PLUS, MEDIA CYBERNETICS) was utilized. Areas of body (a), tail (b), furcae (c), oral (d) and ventral (e) suckers and the distance between them (f) were investigated (Figure). Fifty to 54 cercariae of each sex/strain were analyzed. Measurements were in mm^2, except for the shorter distance between suckers (mm).

Statistical analysis - For statistical analysis, the variance test (one-way Anova) was applied, and significant differences of $p < 0.05$ were considered.



Cercariae of *Schistosoma mansoni*. Investigated morphometric parameters. Areas - a: body; b: tail; c: furcae; d: oral sucker; e: ventral sucker; f: distance between suckers

RESULTS

Sixteen snails (8.3%) were positive for *S. mansoni* cercariae of the CMO strain and 12 (6.2%) for the SJ. Groups of snails infected with miracidia of either the CMO or the SJ strains, were those that presented more specimens shedding male cercariae: 10 in the CMO and 7 in the SJ.

After comparison of strains, it was observed that all morphometric values were higher for males of the BH strain. Males of CMO strain showed higher values than those of SJ, except for the distance between suckers that was the same for both strains (Table I).

By means of statistical analysis of these data it was verified that the strains BH and SJ presented significant differences ($p<0.05$) when compared to one another, regarding all characters, whereas in the comparison of BH with CMO, differences were not observed in the oral sucker of both sexes and in the ventral sucker of females (Table II). Comparison of male specimens of CMO and SJ strains showed that there are no significant differences in morphometric values related to tail, furcae and ventral sucker areas and to the distance between suckers. Females of the BH strain also presented the highest morphometric values, except for the area of suckers that increased in the CMO strain (Table I).

Excluding the body area, lower values were those obtained with females of the SJ strain. Generally, females presented greater dimensions than those of the males; nevertheless, this did not occur for body area, furcae and oral sucker (CMO strain), furcal area (SJ strain) and ventral sucker (BH and SJ strains) (Table I).

All female characters showed significant differences ($p<0.05$) when comparing BH x SJ and SJ x CMO strains. Nevertheless, between BH and CMO strains these differences were not observed in the areas of the suckers (Table II). Males and females of the BH strain presented significant differences ($p<0.05$) related to all characters (Table III).

DISCUSSION

Traditionally, sex identification in cercariae has been achieved by means of their innoculation in laboratory animals and further recovery of adult worms 3-4 weeks later; nevertheless, even with this

TABLE I

Morphometric data, means and standard deviation (sd) of the characters in BH (Belo Horizonte, MG), CMO (Ceará-Mirim, RN) and SJ (São José dos Campos, SP) *Schistosoma mansoni* cercariae strains

Characters	Strains					
	BH		CMO		SJ	
	Mean	sd	Mean	sd	Mean	sd
Body area						
Females	1.24	0.28	0.49	0.10	0.54	0.17
Males	1.04	0.33	0.54	0.14	0.52	0.14
Caudal area						
Females	8.99	1.29	5.49	0.59	3.41	0.70
Males	7.40	0.63	4.96	0.60	3.90	0.97
Furcae area						
Females	7.85	1.23	3.16	0.68	2.27	0.50
Males	5.85	1.08	3.25	0.61	2.91	0.86
Oral sucker area						
Females	0.41	0.13	0.47	0.16	0.28	0.09
Males	0.61	0.24	0.49	0.09	0.31	0.10
Ventral sucker area						
Females	0.29	0.08	0.31	0.11	0.19	0.05
Males	0.38	0.10	0.30	0.07	0.20	0.06
Shorter distance between suckers						
Females	0.07	0.01	0.05	0.01	0.04	0.01
Males	0.06	0.02	0.04	0.01	0.04	0.02

TABLE II

Statistical analysis (variance test: one-way Anova) of intraspecific variances in the comparison of male and female *Schistosoma mansoni* cercariae of different strains

Characters	Strains					
	BH x CMO		BH x SJ		SJ x CMO	
	m	f	m	f	m	f
Body area	S	S	S	S	S	S
Caudal area	S	S	S	S	N	S
Furcae area	S	S	S	S	N	S
Oral sucker area	N	N	S	S	S	S
Ventral sucker area	S	N	S	S	N	S
Shorter distance between suckers	S	S	S	S	N	S

S: significant; N: not significant; m: male; f: female; $p<0.05$; BH: Belo Horizonte, MG; CMO: Ceará-Mirim, RN; SJ: São José dos Campos, SP

TABLE III

Statistical analysis (variance test: one-way Anova) of intraspecific variances in male and female *Schistosoma mansoni* cercariae of Belo Horizonte, MG (BH), Ceará-Mirim, RN (CMO) and São José dos Campos, SP (SJ) strains

	Characters					
	Body area	Caudal area	Furcae area	Oral sucker area	Ventral sucker area	Shorter distance between suckers
Strains						
BH	S	S	S	S	S	S
CMO	S	N	S	N	N	N
SJ	N	S	N	S	N	N

S: significant; N: not significant; $p<0.05$

methodology, sex of adult worms is not always identified, for according to previous experiments, even during this interval, some worms do not reach sexual maturity (Paraense & Malheiros-Santos 1949).

Taking into account the asynchronism regarding maturation (Barbosa et al. 1978) worms may not show, after 3-4 weeks reliable morphological differences to induce sex identification.

Moreover, this procedure is slow, expensive and should not be applied in situations that require a fast and reliable sexual identification of larval *S. mansoni*. Although with the employment of molecular biology techniques this identification is easily obtained (Barral et al. 1993, Mc Cutchan et al. 1994), their access may be restricted, due to the necessity of a special equipment (Pino et al. 1988).

Few are the data on morphological analysis aiming at sex identification in *S. mansoni* cercariae. Pino et al. (1988, 1997) verified that this can be achieved by means of chetotaxic studies.

During the present investigation it was observed that morphometric differences in larval specimens have similarities with those referred for adult worms. Some reports indicate that the BH strain shows bigger dimensions than the SJ (Magalhães & Carvalho 1973, Paraense & Corrêa 1981, Zannoti-Magalhães et al. 1993, Machado-Silva et al. 1995); this same situation occurs in the comparison between CMO and SJ strains (Machado-Silva et al. 1995). The present findings demonstrate that significant differences are not restricted to the dimensions of the body and tail as previously referred (Zanotti-Magalhães et al. 1993), but are also observed when other characters are analyzed (Table I).

Taking this into account, the present approach aims at the establishment of a simple, practical and reliable method, based on morphological evaluation, to properly identify the sex and the Brazilian strains of *S. mansoni* cercariae, just after their shedding from infected snails.

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