Host-induced Morphological Changes of *Schistosoma mansoni* Sambon, 1907 Male Worms

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In order to evaluate the permissiveness of Nectomys squamipes *to* Schistosoma mansoni *and the influence of the albino mice on the morphological aspects of adult worms derived from a population isolated from* N. squamipes, *the morphology of adult* S. mansoni *Sambon, 1907 male worms was studied using a digital image analyser (MOP VIDEOPLAN) and light microscopy. Their sources were as follows: (1) recovered from the wild rodent* N. squamipes *Brants naturally infected from Sumidouro, RJ, Brazil; (2) recovered from albino mice experimentally infected with the strain derived from N. squamipes; (3) recovered after the isolation of a strain derived from aboriginal human infections in Sumidouro. Worms recovered from* N. squamipes *(group 1) showed body length and distance between suckers significantly bigger than those of the specimens maintained in mice (groups 2 and 3). The number of testes in group 1 was statistically less than that of groups 2 and 3. Group 2 strains which were maintained in mice, presented the length of the worms as the only significant different character. Data show that: (1)* N. squamipes *is a more suitable host for the development of* S. mansoni *when compared to the albino mice; (2) a strain of* S. mansoni *isolated from a natural host undergoes morphological changes after its passage in the white mouse.*

Key words: *Schistosoma mansoni* - morphology - strains - *Nectomys squamipes* - sw mice

Schistosoma mansoni Sambon, 1907 develops to its adult form in several rodents: mouse, hamster and albino rat (Stirewalt et al. 1951, Warren & Peters 1967). In some endemic areas for schistosomiasis mansoni, species of wild rodents have been found naturally infected and under laboratory conditions these hosts properly reproduce the schistosome infection (Bastos et al. 1984, Campos et al. 1984, Machado e Silva et al. 1991, Souza et al. 1992, Picot 1992). This situation has been recently reviewed (Mello 1991, Rey 1992).

The host has a strong influence on the phenotype of *S. mansoni* adult worms (Senft et al. 1978) and in permissive hosts such as *Mus musculus* and *Cricetus auratus*, the worms reach full maturation (Cioli et al. 1977). Conversely, in a non-permissive host the worms are atrophied (Jourdane & Imbert-Establet 1980). The reproductive organs of adult male worms vary according to the host. In a same sample of *S. mansoni* differences in the number of testes were observed even though the specimens were recovered from either mice or hamsters (Saoud 1966).

In this paper the permissiveness of *N. squamipes* to *S. mansoni* and the influence of the albino mice on the morphological aspects of adult worms, derived from a population isolated from *N. squamipes*, were evaluated. Data on a sympatric strain of *S. mansoni* isolated from human infections further transferred to albino mice were used for comparison.

MATERIALS AND METHODS

Helminths from natural infection - Twelve *N. squamipes* were captured in a transmission area of schistosomiasis mansoni in Sumidouro, RJ, Brazil. They were sacrificed under laboratory conditions in ether chamber and perfused (Rodrigues e Silva et al. 1992).

All specimens were fixed in alcohol-formalin-acetic acid (AFA), stained with Mayer's Carmalum, cleared with beechwood creosote and preserved in Canada balsam. Voucher specimens were deposited in the Helminthological Collec-

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tion of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

The natural host (human or wild rodent) and the transfer to the mouse (*M. musculus*) were considered in designating the strains. The strain from *N. squamipes* was designated as SN and identified as CHIOC No. 33064 a-f.

Helminths from experimental infection - A sample from sympatric human cases was isolated and maintained in *Biomphalaria glabrata* and mice. The sample was designated as SHM and identified under CHIOC No. 33065 a-c.

A sample of *S. mansoni* was isolated from naturally infected *N. squamipes* and maintained under the same conditions as stated previously. It was designated as SNM, and identified under CHIOC No. 33066 a-c.

All the experimentally infected animals were sacrificed and perfused seven weeks after exposure to infecting larvae. Procedures regarding fixation and staining of worms from experimental infections were similar to those adopted for the SN strain. The worms from the two strains were derived from their first passage in mice.

Morphological analysis - For the examination through a digital image analyser MOP VIDEOPLAN, 50-68 male specimens recovered from heterologous infections were used and the considered morphological parameters are: (1) total length of specimens; (2) distance between the posterior end of oral sucker and anterior end of acetabulum (distance between suckers); (3) extention of testes grouping. The number of testes was determined through light microscopy.

For statistical analysis either Mann-Whitney or Kruskal-Wallis tests were applied, considering as signicative differences values of $p \leq 0.05$ (Siegel 1975).

RESULTS

Statistical analysis of the several evaluated parameters regarding the different strains revealed that in all situations the differences were significative ($p<0.01$) considering total length of adult male worms, varying from 5 to 14.9 mm (Fig. 1a). Mean length (11.0 ± 1.6 mm) of worms isolated from *N. squamipes* (SN strain) was greater than those of strains SNM and SHM (10.2 ± 1.8 and 9.1 ± 1.3 mm), respectively. In these two strains a higher frequency of specimens occurring in determined ranges regarding body length was observed and proved to be different when compared between themselves (Fig. 1a).

As for the number of testes (Fig. 1b) some aspects are outstanding: (1) about 80% of the specimens derived from the three strains presented six-eight testes; (2) about 10% of the specimens from SN strain had less than six testes; (3) in 21% of the specimens from SHM more than eight testes were observed and there was no specimen with less than six; (4) in 16% of the specimens from SNM strain there were more than eight testes and only 2% presented less than six.

In only 5% of the worms recovered from *N. squamipes* the localization of the testes was atypical. The distance between suckers also was different considering each strain: SN (0.36 ± 0.09 mm), SMN (0.34 ± 0.11 mm) and SHM (0.31 ± 0.08 mm). In the comparison of SN x SHM strains there was a more significative difference ($p<0.001$) than that observed in the total ($p<0.05$). Only in the SN strains a higher frequency of worms occurred in a same range of length (Fig. 2a). Among the strains there were no significant difference regarding the extension occupied by testes. Actually, this parameter varied between 0.3 and 0.49 mm (Fig. 2b).

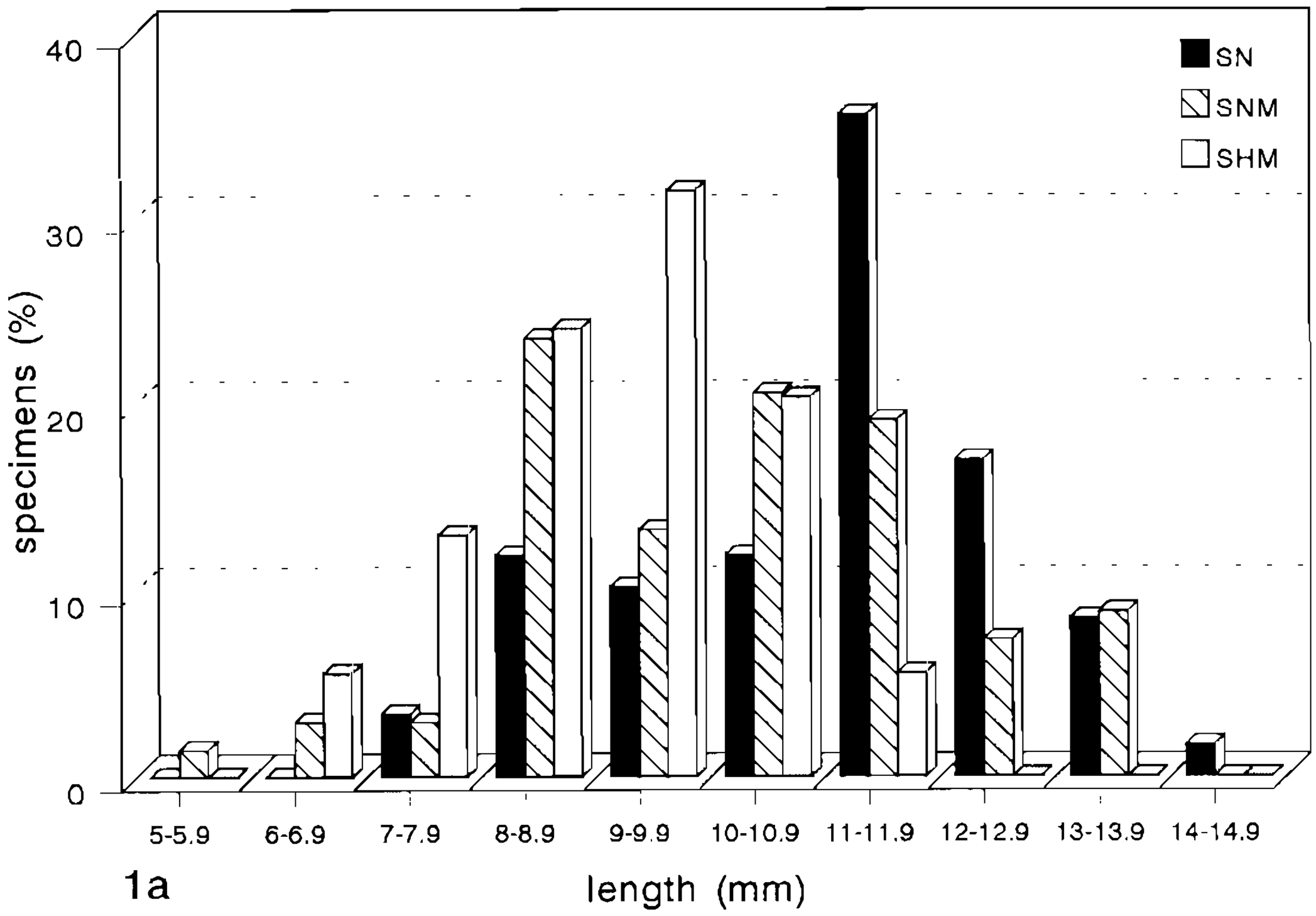
DISCUSSION

It is accepted that a permissive host to *S. mansoni* is that where adult worms reach "normal" dimensions and their eggs pass through the feces of the host as occur in mice and hamsters (Senft at al. 1978). *N. squamipes* also can be included in this group since it eliminates a great number of eggs in the feces (Rodrigues e Silva et al. 1992, Souza et al. 1992, Picot 1992) as well as with respect to the morphological aspects of male adult worms (Figs 1, 2).

Total length of *S. mansoni* specimens recovered from *N. squamipes* was significantly bigger than those of mice (Fig. 1a). Thus, we consider that the physiological conditions of *N. squamipes* are better for the somatic development of *S. mansoni* than those observed in mice and taking this into account, the wild rodent is more permissive than the mouse. It was verified previously that worms recovered from a natural host (*Rattus rattus*) in Guadeloupe (Caribbean Islands) were significantly bigger than those recovered from the albino rat *R. norvegicus* infected with the same *S. mansoni* strain (Imbert-Establet 1982).

There are few reports on the morphology of *S. mansoni* adult worms recovered from natural hosts in Brazil. We observed about 80% of specimens of the SN strain presented six-eight testes. These values accord with those referred to worms recovered either from the wild rodent *Holochilus brasilienis leucogaster* (Dias & Piedrabuena 1980) or from humans (Kastner et al. 1975). The frequency of distribution under or above the reported limits was also almost the same 10 and 9%, respectively.

In the diagnosis of *S. mansoni* it is defined that male adult worms possess eight or nine testes (Travassos et al. 1969). Based on previous data (Magalhães & Carvalho 1973, Paraense & Corrêa 1981) and on the present findings (Fig. 1b) it is suggested that the limits regarding such morphological character should be in the range of 3-13 testes.



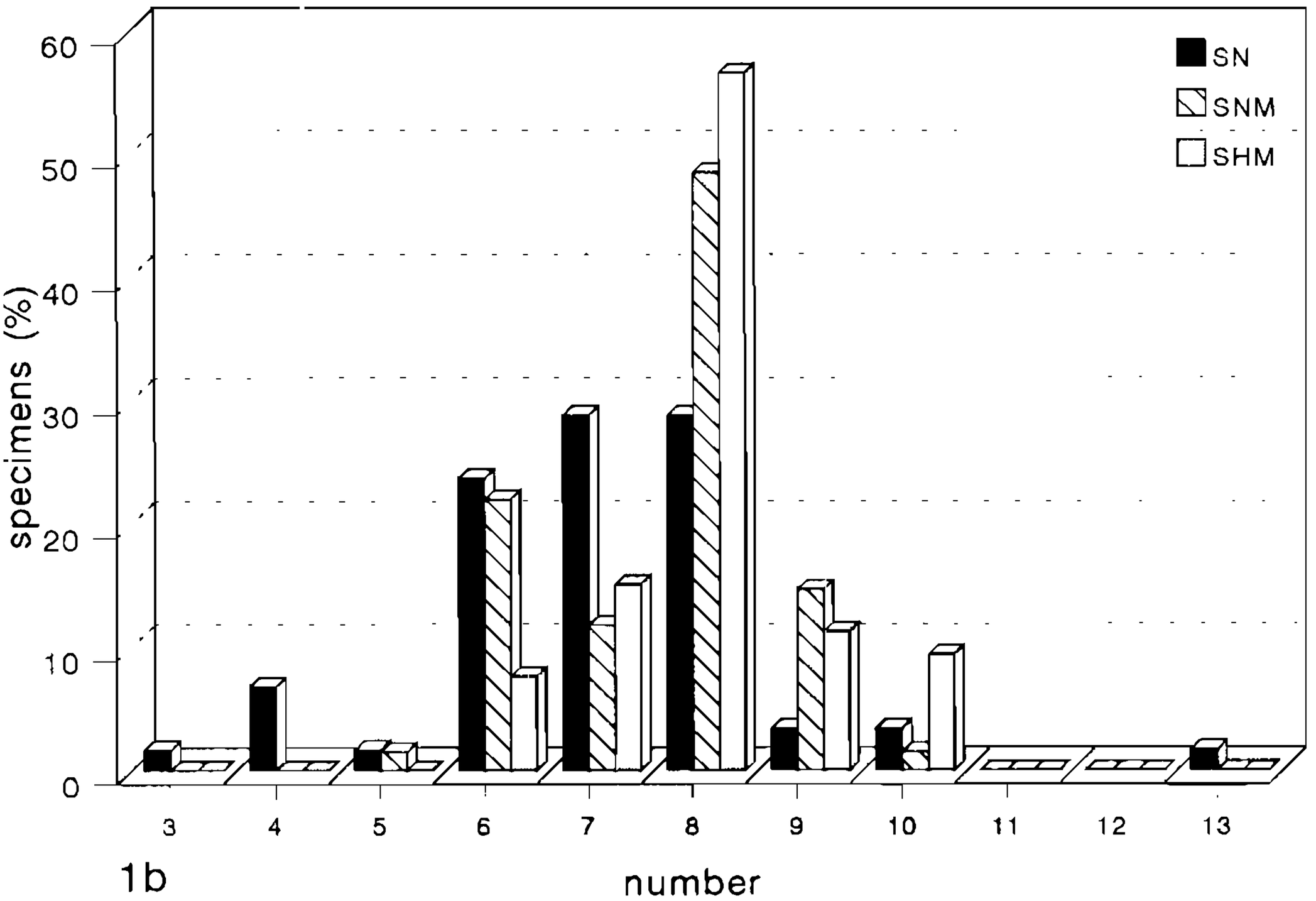
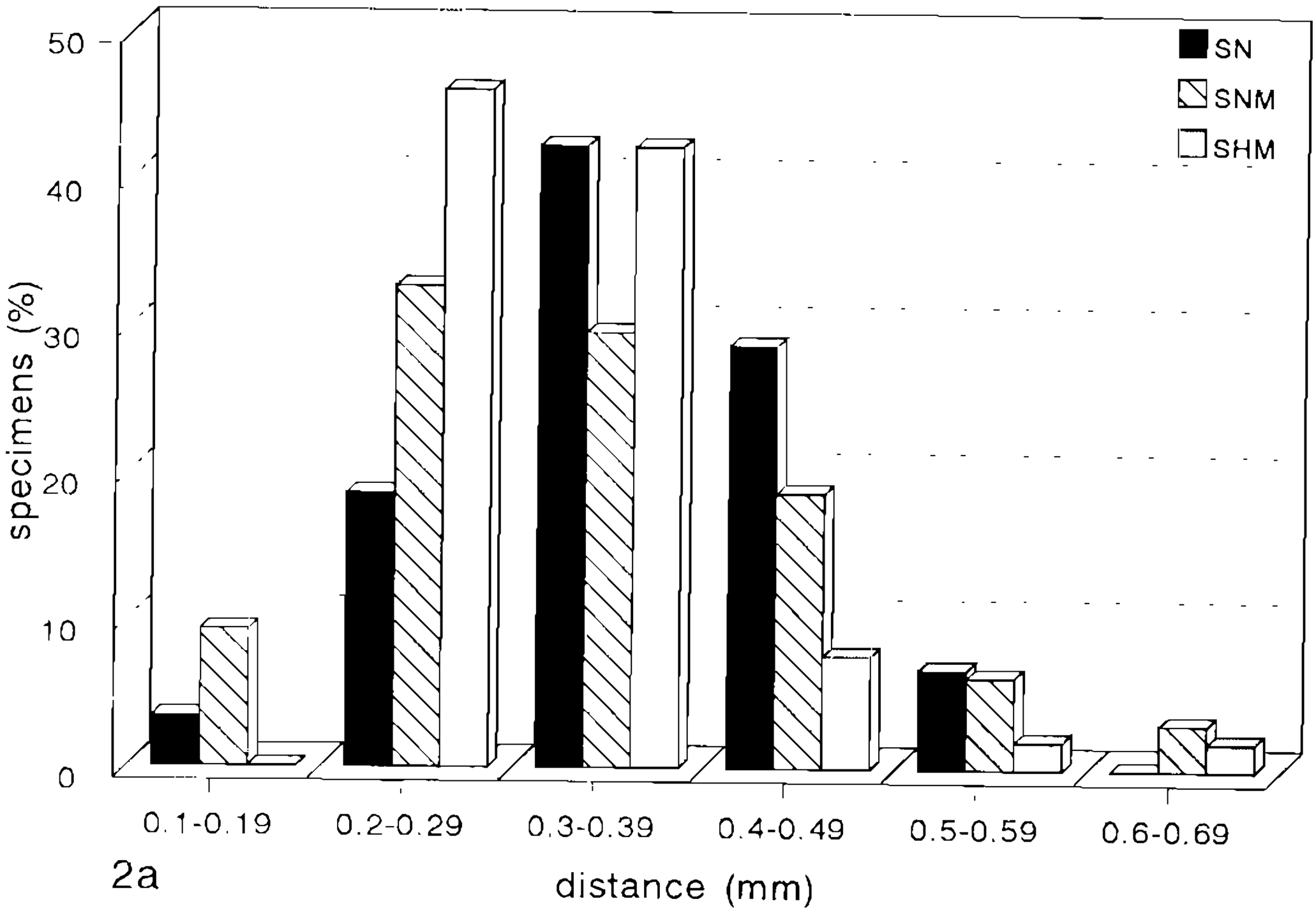


Fig. 1: morphological comparison of strains isolated from *Nectomys squamipes* (SN), after passage in mice (SNM) and another isolated from human cases and passed in mice (SHM), a - total length (mm): b - testes number.



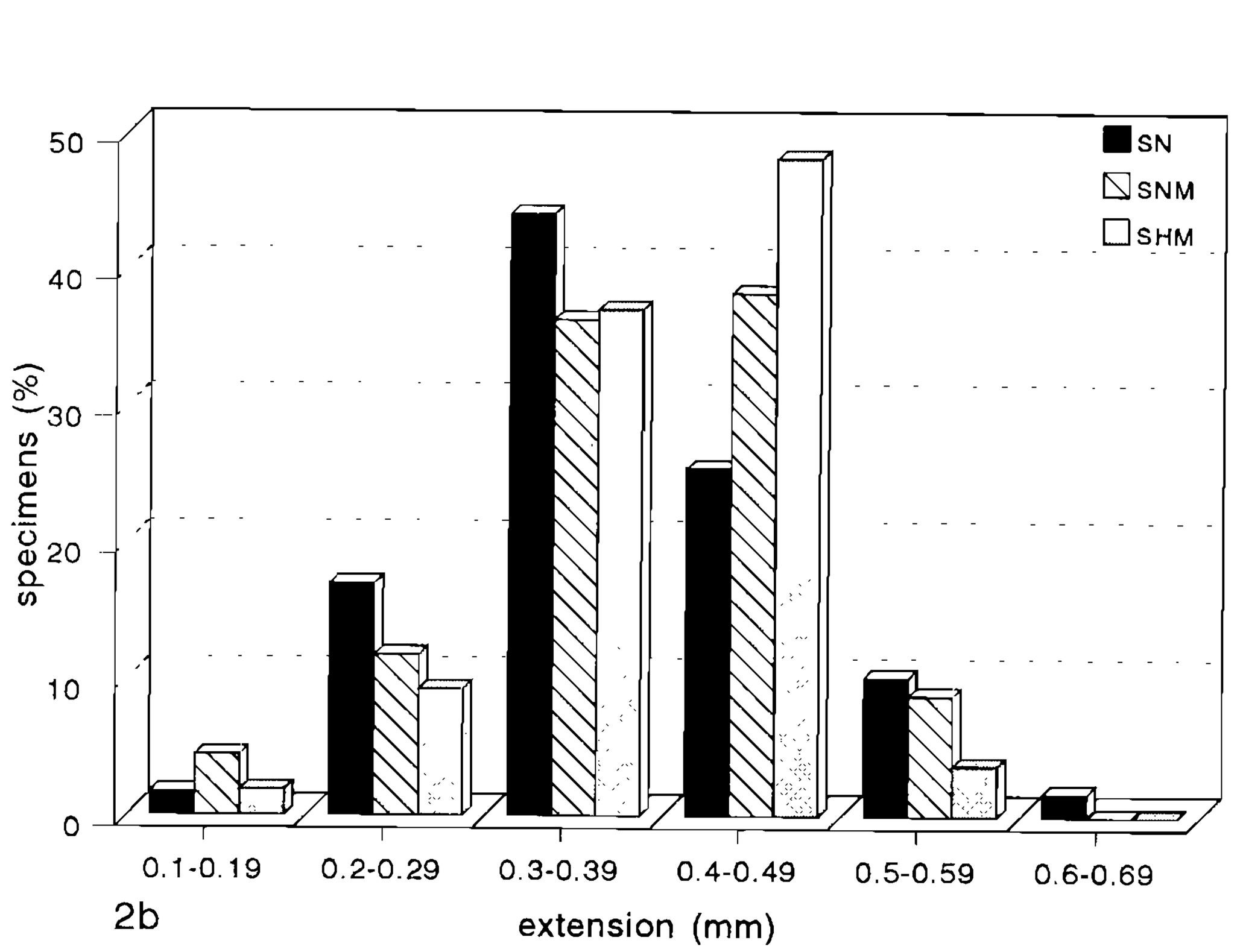


Fig. 2: morphological comparison of strains isolated from *Nectomys squamipes* (SN), after passage in mice (SNM) and another isolated from human cases and passed in mice (SHM), a - distance between oral and ventral sucker (mm); b - extension of testes grouping (mm).

In the strain isolated from *N. squamipes* naturally infected (SN strain) 5% of the specimens presented atypical localization of testes. Probably this phenomena is due neither for permissiveness of host nor for the *S. mansoni* strain considering that it was described occuring in adult worms recovered from the opossum *Didelphis aurita* experimentally infected with a Brazilian strain (Travassos 1953) and also in those from albino mice infected with African or Puerto Rico strains (Najim 1951, Saoud 1965, Coles & Thurston 1970, Soliman et al. 1984).

Vertebrate hosts strongly determine phenotypical characteristics of *S. mansoni* adult worms (Cioli et al. 1977). This fact has been presently observed after morphometric comparison of worms derived from the three strains (Figs 1,2). Three of the morphological characters of worms from SN strains proved to be significantly different from those of the SHM and SNM strains. In fact, morphological analysis of worms isolated from a natural host reproduces the phenotypical population characteristics of *S. mansoni* with more accuracy than those from an artificial host.

Comparison of the morphology concerning SN and SNM strains revealed significative differences related to total length and number of testes after passage in mice. Continuous passages in this host are responsible for morphological changes (Thompson & Lymbery 1990) and loss of genetic variability of the parasite (Loverde et al. 1985, Vieira et al. 1992, Dias Neto et al. 1993) and differences in the behavior of these parasites, regarding the prepatent period and infectivity (Kassim et al. 1979). In this opportunity morphological alterations in the worms were detected already in the first passage.

Actually there are no doubts regarding the existence of intraspecific variations in the *S. mansoni* (Thompson & Lymbery 1990). Considering that the strains derived from humans are perfectly adapted to mice and hamsters (Moore & Meleney 1951) if one find any difference between the strains this may be related to genotypic or phenotypic features of the strains (Magalhães & Carvalho 1973, Paraense & Corrêa 1981).

Among the strains we inoculated in mice the only significative difference was that concerned to the total length of worms (Fig. 1a), for the data, partially, do not reproduce those obtained for the BH and SJ strains which present differences regarding length, number of testes and distance between suckers (Magalhães & Carvalho 1973, Paraense & Corrêa 1981). Geographical origin was responsible for the different data. These authors dealt with allopatric strains while we used in this study sympatric strains. It is known nowadays that sympatric strains isolated from either humans or wild rodents show common antigenic fractions (Carneiro et al. 1991) and isoenzymatic profile (Rodriguez et al. 1991). Conversely, there are evidences that these strains show phenotypical differences. Morphological studies utilizing cercariae from the same strains used after comparison, revealed that their chaetotaxic pattern shows slight differences (Freire 1987). Taking into account that *S. mansoni* genetic variability decreases after sucessive passages in mice, we consider that strains known to be derived from human cases express phenotypical features strongly related to this vertebrate host. It is suggested therefore that morphological studies regarding *S. mansoni* worms must be undertaken on basis on those recovered from wild rodents.

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