Biotemas, 35 (1): 1-12, março de 2022
ISSNe 2175-7925
http://dx.doi.org/10.5007/2175-7925.2022.e82713

Histological aspects of the penial complex and vagina of taxa of the *Biomphalaria tenagophila* complex (Mollusca: Planorbidae)

Tatiane Eni da Silva Tavares *

Fernanda Pires Ohlweiler

Malacology Laboratory, Special Programs Division
Superintendency for the Control of Endemic Diseases
Rua Cardeal Arcoverde, 2878, Pinheiros, CEP 05.400-003, São Paulo – SP, Brazil
* Corresponding author
tatieni.ts@gmail.com

Submetido em 08/07/2021
Aceito para publicação em 17/01/2022

Resumo

Aspectos histológicos do complexo peniano e vagina de táxons do Complexo *Biomphalaria tenagophila* (Mollusca: Planorbidae). Foi realizado estudo histológico de 49 espécimes: 18 *Biomphalaria tenagophila guaibensis*, 19 *B. t. tenagophila* e 12 *B. occidentalis*, coletados em 10 municípios dos estados de São Paulo e Rio Grande do Sul, Brasil. O estudo considerou aspectos histológicos da vagina, bolsa vaginal e complexo peniano. Neste estudo, descrevemos, pela primeira vez, os caracteres histológicos da vagina, bolsa vaginal e complexo peniano de *B. t. tenagophila*, *B. t. guaibensis* e *B. occidentalis*. O tecido epitelial de revestimento e o tecido muscular da vagina, bolsa vaginal e complexo peniano mostram um padrão semelhante em *B. t. tenagophila*, *B. t. guaibensis* e *B. occidentalis*, exceto pela camada muscular intermediária do prepúcio, que distingue *B. t. guaibensis* dos outros dois táxons. Estudos histológicos do complexo peniano e da região da vagina de outras espécies de *Biomphalaria* são necessários para confirmar o valor taxonômico dessas duas regiões, de forma a contribuir para a sistemática do grupo.

Palavras-chave: Água doce; Microanatomia; Sistema Reprodutor

Abstract

Histological studies were carried out with 49 specimens; 18 *Biomphalaria tenagophila guaibensis*, 19 *B. t. tenagophila* and 12 *B. occidentalis*, collected in 10 municipalities in the states of São Paulo and Rio Grande do Sul, Brazil. The study considered histological aspects of the vagina, vaginal pouch and penial complex. In this study, we describe, for the first time, the histological features of the vagina, vaginal pouch and penial complex of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis*. The lining epithelial tissue and muscular tissue of the vagina, vaginal pouch and penial complex show a similar pattern in *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis*, except for the intermediate muscular layer of the prepuce, which distinguishes *B. t. guaibensis* from the other two taxa. Histological studies on the penial complex and vagina region of other *Biomphalaria* species are necessary to confirm the taxonomic value of these two regions, in order to contribute to the group's systematics.

Key words: Freshwater; Microanatomy; Reproductive System

Introduction

The *Biomphalaria tenagophila* complex, proposed by Spatz et al. (1999), is represented by *Biomphalaria tenagophila tenagophila* (d'Orbigny, 1835), *Biomphalaria tenagophila guaibensis* Paraense, 1984 and *Biomphalaria occidentalis* Paraense, 1981. Of the three taxa mentioned above, only *B. t. tenagophila* is susceptible to *Schistosoma mansoni* Sambon, 1907, mainly, in the south and southeast regions of Brazil (CARVALHO et al., 2008). The correct determination of the three taxa of the *Biomphalaria tenagophila* complex is important to identify the intermediate host species of *S. mansoni*, allowing the recognition of vulnerable areas for the transmission of schistosomiasis and, thus, guiding health agents in surveillance and environmental control measures (BRASIL, 2008; PARAENSE, 2008).

The morphological similarities among *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* can lead to taxonomic errors. The penial complex and the vagina are the two regions of the reproductive system of greatest taxonomic importance in *Biomphalaria* (BAKER, 1945; ABDEL-MALEK, 1952); however, there are still many knowledge gaps surrounding the microanatomy of reproductive organs in these planorbid mollusks.

The comparative analysis of the musculature of reproductive structures has proved to be a promising tool to resolve impasses in the phylogeny of *Hygrophila*, the superorder that includes the family Planorbidae (SOLDATENKO; PETROV, 2019).

Histological details of the musculature of reproductive organs in Planorbidae, available in the consulted bibliography, refer to the genera *Anisus* Studer, 1820, *Bathyomphalus* Charpentier, 1837, *Choanomphalus* Gerstfeldt, 1859, *Gyraulus* Agassiz, 1837, *Helisoma* Swainson, 1840, *Planorbella* Haldeman, 1842, *Planorbis* Muller, 1773 and *Segmentina* Fleming, 1818 (ABDEL-MALEK, 1952; 1954a; SOLDATENKO; PETROV, 2009; 2012; 2019; SOLDATENKO; SHATROV, 2013). However, the histological studies on the reproductive organs of *Biomphalaria* are not very comprehensive and are restricted to the *Biomphalaria alexandrina* (Ehrenberg, 1831) (ABDEL-MALEK, 1954b), *Biomphalaria glabrata* (Say, 1818) (PARAENSE; DESLANDES, 1955; PAN, 1958; SOLDATENKO; PETROV, 2019), *Biomphalaria intermedia* (Paraense & Deslandes, 1962) (PARAENSE, 1988), *Biomphalaria kuhniana* (Clessin, 1883) (PARAENSE, 1988) and *Biomphalaria straminea* (Dunker, 1848) (PARAENSE, 1988).

Microanatomical studies of the penial complex and vagina region are important for the taxonomic delimitation of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis*. The present work aims to study the histological aspects of the penial complex and the vagina region of these three taxa, to contribute to the systematic of Planorbidae.

Materials and Methods

Study material

Specimens of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* were collected from water bodies in 10 municipalities in the states of São Paulo and Rio Grande do Sul, Brazil (Table 1). The collected snails were cataloged and deposited in the malacological collection of the Special Programs Division (DPE) of Superintendency for the Control of Endemic Dieses (SUCEN) as testimony material under batch numbers 615 to 3249, not necessarily consecutive.

Collection procedures

Manual collections were carried out from 2018 to 2019 by using tweezers and a perforated metal ladle attached to a wooden handle, following the technique used by Ministry of Health (BRASIL, 2008). The geographic coordinates of collection points were obtained with a Global Positioning System (GPS) device. The snail collections were authorized by the Biodiversity Authorization and Information System (SISBIO) under No. 61020-1.

TABLE 1: *Biomphalaria tenagophila guaibensis*, *Biomphalaria tenagophila tenagophila* and *Biomphalaria occidentalis* used in the histological study of the penial complex and vagina region, from the states of Rio Grande do Sul and São Paulo, in Brazil.

Species	State	Municipality	Lot Number	Exemplary	Histology	Latitude (S)	Longitude (W)
B. tenagophila guaibensis	Rio Grande do Sul	Pelotas	3127	15	08	31º45'08.9"	52º17'23.8"
			3128	14	10	31º44'33.0"	52º19'31.0"
		Number of exemplary		**29**	**18**		
B. tenagophila tenagophila	São Paulo	Barueri	3002	20	05	23°28'49.5"	46°52'17.2"
		Biritiba Mirim	3089	23	03	23°35'09.0"	46°02'08.1"
		Carapicuíba	3179	10	01	23º30'53.5"	46º50'22.0"
			3180	20	02	23º30'50.6"	46º50'23.0"
		Itaquaquecetuba	3177	12	05	23º29'34.6"	46º17'51.0"
		Guarulhos	3166	09	01	23º24'50.0"	46º28'33.4"
		Peruíbe	3145	06	01	24°18'13.5"	47°01'05.9"
	Rio Grande do Sul	Arambaré	9011	04	01	31°03'12.7"	51°30'22.6"
		Number of exemplary		**104**	**19**		
B. occidentalis	São Paulo	Andradina	3249	02	02	20º53'09.6"	51º22'52.3"
		Itaquaquecetuba	3132	30	02	23º30'13.68"	46º20'1.32"
		Santa Isabel	615	09	03	23º17'27.5"	46º12'27.6"
			3174	07	01	23º17'21.4"	46º13'11.4"
			3176	04	04	23º17'4.75"	46º12'59.12"
		Number of exemplary		**52**	**12**		
Total number of exemplary				**185**	**49**		

Treatment of collected snails

Live snails were subjected to parasitological examination by exposure to artificial light (60 W) for up to 4 h to verify trematodes larvae infection. All snails were identified, but only infection-free snails with observable and intact anatomical structures were used in the histological study of the penial complex and vagina region. The infection-free snails were anesthetized with menthol crystals for 24 to 48 h at 4ºC. Then, their bodies and shells were separated following the methodology described by the Ministry of Health (BRASIL, 2008). The shells were cleaned and stored dry for better conservation and the bodies kept in the Raillet-Henry fixative. A total of 104 *B. t. tenagophila*, 29 *B. t. guaibensis* and 52 *B. occidentalis* were identified.

Histology of the penial complex and vagina region

Histological aspects of the epithelial lining and muscle tissue of the penial complex and vagina region of 49 snails – 19 *B. t. tenagophila*, 18 *B. t. guaibensis* and 12 *B. occidentalis* – were observed. (Table 1). The snails were included in paraffin, and serial histological sections with a thickness of 7 to 10 µm were made

on a YD-335 semiautomatic rotary microtome. The sections were stained with Mayer's Hematoxylin and 1% aqueous Eosin Y. The histological slides were mounted on Entellan, analyzed and photographed using a Leica DFC 290 optical microscope. Snails with shell diameter under 10 mm were discarded to avoid analysis errors in young specimens.

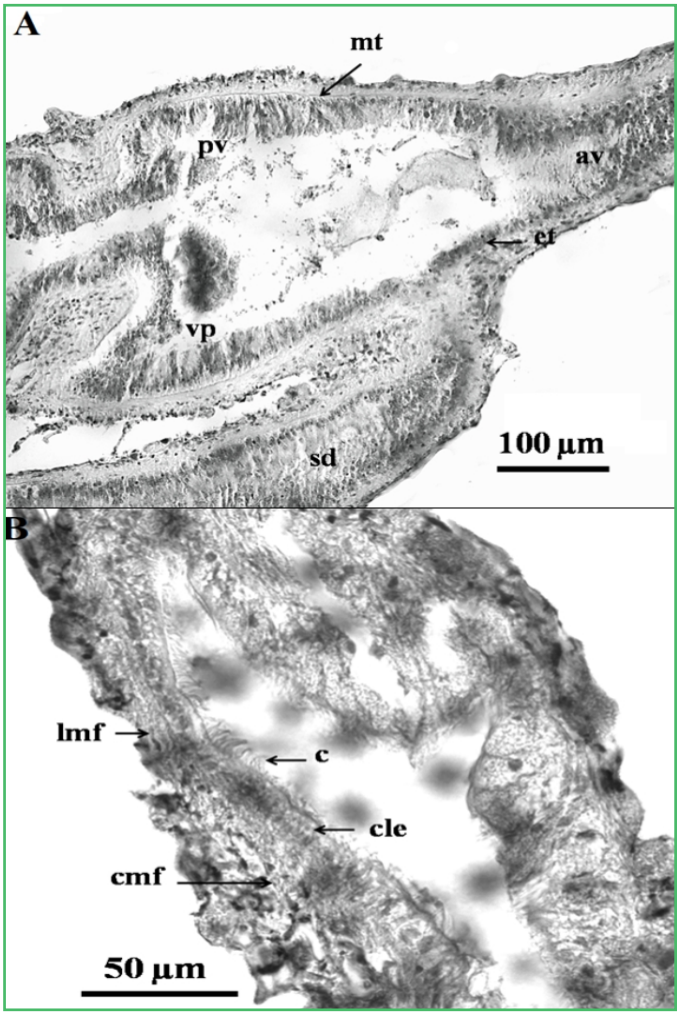
Results

The vagina region includes anterior vagina and posterior vagina. The posterior vagina is the portion between the uterus and the insertion of the duct of the spermatheca, while the anterior vagina includes the portion between the insertion of the duct of the spermatheca and the vaginal opening. The ventral wall of the posterior vagina of *B. t. tenagophila* and *B. t. guaibensis* has an enlarged and bulging area that corresponds to the vaginal pouch, which is absent in *B. occidentalis* (OHLWEILER et al., 2020: Figures 3A and 3B). The penial complex has prepuce, penis sheath, penis and ejaculatory duct.

Vagina and vaginal pouch

Lining epithelial tissue and muscular tissue with similar pattern are present in the vaginas of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* and in the vaginal pouch of the two first taxa (Figure 1A).

FIGURE 1: Vagina in longitudinal sections. **A.** Anterior and posterior vagina of *Biomphalaria tenagophila tenagophila*. **B.** Posterior vagina of *Biomphalaria tenagophila guaibensis*. Abbreviations: av, anterior vagina; c, cilia; cle, columnar lining epithelium; cmf, circular muscular fiber; et, epithelial tissue; lmf, longitudinal muscular fiber; mt, muscular tissue; pv, posterior vagina; sd, spermatheca duct; vp, vaginal pouch.



The vagina and the vaginal pouch have a columnar lining epithelial tissue with ciliated cells and basal nucleus, which rests on a basal membrane. Below the basal membrane is a layer of connective tissue, followed by muscle tissue (Figures 1B; 2A; 2B).

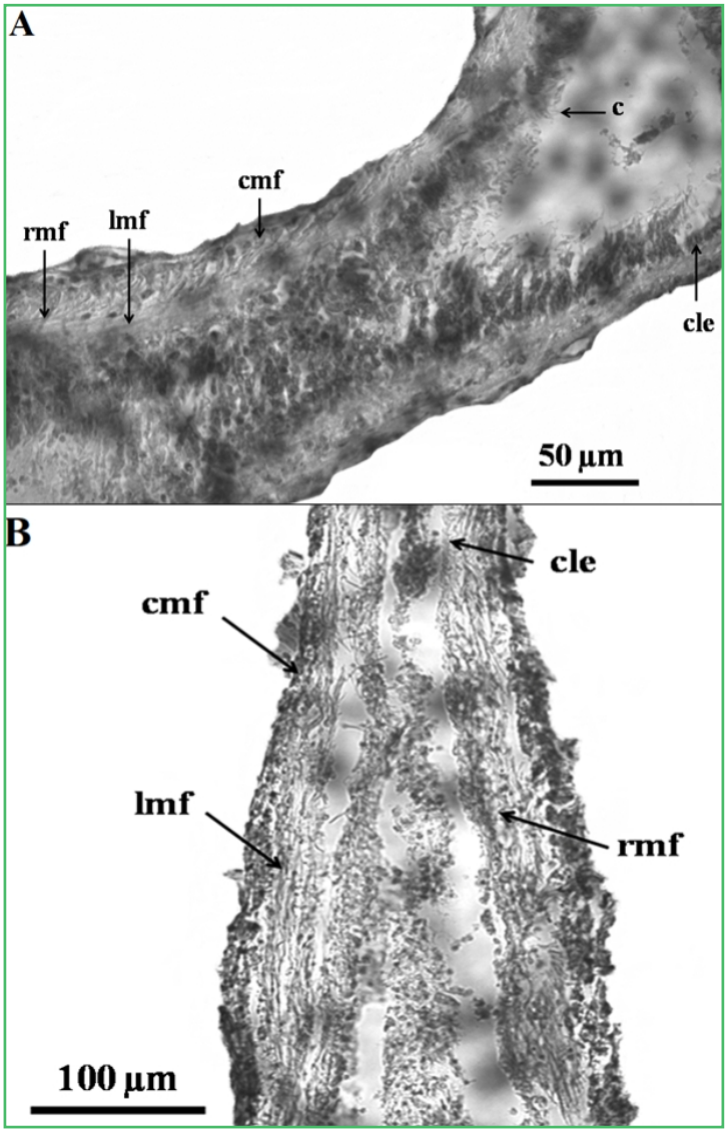
The muscular tissue of the vagina and vaginal pouch consists of an inner layer of longitudinal muscular fibers and an outer layer of circular muscular fibers. Radial muscular fibers are seen in both muscular layers, as well as circular fibers in the inner layer and longitudinal fibers in the outer layer (Figures 1A; 1B; 2A; 2B).

Penial complex

The lining epithelial tissue and the muscular tissue of the penial complex are, generally, similar in *B. t. guaibensis*, *B. t. tenagophila* and *B. occidentalis*.

The prepuce has a layer of columnar lining epithelial tissue with ciliated cells and basal nucleus, which rests on a basal membrane (Figures 3A; 3B; 4A; 4B). As the diaphragm region approaches, the epithelial cells become lower, giving the impression of a ciliated cubic lining epithelium (Figure 3A). After the basal

FIGURE 2: Vagina in longitudinal sections. A. Anterior vagina of *Biomphalaria tenagophila tenagophila*. B. Posterior vagina of *Biomphalaria occidentalis*. Abbreviations: c, cilia; cle, columnar lining epithelium; cmf, circular muscular fiber; lmf, longitudinal muscular fiber; rmf, radial muscular fiber.



membrane there is a layer of connective tissue and another layer of muscle tissue. The muscular tissue predominates in the architecture of the prepuce and consists of three layers of muscular fibers: 1. Inner layer of circular muscular fibers next to the connective tissue. 2. Thick intermediate layer formed by loose longitudinal, circular and radial muscular fibers, arranged in a disorderly manner. In *B. t. guaibensis*, among the loose muscular fibers, two broad bands of longitudinal muscular fibers are observed; this is not seen in the other two taxa. 3. Thin outer layer formed of circular muscular fibers. Dark pigments are observed externally in the muscular tissue, rendering the prepuce a dark aspect (Figures 3B; 4A; 4B; 5A).

FIGURE 3: Prepuce in longitudinal sections. A. *Biomphalaria tenagophila tenagophila*. B. *Biomphalaria tenagophila guaibensis*. Abbreviations: c, cilia; cle, columnar lining epithelium; d, diaphragm; dp, dark pigments; ecm, external circular muscular fiber; icm, internal circular muscular fiber; lce, low columnar lining epithelium; lcm, loose circular muscular fiber; llm, loose longitudinal muscular fiber; lrm, loose radial muscular fiber; mt, muscular tissue; pm, protractor muscle; rm, retractor muscle.

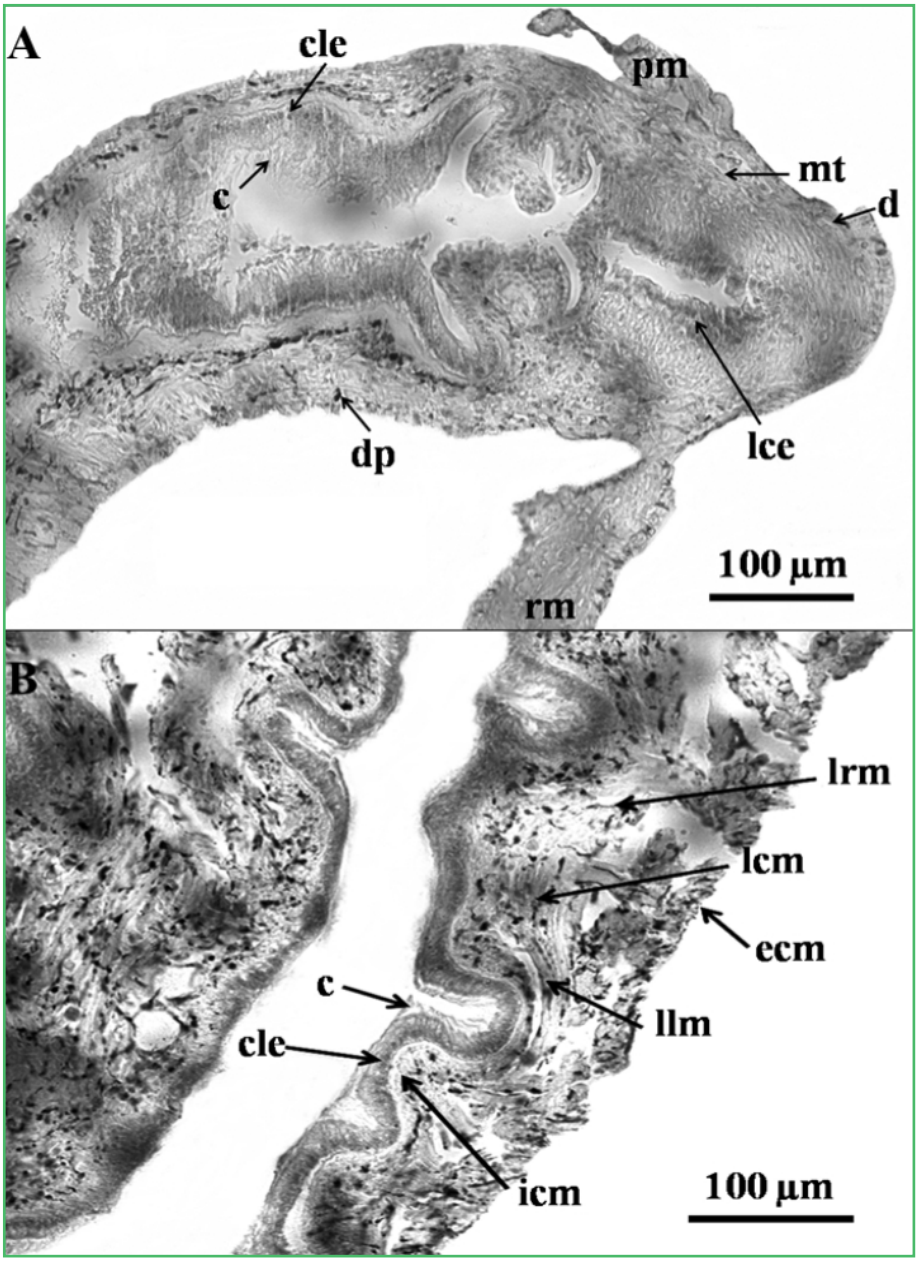
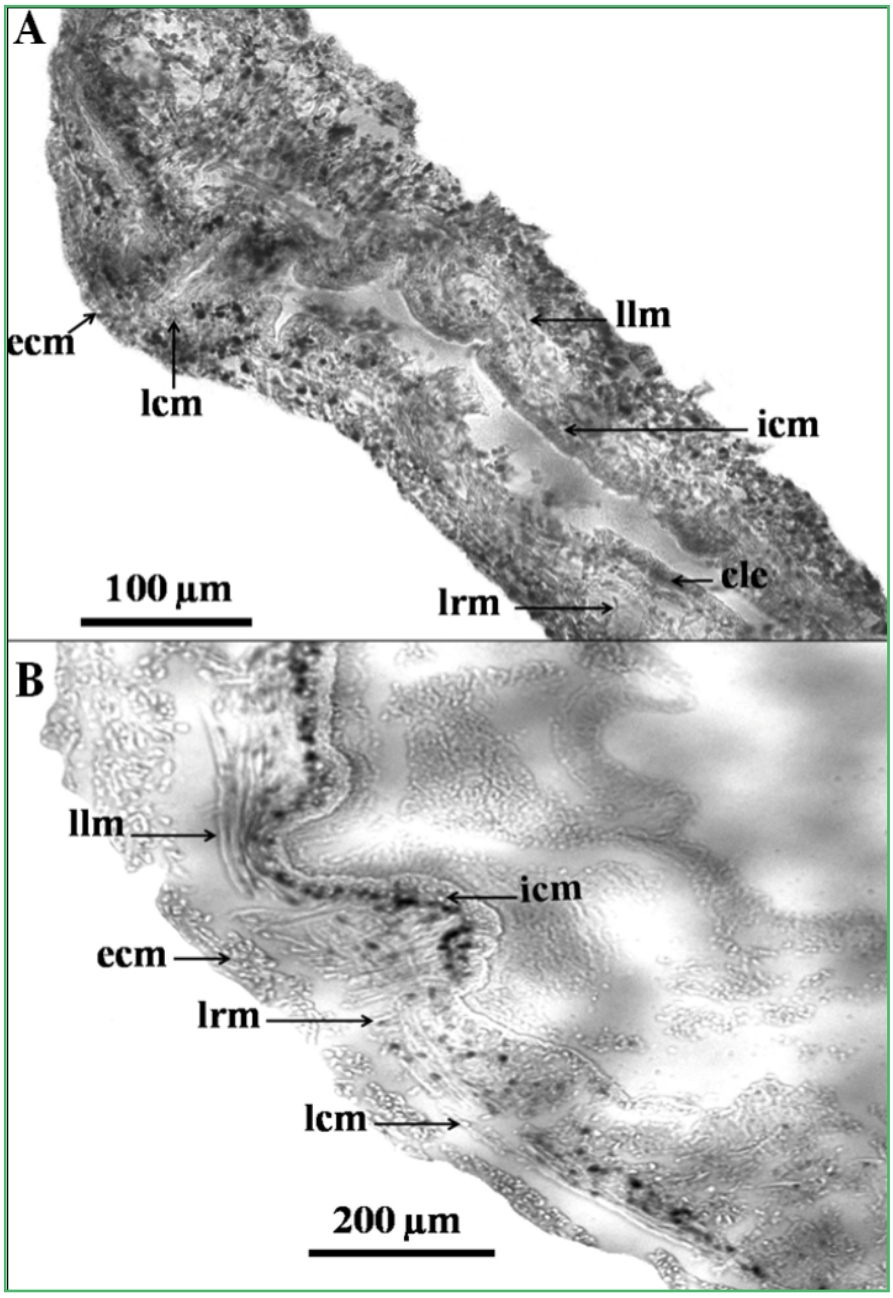
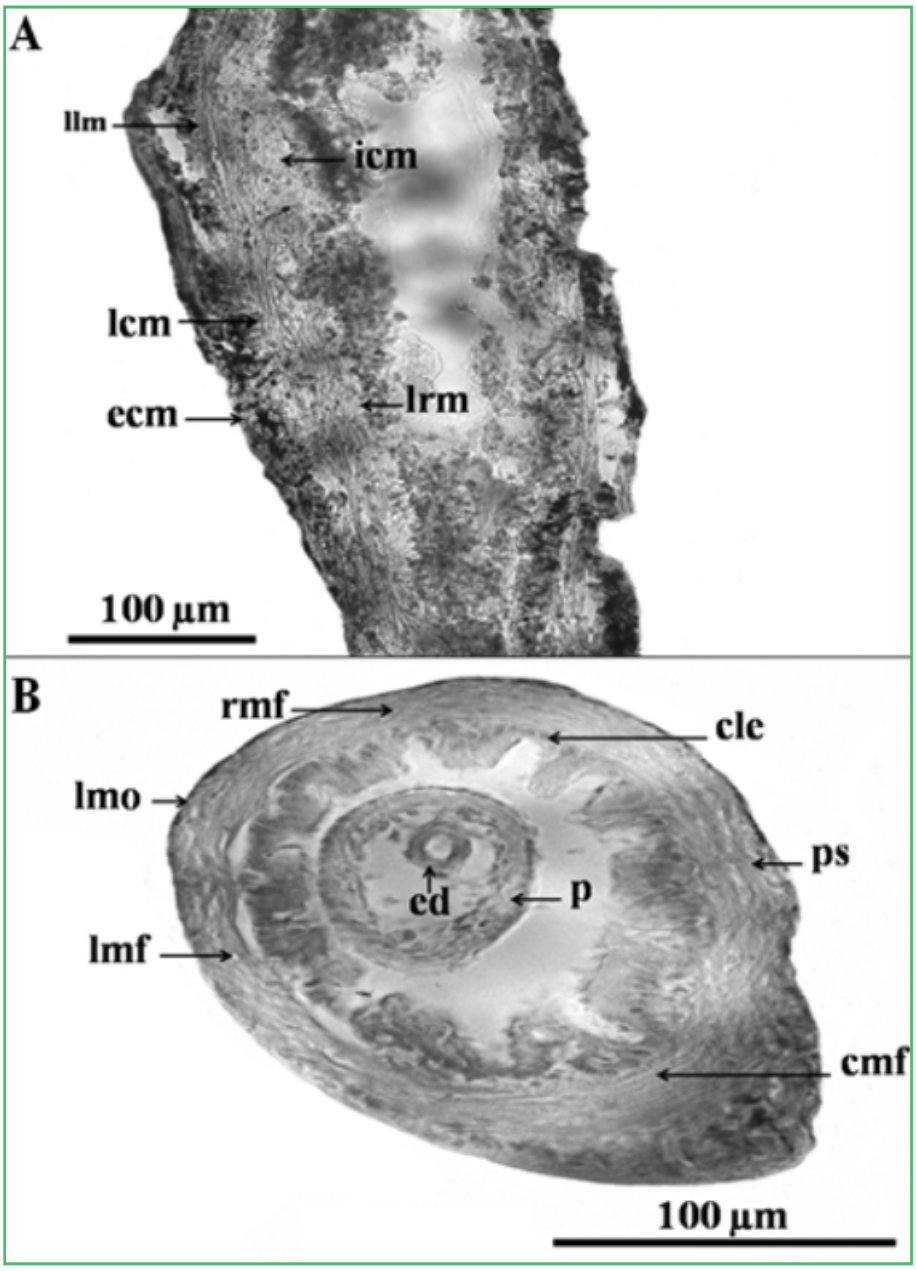


FIGURE 4: Prepuce in longitudinal sections. A. *Biomphalaria occidentalis*. B. *Biomphalaria tenagophila tenagophila*. Abbreviations: cle, columnar lining epithelium; ecm, external circular muscular fiber; icm, internal circular muscular fiber; lcm, loose circular muscular fiber; llm, loose longitudinal muscular fiber; lrm, loose radial muscular fiber.



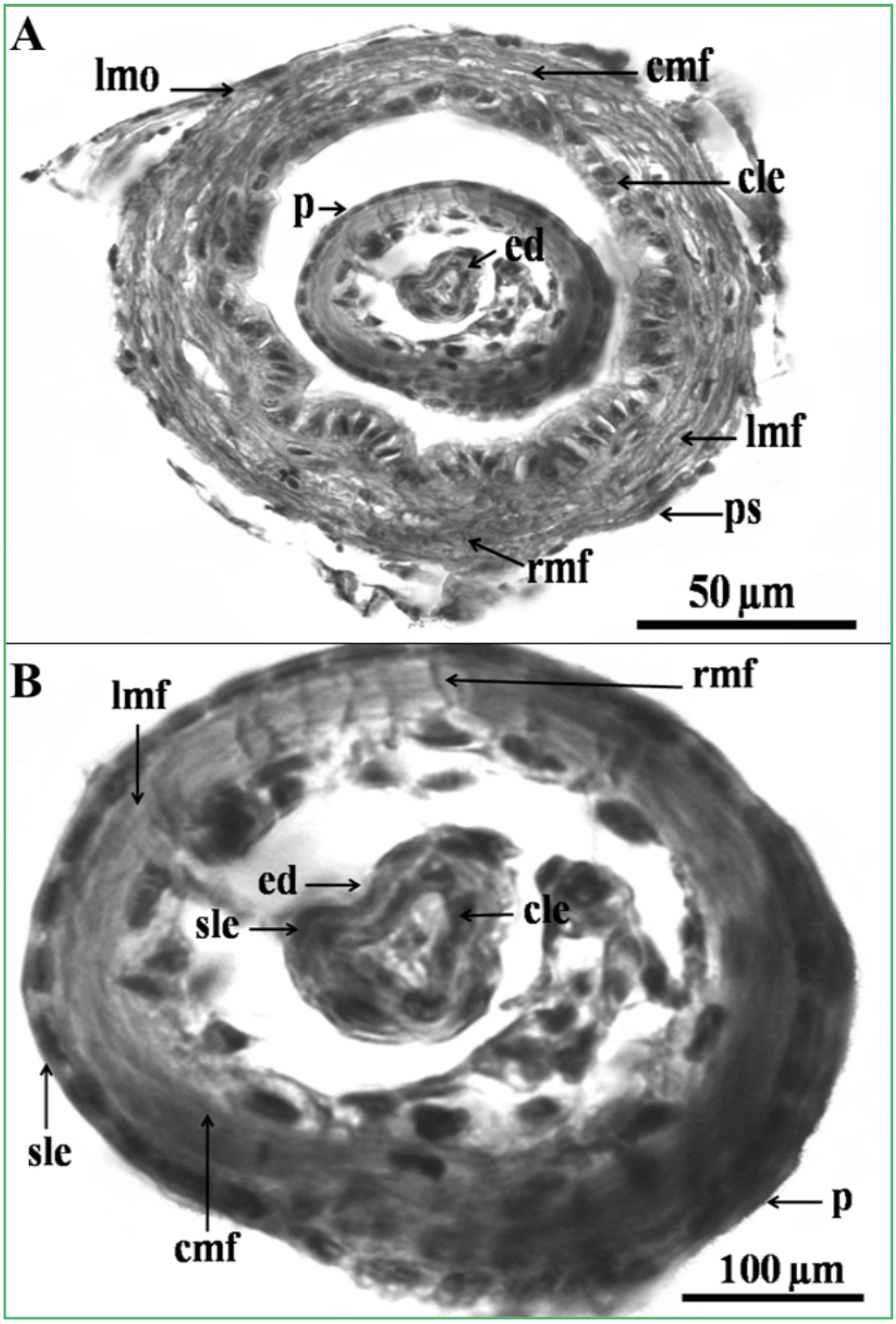
The penis sheath has cubic lining epithelial tissue, with non-ciliated cells and spherical nuclei (Figures 5B; 6A). The epithelium rests on a basal membrane, which is supported by a thin layer of connective tissue. The edge of the cell membrane is observed on the inner surface of epithelial cells. The penis sheath has two layers of muscular tissue: 1. Thick inner layer, the muscular fibers of which are mostly arranged in a circular shape. In this layer, longitudinal and radial muscular fibers are also found. 2. Thin outer layer, with longitudinal muscular fibers. Externally, in the muscular tissue, dark pigments are observed, which, as in the prepuce, renders the penis sheath a dark appearance (Figures 5B; 6A).

FIGURE 5: Penis sheath, penis and ejaculatory duct. A. Longitudinal sections of the penis sheath of *Biomphalaria occidentalis*. B. cross sections of the penis sheath, penis and ejaculatory ducto of *Biomphalaria tenagophila tenagophila*. Abbreviations: cle, cubic lining epithelium; cmf, circular muscular fiber; ecm, external circular muscular fiber; ed, ejaculatory duct; icm, internal circular muscular fiber; lcm, loose circular muscular fiber; llm, loose longitudinal muscular fiber; lmf, longitudinal muscular fiber; lmo, longitudinal muscular fiber of the outer layer; lrm, loose radial muscular fiber; p, penis; ps, penis sheath; rmf, radial muscular fiber.



The penis and the ejaculatory duct (Figure 5B) have a layer of cubic lining epithelial tissue, with non-ciliated cells and a spherical nucleus, and a layer of muscle tissue formed by muscular fibers, mostly circular. In this layer, longitudinal and radial muscle fibers are also found. Outside the layer of muscular tissue, a squamous lining epithelial tissue with cells of elliptical nuclei is observed. The cells in this last layer are characterized by being shorter than tall (Figures 6A; 6B).

FIGURE 6: Penis sheath, penis and ejaculatory duct. A, B. cross sections of the penis sheath, penis and ejaculatory duct of *Biomphalaria tenagophila tenagophila*. Abbreviations: cle, cubic lining epithelium; cmf, circular muscular fiber; ed, ejaculatory duct; lmf, longitudinal muscular fiber; lmo, longitudinal muscular fiber of the outer layer; p, penis; ps, penis sheath; rmf, radial muscular fiber; sle, squamous lining epithelium.



Discussion

Histological studies of the vagina, vaginal pouch and penial complex of the planorbid *Biomphalaria*, besides being little comprehensive, are restricted to few taxa; namely *B. alexandrina* (ABDEL-MALEK, 1954b), *B. glabrata* (PARAENSE; DESLANDES, 1955; PAN, 1958; SOLDATENKO; PETROV, 2019), *B. intermedia* (PARAENSE, 1988), *B. kuhniana* (PARAENSE, 1988) and *B. straminea* (PARAENSE, 1988).

The ciliated columnar lining epithelium found in the vagina of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* and in the vaginal pouch of the first two taxa, although absent in the last taxon, is observed in *B. glabrata* by Paraense and Deslandes (1955) and Pan (1958). The same pattern of epithelial tissue is also reported for the planorbid *B. alexandrina* and *Helisoma trivolvis* (Say, 1817) (ABDEL-MALEK, 1954a; 1954b) and for the limneid *Lymnaea stagnalis apressa* (Say, 1817) (HOLM, 1946). The same number of layers and architecture of the muscular fibers are verified in the region of the vagina of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis.* And they are in accordance with the findings of Pan (1958) for *B. glabrata* and of Abdel-Malek (1954a; 1954b) for *B. alexandrina* and *H. trivolvis*, which present the region of the vagina with two muscular layers, one internal of longitudinal fibers and the other external of circular fibers. However, they differ from what is described for the limneid *L. s. apressa*, which according to Holm (1946), has the vagina with an inner layer of circular muscular fibers and an outer layer of longitudinal muscular fibers.

The columnar lining epithelium with ciliated cells found in the prepuce of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* is also observed in the prepuce of the planorbid *B. glabrata* (PAN, 1958), *B. alexandrina* (ABDEL-MALEK, 1954b) and *H. trivolvis* (ABDEL-MALEK, 1952) and of the limneid *L. s. apressa* (HOLM, 1946).

The structure and the number of muscular layers of the prepuce are similar in the three taxa studied and in *B. alexandrina (*ABDEL-MALEK, 1954b) and *B. glabrata* (PAN, 1958). *Biomphalaria t. guaibensis* differs of *B. t. tenagophila* and B. occidentalis and also of *B. alexandrina* (ABDEL-MALEK, 1954b) and *B. glabrata* (PAN, 1958) regarding the intermediate muscular layer of the prepuce. In *B. t. guaibensis* the intermediate muscular layer of the prepuce has two wide bands of longitudinal fibers, which has not been described for the other aforementioned taxa. When comparing the musculature of the prepuce of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* with the musculature of the prepuce of the planorbid *H. trivolvis* (ABDEL-MALEK, 1952) and of the limneid *L. s. apressa* (HOLM, 1946) we verified differences in the number of layers. The prepuce of *H. trivolvis* contains a layer of longitudinal and circular muscular fibers (ABDEL-MALEK, 1952) and the prepuce of *L. s. apressa* has one of longitudinal muscular fibers and another of circular muscular fibers. On the other hand, in our material, three layers are found, one of circular muscular fibers, one of loose longitudinal, circular and radial muscular fibers and the other of circular muscular fibers.

The non-ciliated cubic lining epithelial tissue found in the penis sheath of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* is in accordance with the reports of Abdel-Malek (1954b) for *B. alexandrina* and of Paraense and Deslandes (1955) and Pan (1958) for *B. glabrata*. However, it differs from the epithelium presented by planorbid of the genera *Helisoma*, *Anisus* and *Planorbis* (ABDEL-MALEK, 1954a; SOLDATENKO; SHATROV, 2013) and by limneid of the genus Lymnaea (HOLM, 1946), in which the lining epithelial tissue of the penis sheath consists of columnar cells.

The number and arrangement of muscular fibers found in the penis sheath of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* is in accordance with the description of *B. alexandrina* (ABDEL-MALEK, 1954b) and *B. glabrata* (PAN, 1958). In these five taxa, the muscular layer of the penis sheath is represented by an inner layer of circular fibers and an outer layer of longitudinal fibers. The comparison of the musculature of the penis sheath of the three taxa studied with planorbids of the genera *Anisus*, *Bathyomphalus*, *Choanomphalus*, *Gyraulus*, *Helisoma*, *Planorbella* e *Segmentina* (ABDEL-MALEK, 1954a; SOLDATENKO; PETROV, 2012; 2019), acroloxids of

the genus *Acroloxus* (SOLDATENKO; PETROV, 2019) and limneids of the genera *Lymnaea* and *Radix* (HOLM, 1946; SOLDATENKO; PETROV, 2019), it shows differences in the number of muscular layers, which varies from one to four in the genera mentioned by the authors, whereas in *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* are observed only two muscular layers.

The penis and ejaculatory duct of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* differ from those of *B. alexandrina* (ABDEL-MALEK, 1954b) and *B. glabrata* (PAN, 1958). In the first three taxa, both structures have a non-ciliated cubic lining epithelium, instead of the ciliated cubic lining epithelium present in the last two species. The presence of a single muscular layer in the penis and ejaculatory duct of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* differs them from *B. alexandrina* (ABDEL-MALEK, 1954b), *B. glabrata* (PAN, 1958; SOLDATENKO; PETROV, 2019), *B. intermedia* (PARAENSE, 1988) and *B. kuhniana* (PARAENSE, 1988) which have two muscular layers and from *B. straminea* (PARAENSE, 1988) which has three muscular layers. And also of the planorbids of the genera *Anisus*, *Bathyomphalus*, *Choanomphalus*, *Gyraulus*, *Helisoma*, *Planorbella* e *Segmentina* (SOLDATENKO; PETROV, 2009; 2012), of the acroloxid of the genus *Acroloxus* (SOLDATENLO; PETROV, 2019), of the lymneid of the genus *Radix* (SOLDATENKO; PETROV, 2019) and of the fisid of the genus *Physella* (SOLDATENKO; PETROV, 2019), which have two to three layers. Soldatenko and Petrov (2019) observed thin muscular fibers perpendicular among the ejaculatory duct and the penis of *B. glabrata*. This feature was not verified in our material nor was it mentioned by Paraense (1988) for *B. intermedia*, *B. kuhniana* and *B. straminea*.

In this study, we describe, for the first time, the histological aspects of the vagina, vaginal pouch and penial complex of *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis*. The lining epithelial tissue and muscular tissue of the vagina, vaginal pouch and penial complex show a similar pattern in *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis*, except for the intermediate muscular layer of the prepuce, which distinguishes *B. t. guaibensis* from the other two taxa. The architecture of the musculature of the penial complex in *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* differs from that presented by some freshwater snails from the families Planorbidae, Acroloxidae, Lymnaeidae and Physidae.

Studies related to the histological aspects of the penial complex and vagina region of other *Biomphalaria* species are necessary in order to confirm the taxonomic importance of the characters in determining the taxa belonging to the genus.

Acknowledgements

The authors are grateful for the financial supported received from FAPESP (process number 2018/05360-9).

References

ABDEL-MALEK, E. T. The preputial organ of snails in the genus *Helisoma* (Gastropoda. Pulmonata). **American Midland Naturalist**, Notre Dame, v. 48, p. 94-102,1952.

ABDEL-MALEK, E. T. Morphological studies on the family Planorbidae (Mollusca: Pulmonata). I. Genital organs of *Helisoma trivolvis* (Say) (Subfamily Helisomatinae FC Baker, 1945). **Transactions of the American Microscopical Society**, San Francisco, v. 73**,** p. 103-124, 1954a.

ABDEL-MALEK, E. T. Morphological studies on the family Planorbidae (Mollusca: Pulmonata). II. The genital organs of *Biomphalaria boissyi* (subfamily Planorbinae, HA Pilsbry, 1934). **Transactions of the American Microscopical Society**, San Francisco, v. 73, p. 285-296, 1964b.

BAKER F. C. **The molluscan Family Planorbidae.** Chicago: University of Illinois Press, 1945. 530 p.

BRASIL. **Vigilância e controle de moluscos de importância epidemiológica, diretrizes técnicas:** Programa de Vigilância e Controle da Esquistossomose (PCE). Brasília: Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica, 2008. 178 p.

CARVALHO, O. D. S.; SCHOLTE, R. G. C.; AMARAL, R. S.; DUTRA, L. V.; GUERRA, M. A. M. Distribuição espacial de *Biomphalaria glabrata*, *B. straminea* e *B. tenagophila*, hospedeiros intermediários do *Schistosoma mansoni* no Brasil. In: CARVALHO, O. S.; COELHO, P. M. Z.; LENZI, H. L. (Ed.). ***Schistosoma mansoni* e esquistossomose:** uma visão multidisciplinar. Rio de Janeiro: Fiocruz, 2008. p. 393-418.

HOLM, L. W. Histological and functional studies on the genital tract of *Lymnaea stagnalis appressa* Say. **Transactions of the American Microscopical Society**, San Francisco, v. 65, p. 45-68, 1946.

OHLWEILER, F. P.; ROSSIGNOLI, T. J.; PALASIO, R. G. S.; TUAN, R. Taxonomic diversity of *Biomphalaria* (Planorbidae) in São Paulo state. Brazil. **Biota Neotropica**, Campinas, v. 20, e20200975, 2020.

PAN, C. T. The general histology and topographic microanatomy of *Australorbis glabratus*. **Bulletin of the Museum of Comparative Zoology at Harvard**, Harvard, v. 119, p. 237-300, 1958.

PARAENSE, W. L. *Biomphalaria kuhniana* (Clessin, 1883), planorbid mollusc from South America. **Memórias do Instituto Oswaldo Cruz**, Rio de Janeiro, v. 83, p. 1-12, 1988.

PARAENSE, W. L. Histórico do gênero *Biomphalaria*, morfologia e sistemática morfológica. In: CARVALHO, O. S.; COELHO P. M. Z.; LENZI, H. L. (Ed.). ***Schistosoma mansoni*** **e esquistossomose –** Uma visão multidisciplinar. Rio de Janeiro: Fiocruz, 2008. p. 285-308.

PARAENSE, W. L.; DESLANDES, N. Observation on the morphology of *Australorbis glabratus*. **Memórias do Instituto Oswaldo Cruz**, Rio de Janeiro, v. 53, p. 87-103,1955.

SOLDATENKO, E. V.; PETROV, A. A. The characteristics of copulation in *Segmentina oelandica* (Westerlund, 1885) (Gastropoda: Pulmonata: Planorbidae). **Zoosystematica Rossica**, San Petersburgo, v. 8, p. 196-204, 2009.

SOLDATENKO, E. V.; PETROV, A. A. Mating behaviour and copulatory mechanics in six species of Planorbidae (Gastropoda: Pulmonata). **Journal of Molluscan Studies**, London, v. 78, p. 185-196, 2012.

SOLDATENKO, E. V.; PETROV, A. A. Musculature of the penial complex: A new criterion in unravelling the phylogeny of *Hygrophila* (Gastropoda: Pulmonata). **Journal of Morphology**, Temuco, v. 280, p. 508-525, 2019.

SOLDATENKO, E. V.; SHATROV, A. B. Comparative ultrastructure of the terminal portions of the male copulatory apparatus in Planorbidae (Gastropoda: Pulmonata**). Invertebrate Reproduction & Development**, London, v. 57, p. 224-236, 2013.

SPATZ, L.; VIDIGAL, T. H.; CALDEIRA, R. L.; NETO, E. D.; CAPPA, S. M. G.; CARVALHO, O. S. Study of *Biomphalaria tenagophila tenagophila*, *Biomphalaria tenagophila guaibensis* and *Biomphalaria occidentalis* by polymerase chain reaction amplification and restriction enzyme digestion of the ribosomal RNA intergenic spacer regions. **Journal of Molluscan Studies**, London, v. 65, 143-149, 1999.