



HISTOLOGICAL STRUCTURE OF THE DISTAL PORTION OF GENITALIA IN *TROGLAEGOPIS MOSORENSIS* (KUŠČER, 1933) (GASTROPODA: PULMONATA: ZONITIDAE)

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ABSTRACT: The histological structure of vagina with perivaginal gland, penis, bursa copulatrix and sarcobelum of *Troglaeopis mosorensis* (Kuščer) is described.

KEY WORDS: land snails, Zonitidae, *Troglaeopis mosorensis*, genitalia, histological structure

INTRODUCTION

Distal portions of female genitalia of most zonitids bear various glandular structures. The so called perivaginal gland is well-developed in most species. It usually surrounds vagina, though in some species it also envelopes spermatheca and oviduct (RIEDEL 1980, 1998). However, members of some genera have more complicated accessory organs of glandular nature (RIEDEL 1980, 1982, 1993).

Transformations of glandular structures within the Zonitidae are of great importance for phylogenetic inferences. SHILEYKO (1991) regards such structures as the ancestral morphological material for evolutionary development of a variety of accessory

organs. However, it is difficult to homologize any glandular structures and organs without histological evidence.

The few existing studies on the histological structure of reproductive organs in members of the Zonitidae (RIGBY 1963, SELIVANOVA & ZHILTSOV 1999) do not cover all the morphological diversity within the group. The main goal of this paper is to describe the histological structure of the distal portion of genitalia (penis, vagina, spermatheca and sarcobelum) in a member of a monotypic genus *Troglaeopis* – *T. mosorensis* (Kuščer, 1933).

MATERIAL AND METHODS

Three specimens of *T. mosorensis* were collected by Dr. A. RIEDEL in the Mosor Mts, Croatia: Kotlenica near Split, a cave Spilja Vranjaca, on May 11th 1999. The specimens were preserved in 4% formaldehyde, dehydrated in a graded alcohol series and embedded in paraffin. Serial sections 6–7 µm thick were mounted on slides, stained with

hematoxylin and eosin and examined in optic microscope.

The following abbreviations have been used in the figure captions: BC – spermatheca, SMF – circular muscle fibres, ED – efferent duct of the gland, EPS – external part of sarcobelum sheath, GC – glandular cells, GL – glandular layer, IPS – inner part of

sarcobelum sheath, LMF – longitudinal muscle fibres, LPS – lumen of penial sheath, ML – muscular layer, P – penis, PC – petal-shaped cells, PP – penial papilla,

PS – penial sheath, PV – perivaginal gland, PVD – vas deferens papilla, Sb – sarcobelum, Su – sinuses.

RESULTS AND DISCUSSION

The distal portion of genitalia in *T. mosorensis* is of the following morphological structure (see also RIEDEL & RADJA 1983) (Fig. 1). The penis is 2.5 times longer than the vagina and oviduct combined. It is cylindrical and covered with a well-developed penial sheath (Fig. 2A). The vas deferens opens to the penis apically, piercing the penial sheath, and forms a papilla. There is no epiphallus. The genital atrium is short. The vagina is thicker than the oviduct and partly enveloped by the perivaginal gland. The gland covers also the base of the spermatheca duct. The spermatheca is small; its duct is of medium length, with a strong distal dilation. The sarcobelum opens into the vagina. It is covered with a sheath and its length approximately equals that of the penis. The histological structure of these organs is described below.

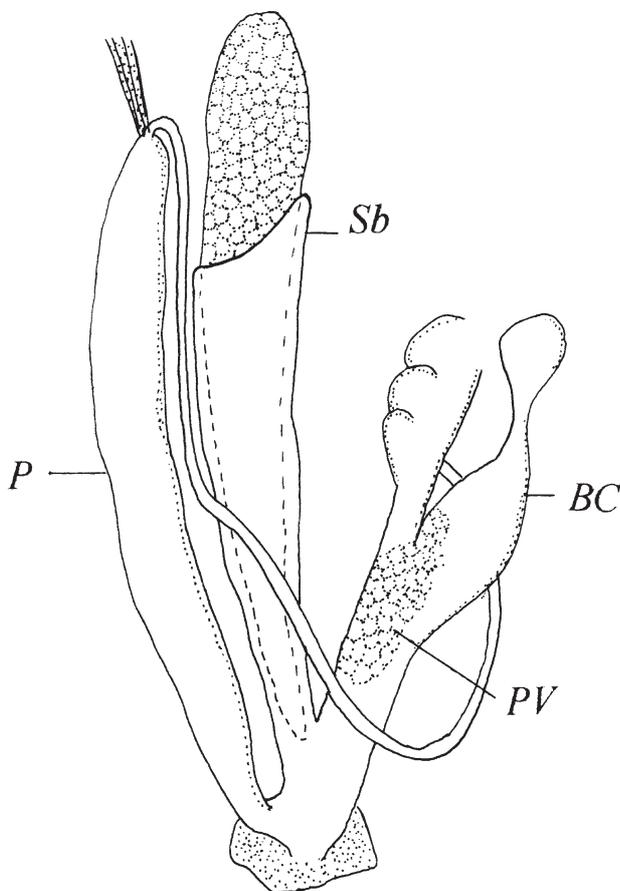


Fig. 1. Distal portion of genital apparatus of *T. mosorensis*, diagrammatic

PENIS

The penial sheath is muscular. Its fibres are variously arranged, though three layers can be distinguished: 1. internal layer with longitudinal muscle fibres, 2. the thickest, intermediate layer with mainly circular and some oblique fibres, 3. external layer with longitudinal fibres.

The penis sheath is closed at its proximal end (proximal-distal is here used in relation to the gonad). It is the thickest in its middle, becomes thinner towards atrium and merges with its wall. The sheath is lined with a squamous epithelium and covered with strengthening connective tissue elements. The portion of penis from its base to the vas deferens papilla is lined with a simple epithelium. The epithelial cells vary in height from tall columnar to cubical; as a result of the varied height of the cells the penial lumen is irregular. The nuclei of the epithelial cells are chromatin-rich. They are large and basally located. In some of the cells mitotic figures could be observed near the basal membrane. The epithelium is surrounded by a layer of connective tissue and muscular elements. Collagen fibres reinforce the penial sheath from the outside.

The differences in height between the epithelial cells become more pronounced in the more distal portions of the penis. The lumen of the latter becomes star-shaped in cross section (Fig. 2B–G). The external layer of the penial sheath becomes thinner.

The epithelium changes its character at the level of the base of vas deferens papilla: the cells become more uniform in height, with vacuolized cytoplasm and corrugated nuclei; the mitotic figures disappear.

The vas deferens papilla increases distally and completely surrounds the most proximal part of the penis (Fig. 2I–J). Its inner surface is lined with a tall columnar epithelium of different character: the nuclei are basally located and chromatin-rich in the wall adjoining penis; some of them are hypertrophied. On the outer wall there are areas of damaged epithelium: groups of cells without a distinct structure.

Two internal longitudinal folds are situated opposite each other in the penis below the vas deferens papilla, one being more massive than the other (Fig. 3A, D). Their presence makes the penial lumen butterfly-shaped. One of the folds flattens distally while the other does not change its shape. Both folds are covered with villi which make their surface irregular (Fig. 2B–G).

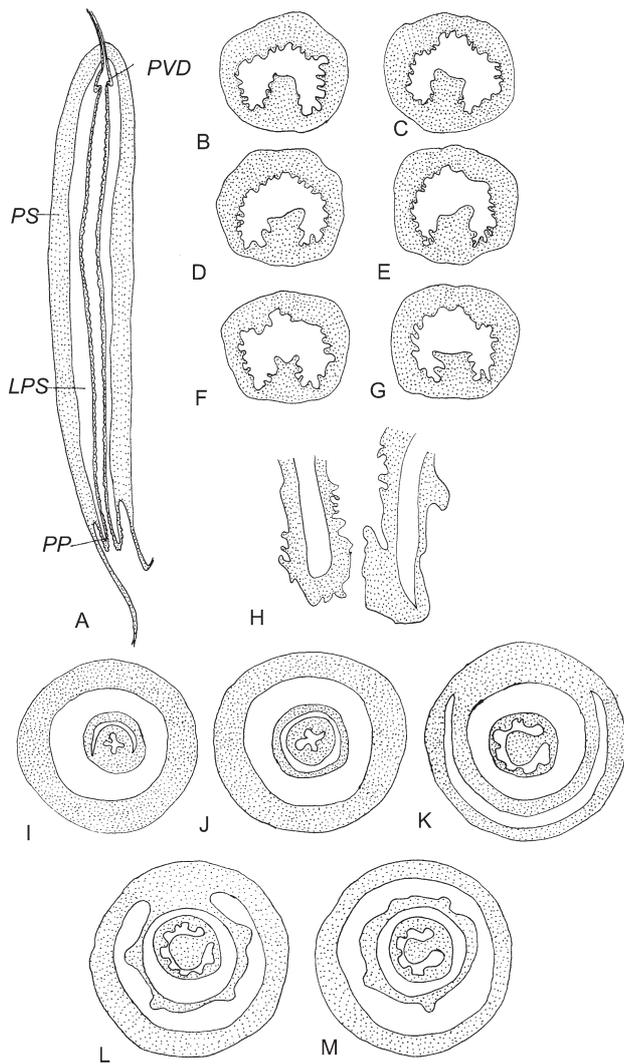


Fig. 2. Penis of *T. mosorensis*, diagrammatic: A – longitudinal section, B–G – consecutive cross sections through the mid part of penis (penial sheath omitted), H – longitudinal section of penial papilla, I–J – consecutive cross sections through the penis at the level of vas deferens papilla, K–M – consecutive cross sections through the penis at the level of papilla base

The inner surface of penis is lined with similar but smaller villi. Their connective tissue base consists of regularly arranged cells separated by collagen fibres. Due to this structure the villus is rather firm.

The lining of penis lumen consists of a simple epithelium. It is cubical in areas between the folds and villi and tall columnar at the base of the folds. The nuclei of the tall columnar cells are obliquely oriented. The cells become flattened on the slopes of the folds while the fold ridge is covered with flat cells which, however, do not constitute a squamous epithelium. The cells are twice wider than high and their nuclei are oriented parallelly to the basal membrane.

The form of the larger fold changes near the middle of the penis length, the fold becoming mushroom-

shaped in cross section. The fold at that level consists of connective tissue elements. There are various cells and muscle fibres of regular arrangement – running across the fold (Fig. 3B, E). The epithelium lining generally resembles that described above. The main difference is that the nuclei of the cells of the fold ridge are complex: cruciform i.e. segmented to various degree.

The direction of the fibres changes in the mushroom-shaped fold of the distalmost portion of the penis (Fig. 2C, F). They are longitudinal at the base of the fold, and running transversely at the ridge;

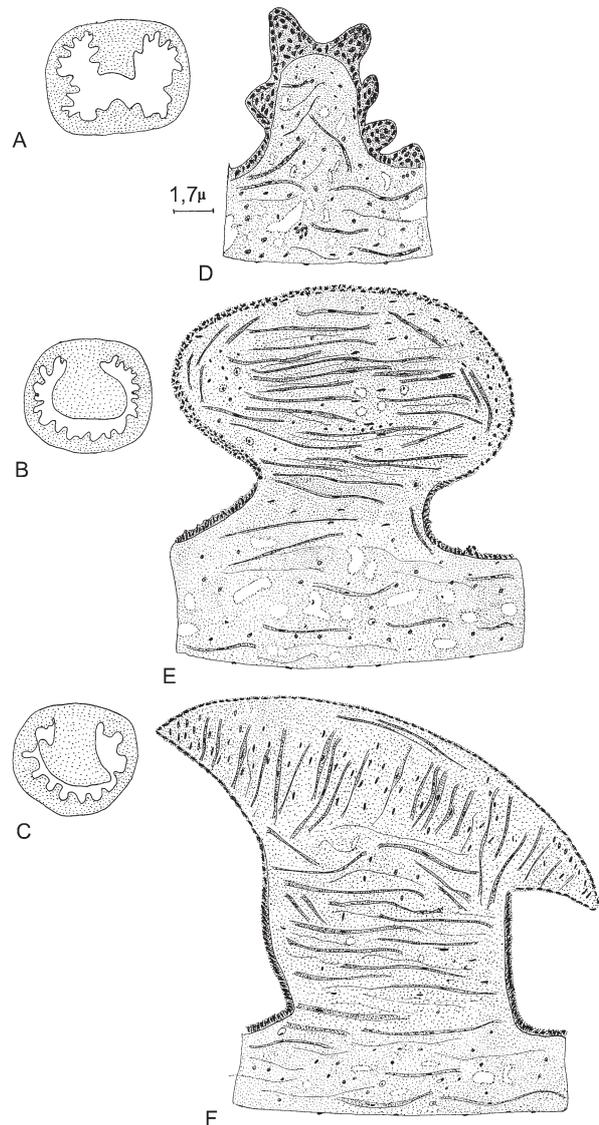


Fig. 3. Internal structure of penis of *T. mosorensis*: A – cross section through the mid part of penis (penial sheath omitted), B – cross section through the distal part of penis (penial sheath omitted), C – cross section through the penis (penial sheath omitted) just next to the base of penial papilla; A–C diagrammatic; D–F – histological structure of the penial fold, cross sections at corresponding levels

there are also some oblique fibres. Likewise, the character of villi is different: they become wider, but the orientation of the cells in the villus stroma remains transverse.

The penis terminates with the penial papilla (Fig. 2A, H, K–M), the mushroom-shaped fold disappearing just before the papilla. The villi become more slender again. They cover not only the inner surface of the papilla (its lumen) but also its outer surface (Fig. 2H).

SPERMATHECA AND VAGINA

The spermatheca lumen is lined with a multi-layer epithelium (Fig. 4A, G), with alternating areas of different height. Tall columnar cells predominate, with processes on their tops. There are also small cells with chromatin-rich nuclei located near the basal membrane. A very thin layer of connective tissue with circular fibres surrounds the lumen.

The epithelium becomes simple on the border between the spermatheca container and duct (Fig. 4B, C, H). Its cells vary in height making the duct lumen irregular. The outer layer of the spermatheca duct wall is thicker due to better developed circular muscles.

In more distal portion of the duct, longitudinal folds appear (Fig. 4D). Their base consists of connective tissue abounding with cells. Some sinuses and numerous muscular fibres appear in the duct wall (Fig. 4H). A layer of loose connective tissue with empty cells appears also below the epithelium.

In the expanded part of spermatheca duct there are also high internal folds which are covered with tall columnar cells of elongated nuclei (Fig. 4E, F, J, K), with connective tissue located at the base of these cells. It is noteworthy that the epithelial lining of the duct is distinctly basophilic. The duct lumen is irregular because of the folds. In the connective tissue below the epithelium there are numerous vesicular cells and sinuses. Many longitudinal muscle fibres appear in the muscular layer externally to circular fibres. Their contents in the expanded part of the spermatheca duct is basophilic.

The folds decrease in height just before junction of the spermatheca duct and the vagina. Cells of the perivaginal gland are developed on the distalmost portion of the duct (Fig. 4F, J). The gland is well-developed on the vagina mostly, but it does not surround it entirely. The gland consists of various secretory cells. Some are large, with hypertrophied, chromatin-rich nuclei. Their cytoplasm is filled entirely or partly with secretion granules. Other cells have degenerative nuclei and no secretion in their cytoplasm. A connective tissue stroma is clearly visible in the gland.

The vaginal lumen is lined with basophilic tall columnar cells. Together with connective tissue they

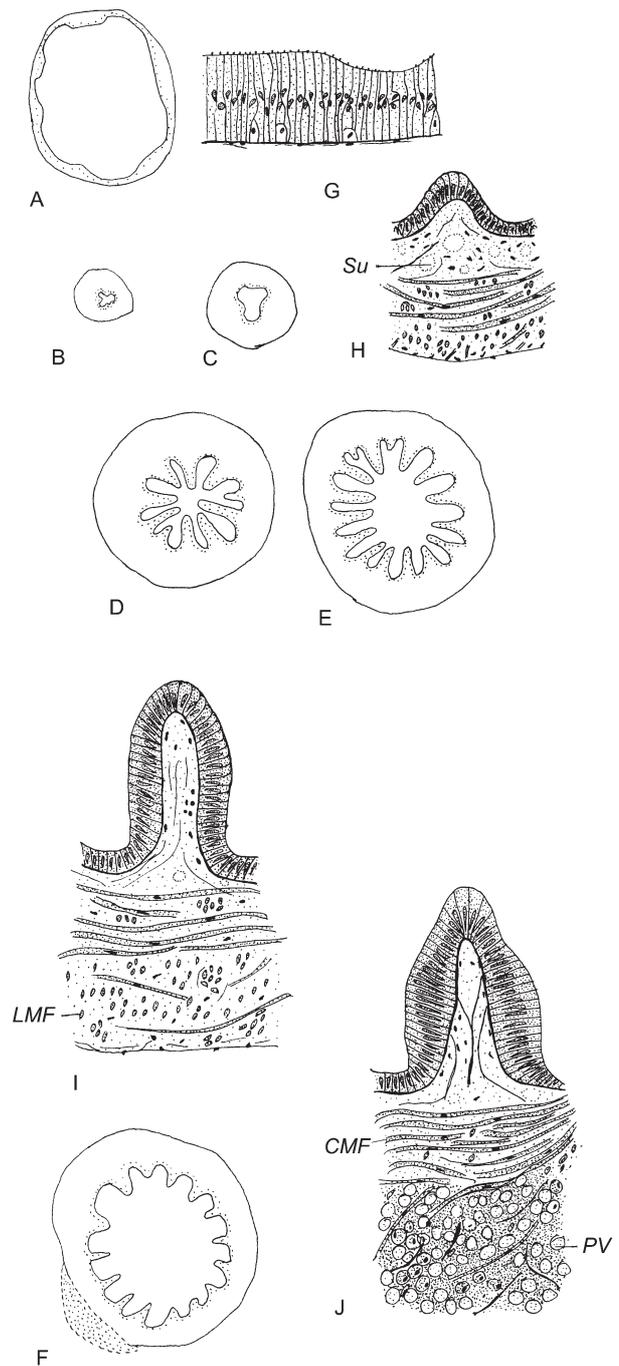


Fig. 4. Structure of spermatheca of *T. mosorensis*: A – cross section through the spermatheca lumen, B–D – cross sections through the proximal part of spermatheca duct, E–F – cross sections through the expanded part of spermatheca duct; A–F diagrammatic; G–J – histological structure of the spermatheca wall at levels corresponding to A–F

form small longitudinal folds. Some basophilic contents is visible in the vaginal lumen. The wall of vagina contains few muscular elements. The perivaginal gland is united with the vagina, both having a common connective tissue base.

SARCOBELUM

The inner wall of the upper part of sarcobelum consists of an accumulation of glandular cells disposed on the basis of connective tissue stroma, the elements of connective tissue being intertwined with the muscular layer of sarcobelum. The stroma consists not only of fibres but also of cells. Some collagen fibres are visible on the outside of the gland, supporting it.

The secretory cells are oval and contain a fine-grained basophilic cytoplasm. Their nuclei are chromatin-rich and pressed to the cell periphery. Other secretory cells have their cytoplasm partly or entirely free of the fine-grained contents. Their nuclei are rounded and centrally located. Some cells have vacuolized cytoplasm (Fig. 5).

The glandular cells are actually united in lobules separated by collagen fibres. Probably each lobule has its own efferent duct piercing the muscular layer and opening into the sarcobelum lumen. Such ducts were quite often observed on the slides. They have no epithelial lining.

Below the glandular layer there is a well-developed muscular layer. It consists mainly of circular muscles but oblique fibres are present as well, so that the layer has a reticulate structure. Sinuses and collagen fibres are developed in that layer, ensuring its elasticity and resistance.

The innermost layer of the sarcobelum wall consists of longitudinal folds whose radially arranged branches reach almost to the centre of sarcobelum lumen and fill it almost entirely. Connective tissue lies at the base of the folds, the cells becoming increasingly more elongate and narrower at base (petal-shaped, especially at the ridges of the folds) towards the lumen centre. The cytoplasm of the fold-forming cells degenerates into a granular basophilic mass. There are low folds between the higher

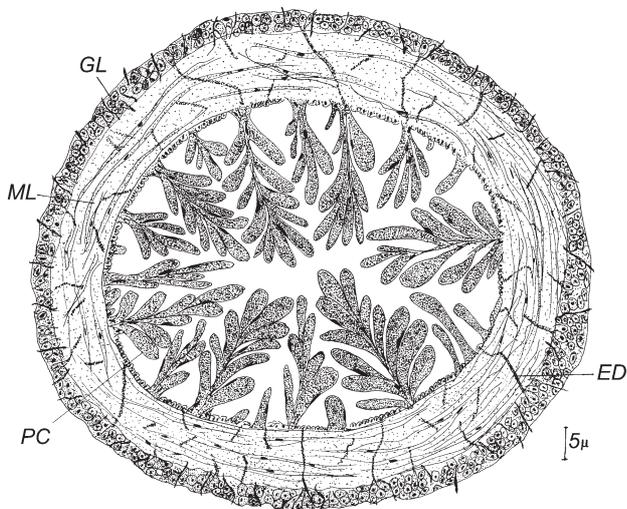


Fig. 5. Histological structure of sarcobelum of *T. mosorensis*; cross section through the apical part

ones, both kinds being radially arranged. The inner surface of the sarcobelum wall between the folds is covered by columnar epithelium.

The sarcobelum sheath starts at one third its length from the apex and consists of two parts. They are directed towards each other, and their epithelium is built of low cubical cells. A layer of connective tissue with isolated muscle fibres lies below the epithelium. Distally the sheath becomes better developed, enveloping the sarcobelum entirely and forming its outer wall (Figs 6–8).

The glandular tissue disappears gradually in distal direction. Some cells or their groups are embedded in the muscular layer, so that the glandular tissue becomes diffuse (Figs 7, 8A). It should be noted that there are no glandular cells. No gland ducts are visible in the distal part of sarcobelum, contrary to its apex.

The distalmost portion of sarcobelum contains no glandular cells (Fig. 8B); its lining is different as well. The folds decrease in height, their bases become thicker and the number of cells in the folds increases; the lumen becomes larger. The granular degeneration of the cell contents is gradually weaker pronounced, to become completely absent at the end of sarcobelum (Fig. 8B). The folds here consist of connective tissue rich with cells, the lining is built of columnar epithelium.

The outer layer of the distalmost portion of sarcobelum consists of the outer part of the sheath adhering to the sarcobelum proper. The distalmost part of sarcobelum (its papilla) is covered with a stratified epithelium of at least two-three cell layers. The nuclei are irregular, like those of the penial cells.

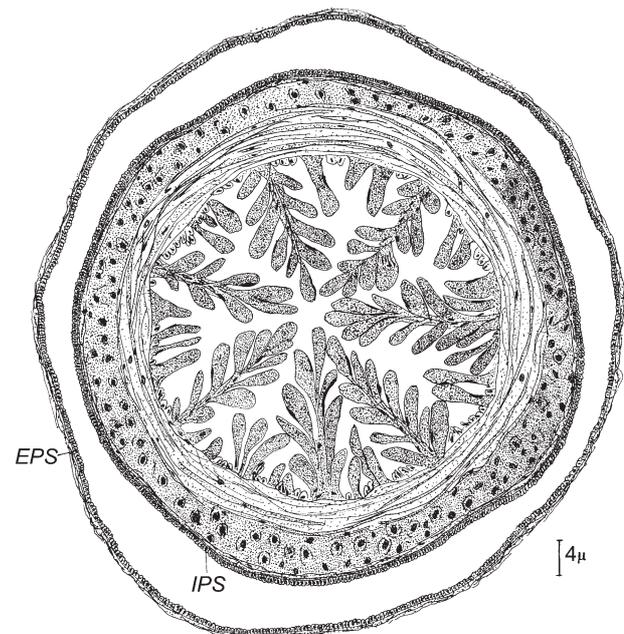


Fig. 6. Histological structure of sarcobelum of *T. mosorensis*, cross section at the level of the upper part of sarcobelum sheath

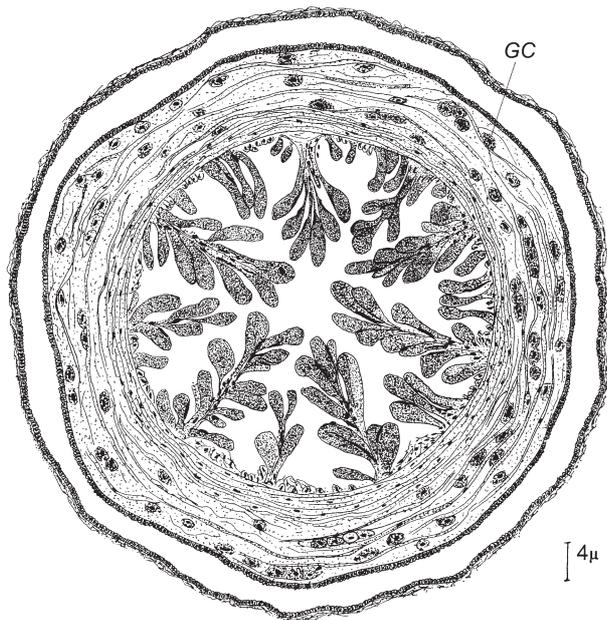


Fig. 7. Histological structure of sarcobelum of *T. mosorensis*, cross section of the mid portion

The outer part of the sheath thickens distally and fuses with the genital atrium wall. It has an epithelial lining that consists of tall cells with nuclei occupying almost entire cell volume and oriented baso-apically.

ACKNOWLEDGEMENTS

We are very grateful to Dr. A. RIEDEL for providing us with the unique material of *Troglaeopsis mosorensis* which he collected especially for this study. We thank Dr. A. N. SUVOROV for his assistance in preparation of this paper, Dr. A. A. SHILEYKO for valuable consultations and O. S. KUGANOVA for her advice regarding histological methods.

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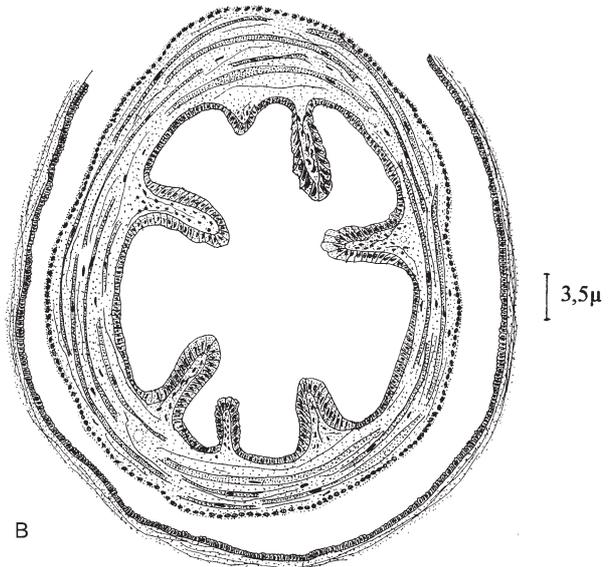
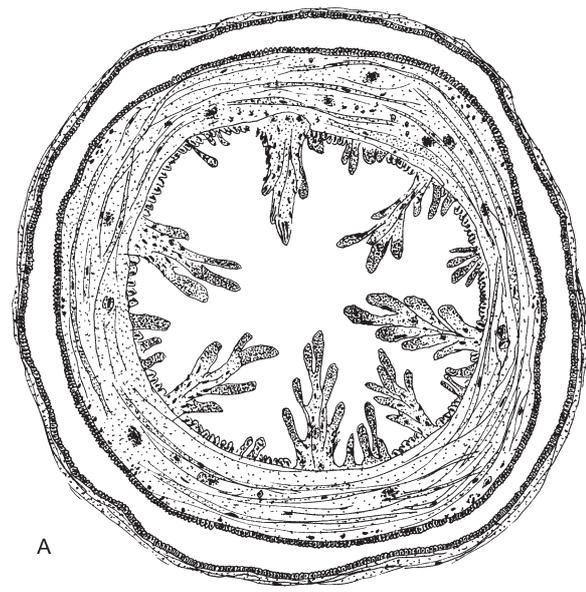


Fig. 8. Histological structure of the distalmost portion of sarcobelum (A) and its papilla (B) of *T. mosorensis*

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received: January 17th, 2000
accepted: March 15th, 2000

