

## MORPHOLOGY OF THE CYSTICERCOID OF HYMENOLEPIS ERINACEI (GMELIN, 1789)

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**Abstract.** The morphology of the cysticeroid of *H. erinacei* has been studied for the first time by histological methods. The cysticeroid consists of a tailed cyst enclosing the neck and scolex of the cestode. The outer limiting layer surrounds the surface of the cyst, neck and scolex. The tegument of the cyst is characterized by circular and longitudinal layers of fine connective tissue fibres. The tegument of the proximal part of neck and scolex is characterized by microtriches. The basement layer is of fibrous character. The subtegument of the cyst, neck and scolex is formed by outer circular and longitudinal muscle and connective tissue layers and a layer of pyriform cells. The subtegument of the cyst is further formed by an inner longitudinal muscle and connective tissue layer with distinctly prevailing connective tissue fibres forming a lamellar structure. Under the subtegument of the cyst is an inner zone consisting of oval nuclei and cytoplasmic processes, without distinct cellular borders. The parenchyma is situated under the subtegument of the neck and scolex. The inner edge of the cyst and neck is bordered by the inner limiting layer.

The cysticeroid of *H. erinacei* develops in various species of beetles of the families Scarabaeidae and Silphidae (Prokopič 1971, Prokopič and Karapchanski 1973, Prokopič and Bílý 1975). The development of larvae was studied by Joyeux (1927) and Prokopič (1971). The latter author studied these larvae both under natural and experimental conditions. This study presents first results concerning the morphology of cysticeroids studied by histological methods. The histochemistry of cysticeroids will be the subject of a further paper.

### MATERIAL AND METHODS

The material was obtained from beetles, *Geotrupes stercorarius* L. (Scarabaeidae), *Oeceoptoma thoracica* (L.), *Necrophorus humator* Goeze, *N. interruptus* Steph. and *N. vespilloides* Herbst (Silphidae). Isolated cysticeroids were fixed in Baker's formaldehyde (Pearse 1968), embedded in paraffin and cut to 6  $\mu\text{m}$  sections. The following histological methods were applied: Mayer's, Böhmer's and Weigert's haematoxylin-eosin, van Gieson's method, Masson's and Goldner's trichrome, Mallory's PTAH method, aldehyde fuchsin, Gomori's impregnation method, PAS reaction and Kossa's method for the detection of calcium (for the description of the methods see Pearse 1968).

### RESULTS

The cysticeroid (Plate I, Figs. 1, 2) consists of a cyst with a characteristic wide, tail-shaped appendage. The cyst cavity contains an invaginated scolex surrounded by neck. The scheme of invagination is shown in the paper by Valkounová and Prokopič (1978).

#### CYST

The cyst (Plate I, Fig. 2) measures 180—288  $\times$  141—248  $\mu\text{m}$ , its wall being 12—20  $\mu\text{m}$  (rarely up to 30  $\mu\text{m}$ ) thick. The tail measures 76—289  $\times$  46—148  $\mu\text{m}$ . The surface of

the cyst and tail is covered by a 1  $\mu\text{m}$  thick outer limiting layer, which is refractive and often damaged or absent. It was not stained by any of the methods used. The tegument is 1  $\mu\text{m}$  thick and consists of an amorphous substance. In the outer part of this substance are fine circular and longitudinal connective tissue fibres reaching approximately 1/3—1/2 of tegument width. They are slightly undulated in section material (Plate III, Fig. 1, Plate IV, Figs. 1, 2) and stained rose by haematoxylin-eosin and van Gieson's method, faint red-violet by Masson's and Goldner's trichrome, black with Gomori's method and red by PAS method. The amorphous substance of the tegument is stained faint pink by haematoxylin-eosin and Masson's trichrome, faint red by Goldner's trichrome, blue by Mallory's PTAH method and deep pink by Gomori's method. The tegument of the tail gradually tapers, being one half thinner at the distance of 20—100  $\mu\text{m}$  from the cyst. At the distance of 150 to 250  $\mu\text{m}$  from the cyst it is sometimes invisible in the light microscope. Also the fibrils of the tail tegument are poorly visible and are stained only slightly. The basement layer situated under the tegument is 0.5  $\mu\text{m}$ \*) thick, of fibrous character and is stained blue by Masson's trichrome and black by Gomori's method. Fine processes of this layer were visible among the circular fibrils of the subtegument in some sections stained with Masson's trichrome. The basement layer in the tail gradually tapers so that it is invisible in its posterior part.

The subtegument consists of outer circular and longitudinal muscle and connective tissue layer, middle cellular layer and inner longitudinal muscle and connective tissue layer. The outer circular fibres and longitudinal fibres situated lower are regularly arranged (Valkounová and Prokopič 1978). The connective tissue fibres (Plate III, Figs. 1, 2, Plate IV, Figs. 1, 2) measure 1—2  $\mu\text{m}$  in diameter, as well as the distance between them (depending on dilatation or contraction of the cyst). They are stained pink by haematoxylin-eosin and by van Gieson's method, blue by Masson's trichrome and green by Goldner's trichrome. The ground substance of these fibres is stained deep pink by Gomori's method, fine fibrils situated in it stain gray-black and fine fibrils on the surface stain black. The muscle fibres run in parallel with connective tissue fibres and reach 0.5  $\mu\text{m}$  in diameter. They are stained red by haematoxylin-eosin and by Masson's and Goldner's trichrome, yellow by the method of van Gieson, pink by PAS method and blue-gray by Gomori's method. The cellular layer consists of a dense layer of pyriform cells (Plate III, Fig. 1) extending up to the tegument by their anterior narrowed part put into the spaces formed by crossing circular and longitudinal fibres (Valkounová and Prokopič 1978). The size of cells depends on the dilatation or contraction of their narrowed part. They measure 8—16  $\times$  4—8  $\mu\text{m}$ , their nucleus 3  $\times$  3—4  $\mu\text{m}$  and nucleolus 1  $\mu\text{m}$ . The central plasma of the widened part of cells is very granulated. The nucleus is situated excentrically near the lower margin of the cell and it is rich in chromatin. The nucleolus is situated excentrically near the lower margin of the nucleus. The pyriform cells are stained like the tegument, i.e., blue by Mallory's PTAH method. Older cysts contain pyriform cells with non-granulated plasma and with picnotic nuclei staining blue-black by haematoxylin-eosin and by Gomori's method. Among the pyriform cells there are calcareous bodies measuring 2—4  $\mu\text{m}$  in diameter, spherical or elliptical nuclei measuring 3  $\times$  2—3  $\mu\text{m}$  with a distinct nucleolus, and plasma situated around the nuclei and without distinct cellular border. The cellular layer contains numerous connective tissue and muscle fibres connected with fibrous layers of the tegument and subtegument. Most numerous are fine fibres occurring in the tegument and forming a dense network on the surface of pyriform cells. The inner longitudinal

\*) Values lower than 1  $\mu\text{m}$  cannot be considered exact, but they may help the reader to make a better idea about the size of the studied material.

muscle and connective tissue layer (Plate II, Figs. 1, 2, Plate III, Figs. 1, 2) situated under the cellular layer is 2—4  $\mu\text{m}$  thick. There prevail distinctly connective tissue fibres forming a lamellar structure. The muscle fibres occur rarely and are of the same thickness as those in the outer fibrous layer. This layer is stained most intensely by Masson's blue trichrome, the other methods produce the same staining as in the outer fibrous layer of the subtegument. Numerous spindle-shaped nuclei of fibroblasts lying in longitudinal direction were observed among the fibres. They measure  $4-8 \times 1-2 \mu\text{m}$  and contain a small nucleolus measuring 1  $\mu\text{m}$  in diameter. A dispersed chromatin is sparsely distributed in the nucleus. The subtegument extends into the tail by the outer circular and longitudinal muscle and connective tissue layer and by the cellular layer, the plasmatic network of which with nuclei and numerous fibres fills its middle part around the longitudinal axis. It is stained more slightly than analogous layers in the cyst wall. In the proximal part of tail, with the increasing length the outer muscle connective tissue layer gradually decreases and the pyriform cells become smaller so that they are no more visible in the distal part of tail when observed in the light microscope. Under the inner longitudinal fibrous layer of the subtegument follows a 1—4  $\mu\text{m}$  thick inner zone (Plate II, Figs. 1, 2, Plate III, Fig. 1) which cannot be exactly identified in the light microscope. It contains an excess of nuclei and plasma, but without distinct cellular borders. The nuclei are oval, measuring  $4 \times 2 \mu\text{m}$ , without chromatin and with centrally situated nucleolus measuring 1  $\mu\text{m}$  in diameter. At the site of invagination the outer part of this layer is of a fibrous character, the inner part forms distinct plasmatic lines. The whole layer is stained rose-red, slight rose or is not stained at all by haematoxylin-eosin, slightly red by Masson's and Goldner's trichrome and slightly blue by Gomori's method. The inner limiting layer lines the cyst cavity, it is strongly refractive, 1  $\mu\text{m}$  thick and is not stained by any of the methods used. Neither the inner zone nor the inner limiting layer reach the tail.

## NECK

The neck (Plate I, Figs. 1, 2) is situated in the cyst cavity in such a manner that it surrounds the scolex and their outer limiting layers are adjacent to one another. It is 11—40  $\mu\text{m}$  long reaching the maximum thickness at the site of invagination. The outer limiting layer has the same properties as the corresponding layer of the cyst and it is 1  $\mu\text{m}$  thick. The tegument (Plate II, Fig. 2) reaches the maximum width (1.5  $\mu\text{m}$ ) in the proximal part of the neck (continuation of scolex tegument); in the distal part it is 1  $\mu\text{m}$  thick. Microtriches were found in the proximal part of the neck. Immediately behind the scolex they are 1  $\mu\text{m}$  long, then they become gradually shorter and disappear approximately in the midlength of the neck. The tegument consists of an amorphous substance only (unlike in the cyst tegument, no fine connective tissue fibrils were observed). The microtriches are obviously on the whole surface of neck tegument, though they are invisible in the light microscope in the distal part (Valkounová and Prokopič 1978). The microtriches are stained purple by haematoxylin-eosin, red by Masson's and Goldner's trichrome and yellow by the method of van Gieson. The amorphous substance is stained in the same way as that in the cyst tegument. The basement layer is approximately 0.5  $\mu\text{m}$  thick, it is stained light blue by Masson's trichrome and dark grey by Gomori's method. The subtegument consist of an outer fibrous and an inner cellular layer. Both layers are arranged in the same way as in the cyst subtegument. Only the connective tissue fibres are 1  $\mu\text{m}$  thick and the pyriform cells measure  $4-8 \times 3-4 \mu\text{m}$ , their nuclei 2  $\mu\text{m}$  and nucleoli approximately 0.5  $\mu\text{m}$ . Longitudinal layer of fine connective tissue fibres measuring about 1  $\mu\text{m}$  was observed on the inner side of subtegument in some sections at high magnification. A layer of parenchyma was

observed under the subtegument. It was 3—30  $\mu\text{m}$  thick in the proximal part of neck and 5—50  $\mu\text{m}$  thick in the distal part of neck (the site of invagination). Branching excretory canals measuring 2  $\mu\text{m}$  in diameter were found in the parenchyma. Among the cells of subtegument and parenchyma are calcareous bodies measuring 2—4  $\mu\text{m}$  in diameter, which are most numerous at the site of invagination and at the border between the scolex and neck.

## SCOLEX

The scolex (Plate I, Figs. 1, 2) is oval or spherical, measuring 114—150  $\times$  110—135  $\mu\text{m}$ . The outer limiting layer is 1  $\mu\text{m}$  thick and often damaged, particularly on the surface of suckers. The tegument is 2—3  $\mu\text{m}$  thick and the microtriches are visible on its whole surface. On the suckers they measure 2  $\mu\text{m}$  and on the remaining parts 1  $\mu\text{m}$ . The amorphous substance of the tegument is 1  $\mu\text{m}$  thick. The microtriches and the amorphous substance are stained in the same manner as in the neck wall. The basement layer is of the same thickness and properties as that in the wall of neck and of cyst. The subtegument of scolex is the same as the subtegument of neck, only the pyriform cells are more sparse. The remaining part of the scolex is filled with a parenchyma containing numerous muscle and connective tissue fibres, particularly in the rostellum (size of rostellum 64—79  $\times$  42—56  $\mu\text{m}$ ) and in the suckers (size of suckers 57—76  $\times$  38—68  $\mu\text{m}$ ). The calcareous bodies measure 3—5  $\mu\text{m}$  and they are situated mostly in the parenchyma of the distal part of scolex, where also branching excretory canals of 2  $\mu\text{m}$  in diameter were observed.

## DISCUSSION

The cysticercoïd of *H. erinacei* resembles those of *H. diminuta* (Rudolphi, 1819) and of *Raillietina cesticiillus* (Molin, 1858) in its morphology and structure. The cysticercoïd of *H. diminuta* was described by Voge (1960a) and Ubelaker et al. (1970) and the cysticercoïd of *R. cesticiillus* was described by Voge (1960b) and Baron (1971). Voge (1960a, b) studied the cysticercoïds in series of histological sections using a light microscope, whereas the other authors, in addition to light microscopic observations studied also their ultrastructure.

The cyst tegument is termed "external membrane" by Voge (1960a, b) and "globular layer" by Baron (1971). Ubelaker et al. (1970) found a thin fibrous layer which they termed "terminal filamentous web" in the cyst tegument. Baron (1971) in his electron microscopic studies described "microtrix-like projections" on the surface of cyst tegument. Ubelaker et al. (1970) termed them "microvilli" (the tegument surface of the mentioned cysticercoïds is dealt with in the paper by Valkounová and Prokopič 1978).

Voge (1960b) observed circular and longitudinal fibrous layer in the outer part of cyst subtegument in *R. cesticiillus*. Baron (1971) found only a circular fibrous layer in the light microscope. During electron microscopic examinations he observed both layers, but the longitudinal layer was on the surface. Neither Voge (1960b) nor Baron (1971) mentioned muscle fibres in these layers. Ubelaker et al. (1970) described "circular muscle bundles" on the surface of the cyst subtegument in *H. diminuta*. The cellular part of the subtegument corresponds to "peripheral layer" and "intermediate layer" in the description by Voge (1960a) and to "intermediate layer" described by the same author (Voge 1960b). The peripheral layer consists of pyriform or flask-shaped cells which have the similar properties as the pyriform cells of *H. erinacei*. Lumsden (1968) found "flask cells" in the subtegument of adult *H. diminuta*; *Lacistorhynchus tenuis* (v. Beneden, 1858) and *Calliobothrium verticillatum* (Rudolphi, 1819), which conform to the

description of pyriform cells of the cysticercoïd of *H. erinacei*. The inner longitudinal fibrous layer and inner zone of the cyst was described by Voge (1960a, b), Baron (1971) and Ubelaker et al. (1970). The term "inner zone" was taken from Ubelaker's description, as it cannot be exactly defined in the light microscope. Voge (1960a, b) terms this layer "lining of the cavity" and Baron (1971) "electron-dense membranes". We have not used the term proposed by Voge, because the lining of the cavity forms the inner limiting layer which was observed under the inner zone. The term used by Baron is suitable for electron microscopic studies.

**Acknowledgement.** We wish to thank Dr. S. Bílý, C.Sc. of the Department of Entomology, National Museum, Prague for his help in collecting the material.

## МОРФОЛОГИЯ ЦИСТИЦЕРКОИДА *HYMENOLEPIS ERINACEI* (GMELIN, 1789)

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**Резюме.** Морфологию цистицеркоида *H. erinacei* изучали в первый раз с помощью гистологических методов. Цистицеркоид состоит из хвостатой цисты, содержащей шейку и сколекс цестоды. Внешний ограничивающий слой окружает поверхность цисты, шейки и сколекса. Тегумент цисты характеризуется круговыми и продольными слоями тонких волокон соединительной ткани; тегумент проксимальной части шейки и сколекса характеризуется микротрихами. Базальный слой фиброзного характера. Субтегумент цисты, шейки и сколекса образован внешним слоем круговых и продольных мышц и соединительной ткани и слоем грушевидных клеток. Субтегумент цисты состоит из внутреннего слоя продольных мышц и соединительной ткани с выразительно преобладающими волокнами соединительной ткани, образующими ламеллярную структуру. Под субтегументом цисты внутренняя зона, состоящая из овальных ядер и плазматических отростков, без выразительного клеточного ограничения. Паренхима под субтегументом шейки и сколекса. Внутренний край цисты и шейки ограничен внутренним ограничивающим слоем.

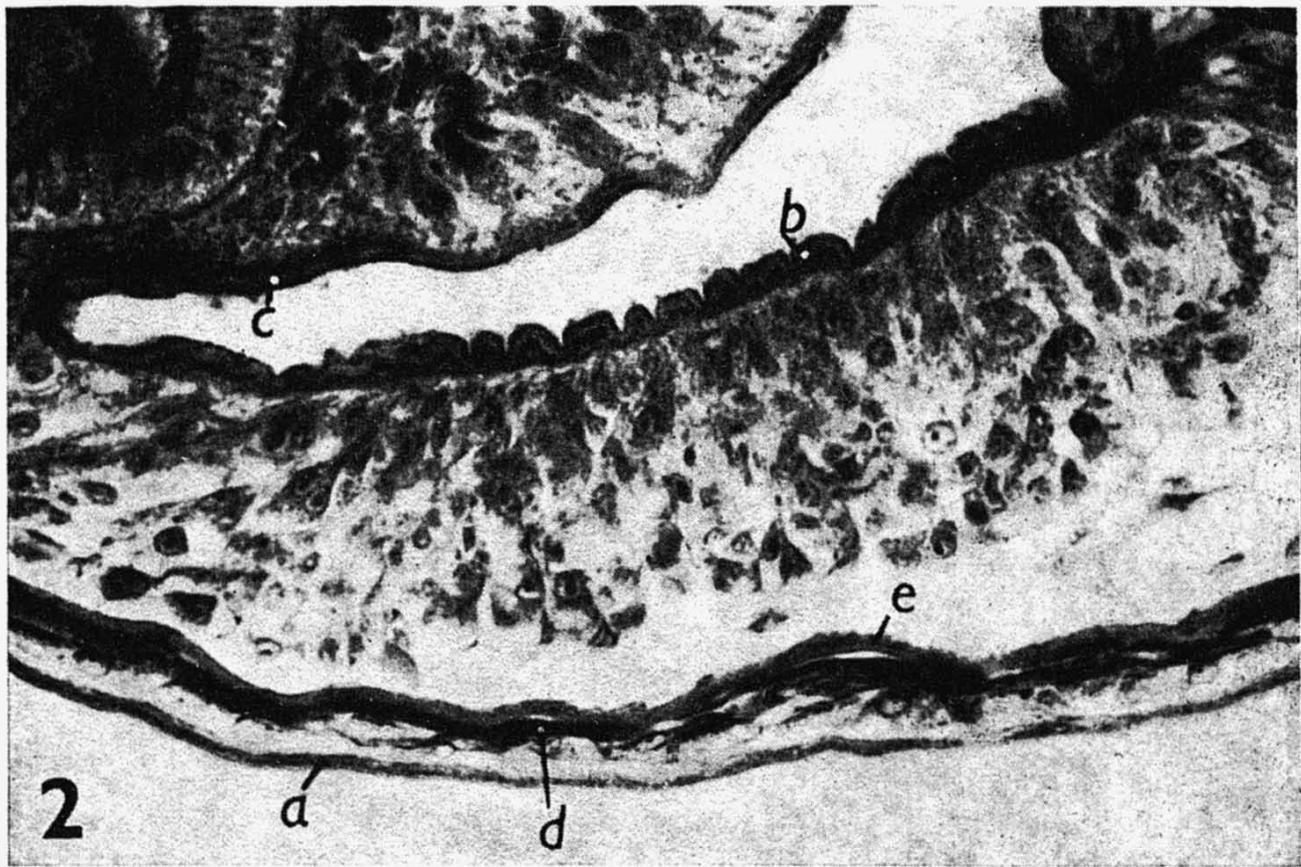
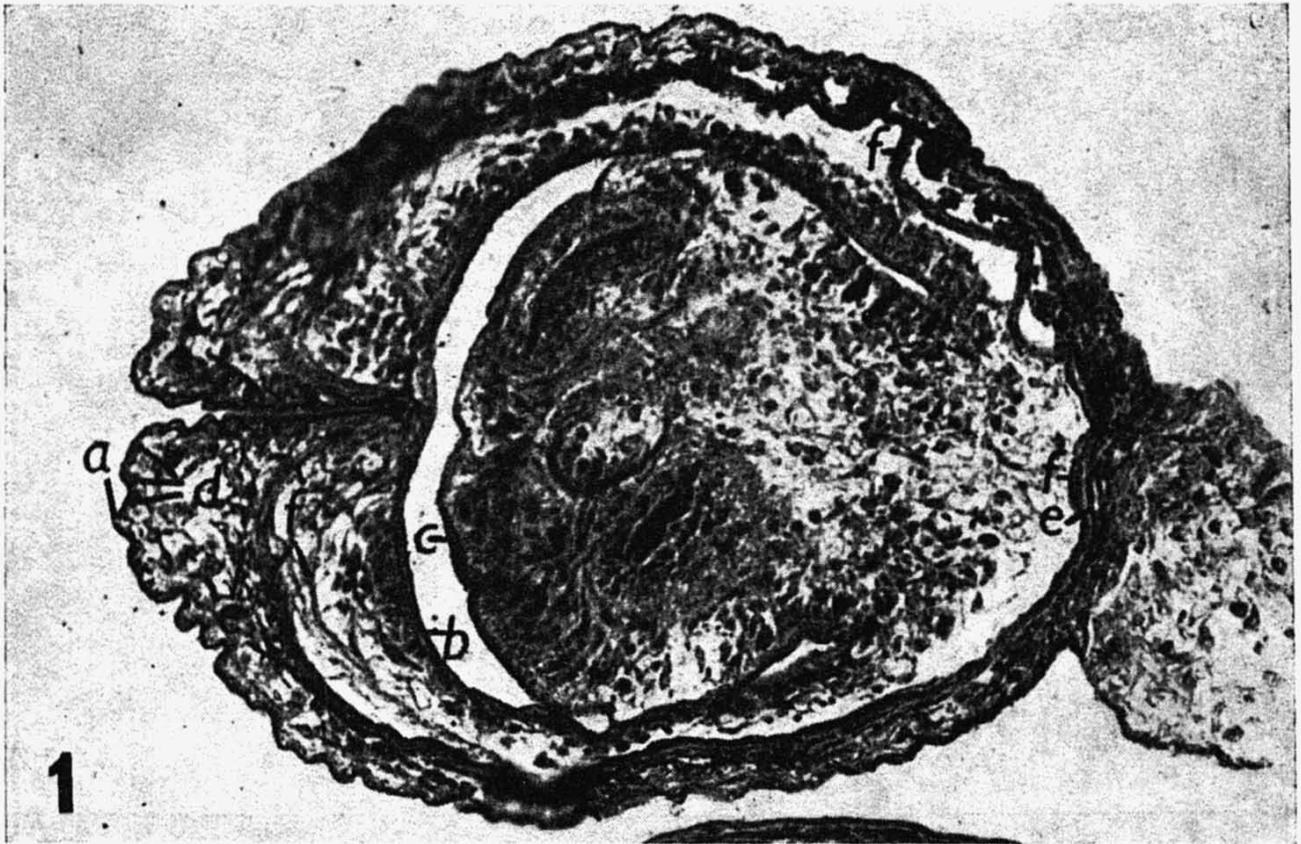
## REFERENCES

- BARON P. J., On the histology, histochemistry and ultrastructure of the cysticercoïd of *Raillietina cestocillus* (Molin, 1858) Fuhrmann, 1920 (Cestoda, Cyclophyllidea). *Parasitology* 62: 233—245, 1971.
- JOYEUX CH., Recherches sur le cycle évolutif d'*Hymenolepis erinacei* (Gmelin, 1789). *Ann. Parasitol. Hum. Comp.* 5: 20—26, 1927.
- LUMSDEN R. D., Cytological studies on the absorptive surfaces of cestodes. I. The fine structure of the strobilar integument. *Z. Parasitenk.* 27: 355—382, 1966.
- PEARSE A. G. E., *Histochemistry theoretical and applied*. V. 1. Churchill Ltd., 759 pp. London 1968.
- PROKOPIČ J., The life cycle of the cestode *Rodentolepis erinacei* (Gmelin, 1789). *Folia parasit.* (Praha) 18: 27—32, 1971.
- , BÍLÝ S., Beetles (Coleoptera) as new intermediate hosts of helminths. *Věst. Čs. spol. zool.* 39: 224—230, 1975.
- , KARAPCHANSKI I., The larval stages of several cestodes from beetles (Coleoptera) in Bulgaria. *Helminthologia* 14: 171—181, 1973.
- UBELAKER J. E., COOPER N. B., ALLISON V. F., The fine structure of the cysticercoïd of *Hymenolepis diminuta*. I. The outer wall of the capsule. *Z. Parasitenk.* 34: 258—270, 1970.
- VALKOUNOVÁ J., PROKOPIČ J., Morphology of cysticercoïd of the cestode *Rodentolepis crassiscolex*. *Věst. Čs. spol. zool.* 42: 303—310, 1978.
- VOGE M., Studies in cysticercoïd histology. I. Observations on the fully developed cysticercoïd of *Hymenolepis diminuta* (Cestoda: Cyclophyllidea). *Proc. Helminthol. Soc. Wash.* 27: 32—36, 1960a.
- , Studies in cysticercoïd histology. III. Observations on the fully developed cysticercoïd of *Raillietina cestocillus* (Cestoda: Cyclophyllidea). *Proc. Helminthol. Soc. Wash.* 27: 271—274, 1960b.

Received 13 March 1979.

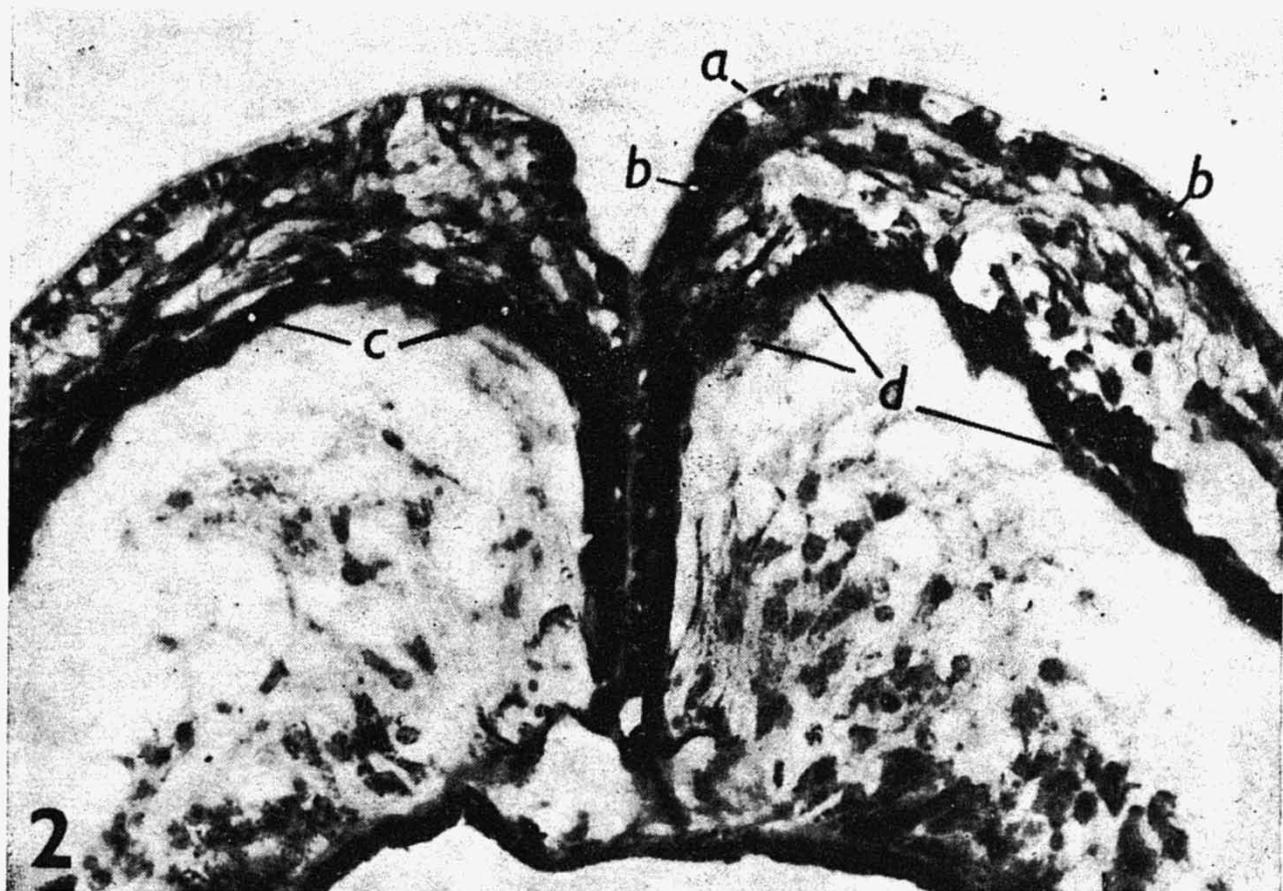
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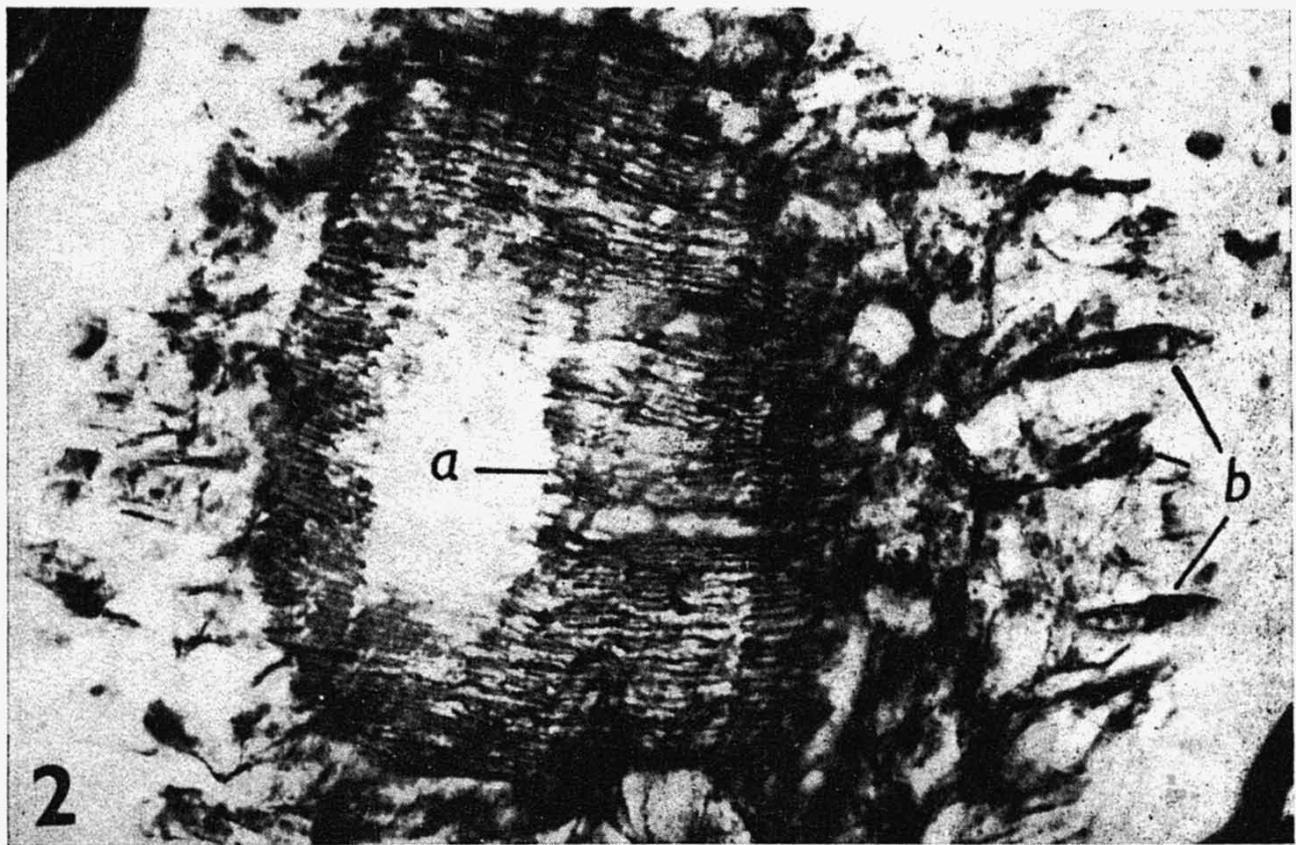
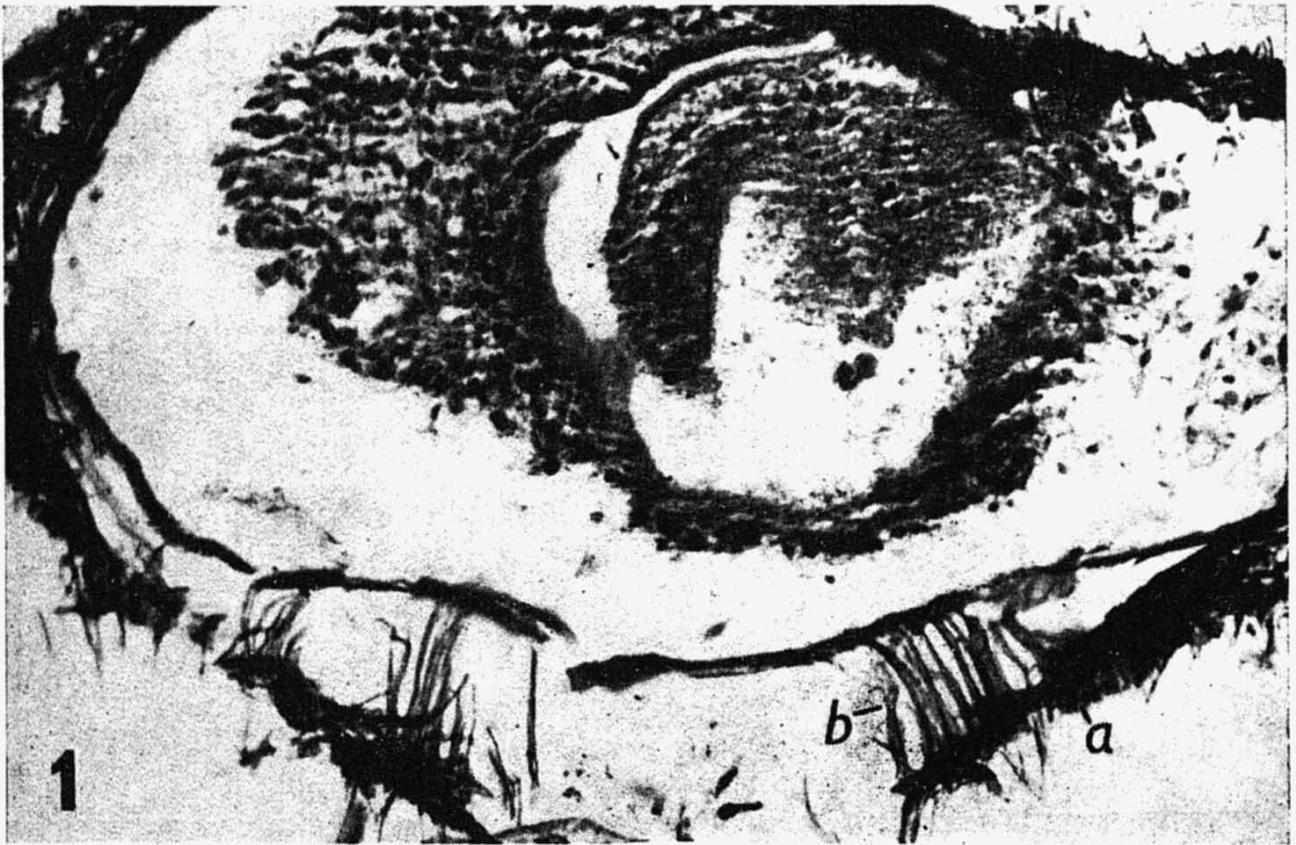


**Fig. 1.** Tegument of cyst (a), neck (b) and scolex (c), circular connective tissue fibres in the outer part of cyst subtegument (d), inner longitudinal fibrous layer of cyst subtegument (e), inner zone of cyst (f). Goldner's trichrome ( $\times 300$ ).

**Fig. 2.** Tegument of cyst (a), neck (b) and scolex (c), inner longitudinal fibrous layer of cyst subtegument (d), inner zone of cyst (e). Masson's trichrome ( $\times 800$ ).



**Fig. 1.** Cyst structure—tegument with distinct longitudinal connective tissue fibres (a), circular connective tissue fibres (b) and longitudinal connective tissue fibres (c) of outer part of tegument, pyriform cells (d), inner longitudinal fibrous layer of subtegument (e), inner zone (f); calcareous bodies of the neck (g). Goldner's trichrome ( $\times 850$ ). **Fig. 2.** Cyst tegument (a), layer of circular connective tissue fibres in the outer part of cyst subtegument (b), inner longitudinal fibrous layer of cyst subtegument (c), inner zone of cyst (d). Gomori ( $\times 850$ ).



**Fig. 1.** Connective tissue fibres of cyst tegument (a), connective tissue fibres of cyst subtegument (g) Gomori ( $\times 400$ ).  
**Fig. 2.** Connective tissue fibres of cyst tegument (a), connective tissue fibres of cyst subtegument (b). Gomori ( $\times 1000$ ).