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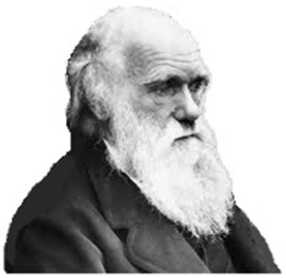

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Histology of marbled crayfish *Procambarus virginalis* (Lyko, 2017): annotated atlas

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ABSTRACT

The educational publication contains microphotographs and description of histological preparations of the structure of marbled crayfish. The above materials may be used both for carrying out laboratory work on disciplines "Histology", "Cytology", "Cell Biology", "Special Practice", and for self-study of relevant educational topics. Designed for specialists in the field of hydrobiology and histology, students and graduate students of institutions of higher education who studying in the field of "091 Biology", "207 Water bioresources and aquaculture" and "162 Biotechnology and bioengineering". The publication contains the results of studies conducted by President's of Ukraine grant for competitive projects Ф75/142 «The reproductive potential of invasive species of Dnieper region reservoirs and their impact on bioproductivity formation» (№ 0118U006319) of the State Fund for Fundamental Research.

Keywords: Marmorkrebs, histology, marbled crayfish, *Procambarus virginalis*, hemolymph, hepatopancreas

1. INTRODUCTION

Marbled crayfish *Procambarus virginalis* (Lyko, 2017) (known as a form *P. fallax f. virginalis* Martin et al., 2010 until 2010) (Decapoda, Cambaridae), are parthenogenetic crustaceans, first discovered in German reservoirs in 1990 [3, 7, 8] were described as a subspecies of the American species *P. fallax* (Hagen, 1870), which natural area covers the waters of Georgia and Florida (USA) [14]. Recent studies by European scientists have allowed to place the marbled crayfish into a separate new species of decapods *P. virginalis* (Lyko, 2017). These crustaceans have come to Europe as aquarium species and got to the reservoirs of Germany [2]. The appearance of parthenogenetic individuals of this species in natural waters

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has began its expansion in different countries, marbled crayfish became a threat to local species in a number of countries: the Netherlands, Italy [9], Slovakia [6], Sweden [1], Czech Republic [11], Ukraine [10] and Japan [4]. Marbled crayfish is a typical invasive hydrobiont [12], which is common in many places and forms populations by means of self-reproduction of only one individual, endangering agriculture and competing with aboriginal species.

A number of scientists conducted preliminary studies to assess the risks of the introduction of marbled crayfish to the reservoirs of Ukraine [5]. They claim that the climatic conditions of the reservoirs of central and southern Ukraine are favorable for the naturalization of marbled crayfish and designate it as species with a high invasiveness level. In 2015, the first marbled crayfish were found in the reservoirs of Dnipropetrovsk and Odessa regions [10], which confirms the expected risks of its introduction and naturalization. High ecological plasticity and the ability to parthenogenetic reproduction allows marbled crayfish to rapidly increase their number in recipient reservoirs and adapt to their conditions. High adaptability of marbled crayfish is associated with epigenetic mechanisms that are implemented in the DNA molecule and help to read genetic information by activating or deactivating the corresponding genes under the influence of environmental factors [7]. Similar adaptations of crustaceans to the conditions of existence occur on the biochemical and cellular levels, which allows them to live in reservoirs with high levels of pollution.

One directions of studying the boundaries of adaptive possibilities of marbled crayfish is the implementation of cytological and histological methods for the studying the structure of crayfish tissues and organs, both in normal state and in pathology.

In modern histology, there are numerous different microscopic methods of tissue research. The classical approach is making a fixed histological preparation. The atlas contains the basic technique principles of making histological preparations from tissues and organs of marbled crayfish, author microphotographs of preparations with their short description. Such fixed preparations are widely used both in scientific research and in the educational process.

2. MATERIALS AND METHODS

Individuals of marbled crayfish *Procambarus virginalis* (Lyko, 2017) (Decapoda), were used as the material for this work; they were obtained in laboratory conditions in the research laboratory of hydrobiology, ichthyology and radiobiology of the biological research institute of the Oles Gonchar Dnipro National University.

2. 1. Preparation of histological sections

Methods of classical histology were used in this work [13]. This method allows to cut marbled crayfish on a microtome and study the structure of their organs and tissues.

The process of making the histological preparation for optical microscopy included the following main steps:

1. Selection of material and its fixation.
2. Condensation of the material.
3. Preparation of paraffin blocks (cassettes).
4. Preparation of sections.

5. Staining or opacification of sections.
6. Fixing sections in balsam.

Marbled crayfish were fixed in a 4% solution of formalin, then treated in accordance with generally accepted histological methods [13]. It was carried out to prevent the processes of autolysis and to maintain the structure of organs close to the intra-vitam state. Samples were labeled and stored in a dark place at room temperature.

Dehydration of tissues and organs was carried out in alcohols of increasing concentration and in xylol in order to condensate them, which is necessary to make thin sections. For condensation and making tissues and organs homogenous to provide high-quality of section, it was poured in paraffin (Fig. 1).

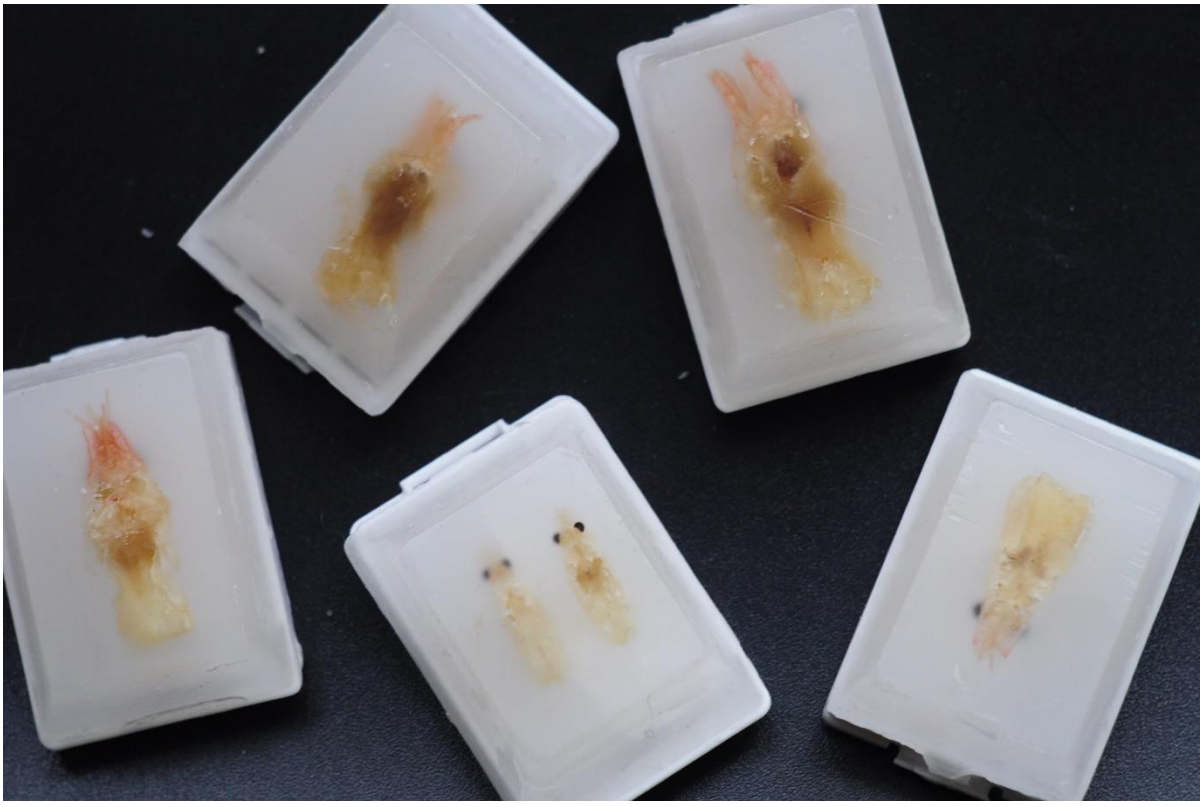

Figure 1. Paraffin blocks for the preparation of histological sections

Microtome "Microm HM 325" was used for the preparation of sections of marbled crayfish. After receiving the sections, they were placed on the slides. Further, the sections were deparaffined and stained with hematoxylin-eosin. After finishing the staining, the sections were filled with Canada turpentine, and covered with a cover glass.

2. 2. Making hemolymph smears

Hemolymph of marbled crayfish was collected intra-vitam. At the same time for the preparation of smears and further morphological evaluation of hemocytes, the influence of

external factors on the crayfish organism was minimized and the following scheme should was clearly followed:

1) Selection of crayfish. Under experimental conditions, crayfish are caught by a net and placed into a container with water prior to manipulation.
2) Fixation. The selected crayfish were fixed on a plane surface with a bandage, which wrapped the carapace of crayfishб leaving ambulatory leg available.
3) Selection of hemolymph. Hemolymph was taken in vivo by amputation of 1/3 of the ambulatory leg (trunk leg Y). After amputation of the part of the ambulatory leg, it is necessary to wait for one second before the appearance of a drop of fluid in the place of the cut of the crayfish leg. It should be noted that hemolymph of crayfish has a high agglutination speed, therefore loses its fluidity after 3-5 s after the cut off of the leg. In this regard, all procedures for the selection of hemolymph must be carried out quickly.
4) Preparation of smears. Hemolymph was applied on a single object glass from a single crayfish, followed by preparation of smear. It is advisable to use the same method of applying hemolymphs to the glass – from the end of the rib through the object glass to the left, to facilitate further microscopy. Then smears are stained by Romanovsky-Giemsa method.
5) Selected hemolymph can be used as a biological indicator of the current state of freshwater crayfish under the influence of environmental factors or for the studies of their physiological responses to anthropogenic impact.

Microphotographs of tissues and organs of marbled crayfish were performed using a Sciencelab T500 5.17M digital header connected to a Jenamed microscope.

3. HISTOLOGICAL PICTURE OF TISSUES AND ORGANS OF MARBLED CRAYFISH *PROCAMBARUS VIRGINAL* (LYKO, 2017)

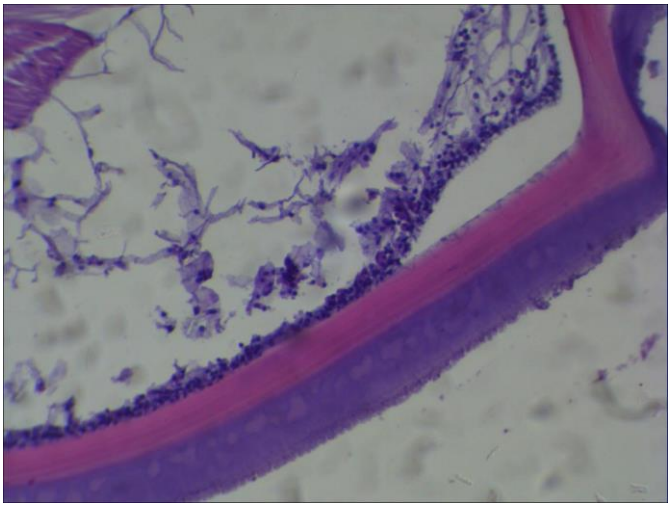

Figure 2. Cuticle (ob. lens × 8, oc. lens × 10). The cuticle has a strained structure. The surface of the cuticle covers the thin layer of the epicuticle, followed by a thick layer of exocuticle, which is stained in a blue-violet color. The lower layer of the cuticle is an endocuticle, stained in a pinkish-red color. Under the endocuticle there are epithelial cells of the epidermis. The epidermis and the cuticle are separated from each other by a thin basal membrane.

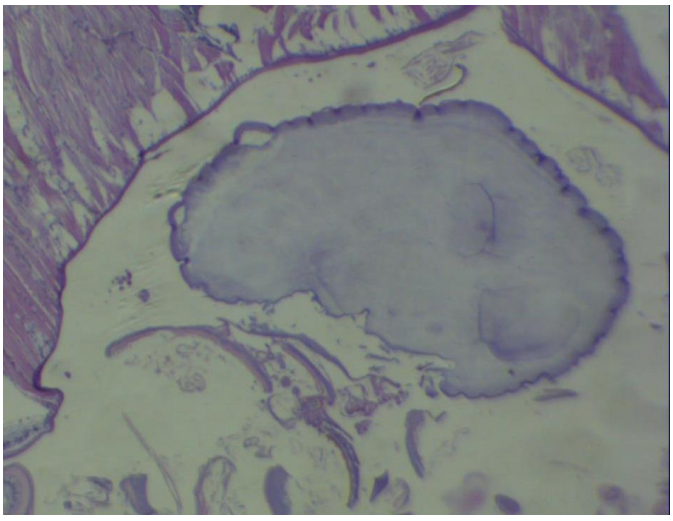

Figure 3. Stomach stones (ob. lens × 8, oc. lens × 10) take an active part in the process of ecdysis of cayfish. This is a depot of calcium for a crustacean body, which, after ecdysis, is spent on the restoration of a solid shell. Stomach stones are located on the sides of the stomach. They have an oval "lens" shape.

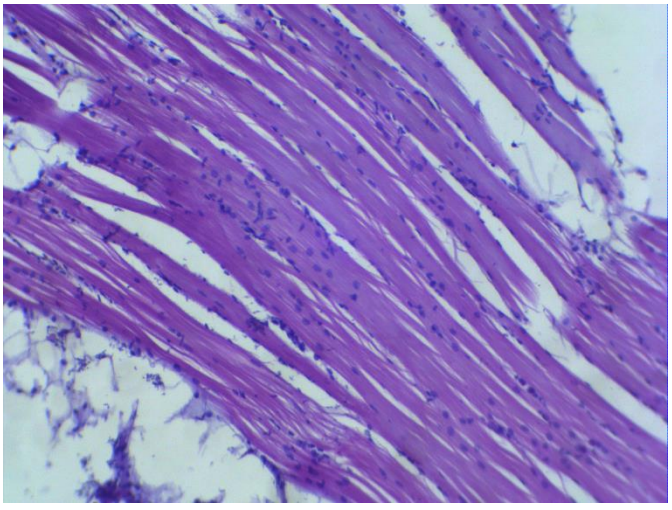

Figure 4. Muscles, longitudinal section (ob. lens × 8, oc. lens × 10). Bundles of strained muscle fibers are parallel to each other, cut along. Between the beams and the separate fibers there are layers of loose connective tissue in which small vessels and capillaries are visible, as well as groups of adipose cells.

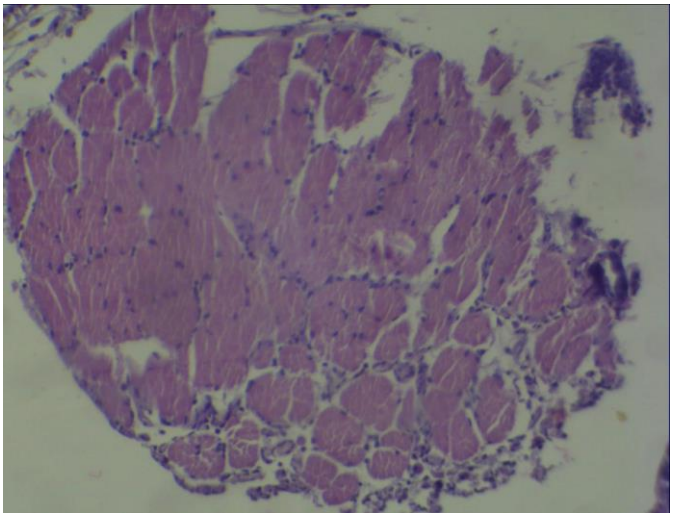

Figure 5. Muscles, cross-section (ob. lens × 8, oc. lens × 10). The central part of the fiber is filled with bundles of myofibrils, which determine the longitudinal and transverse striation of muscle fibers. They form a bundle of continuous fibers that extend from one end of the muscle fiber to the second one in parallel to its axis. The transverse striation of muscle fibers is explained by the special structure of myofibrils.

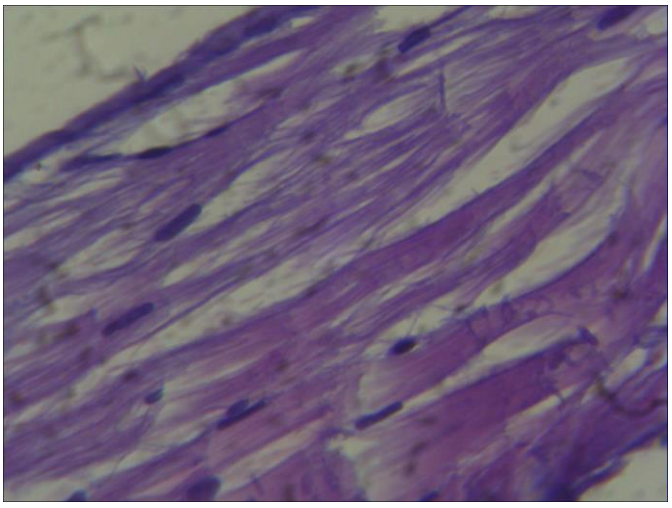

Figure 6. Muscle fiber (ob. lens 40, oc. lens × 10). The striated muscle fiber has a cylindrical shape, uniform width, but narrows slightly at the ends. On the surface, the fiber is covered with a thin, unstructured membrane, called sarcolemma. Under the membrane, the oval basophilic nuclei, which contain relatively little amount of chromatin, are placed unevenly along the periphery of the muscle fiber.

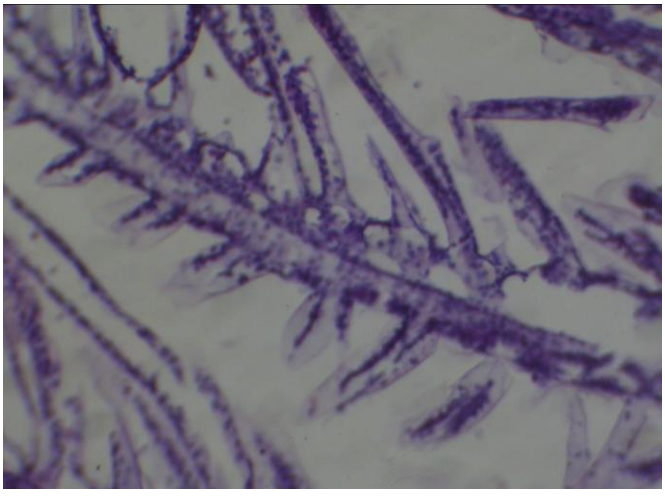

Figure 7. Longitudinal section of the gill (ob. lens × 8, oc. lens × 10). The gills in Decapoda are located in longitudinal rows. They consist of respiratory epithelium and thin cuticle. The body cavity continues in the gills, where the hemolymph gets in. Gas exchange occurs through the thin and gentle cuticle of the gill.

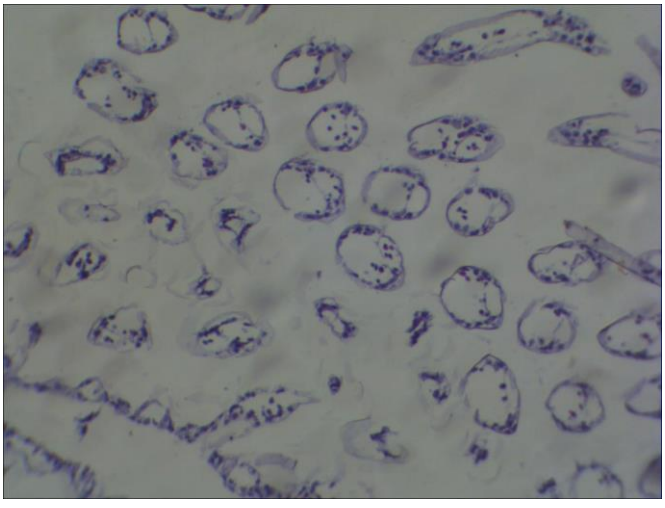

Figure 8. Cross section of the gill (ob. lens × 8, oc. lens × 10). The gill apparatus consists of a thin layer of respiratory epithelium, covered with a thin cuticle. The epithelium contacts with water and is able to absorb oxygen, dissolved in water. The gills have thin vessels that contain hemolymph cells that carry oxygen to the internal organs of the cancer.

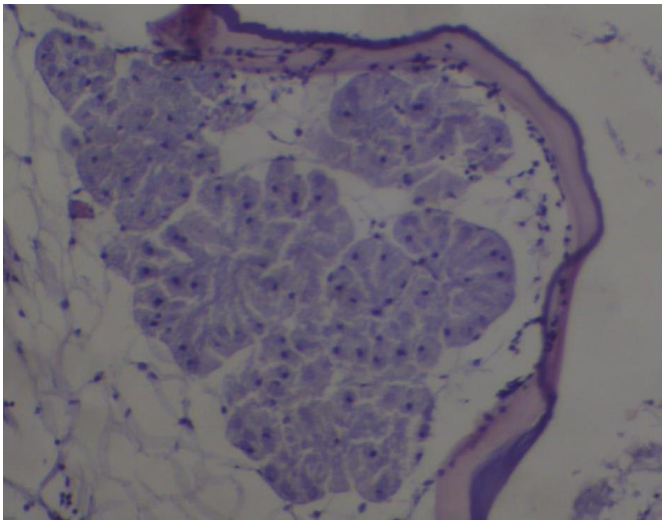

Figure 9. Haemopoietic tissue (ob. lens × 8, oc. lens × 10). The haemopoietic organ of marbled crayfish is hemopoietic nodes located in the connective tissue near the stomach. The cells are mitotic and differentiate in different directions, forming amebocytes and cyanocytes.

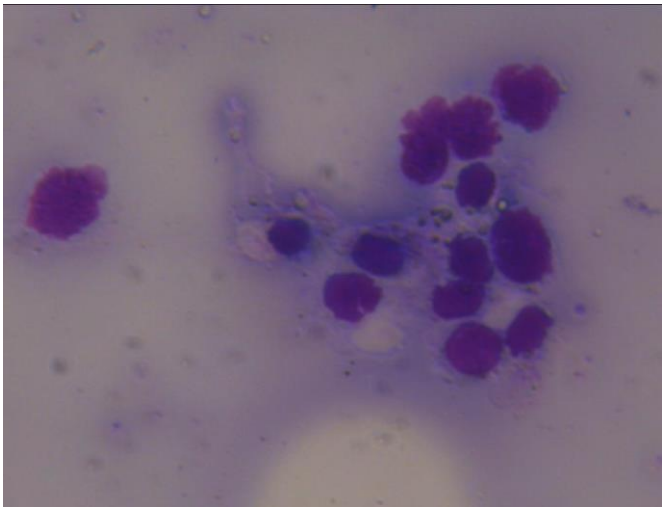

Figure 10. Hemolymph smear (ob. lens × 8, oc. lens × 10). The hemolymph partly moves inside the vessels covered with the epithelium, partly in the areas of the body's cavity, unlimited with special walls, the sinuses. Hemolymph is colorless. The hemolymph contains amoeboid cells. Hemolymph functions are: transport, humoral, respiratory, mechanical and protective.

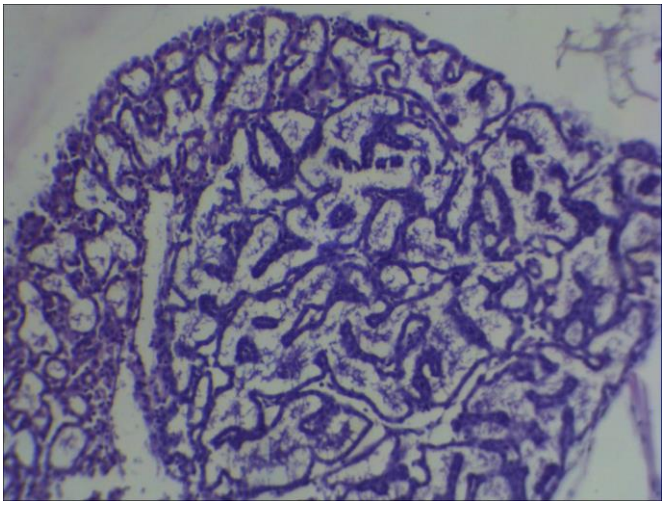

Figure 11. Green (antennal) gland, (ob. lens × 8, oc. lens × 10). These are quite large rounded glands that are located in the head part and open with ducts near the antenna base. Each gland consists of a large coelomic sac, a gyrose duct and a bladder. The secretory part of the antennal gland of marbled crayfish has the form of a sac, divided into multiple chambers, lined with a single-layer glandular epithelium that forms the labyrinth.

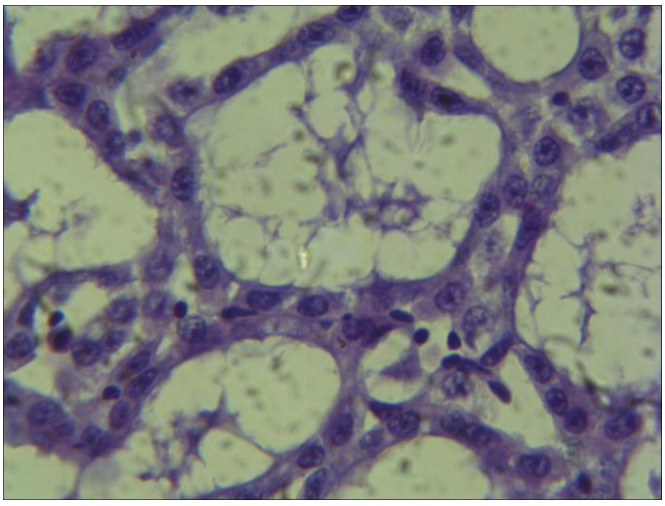

Figure 12. Cells of green gland, glandulocytes, **(ob. lens × 40, oc. lens × 10).** Epithelial cells are located on a thin basal membrane, they are cubic, and contain large spherical nuclei with one or more nucleoli. The secretion is accumulated in the apical part of the cell, resulting in the cytoplasm dilution and participation in secretion; cells are partially destroyed in the process of secretion.

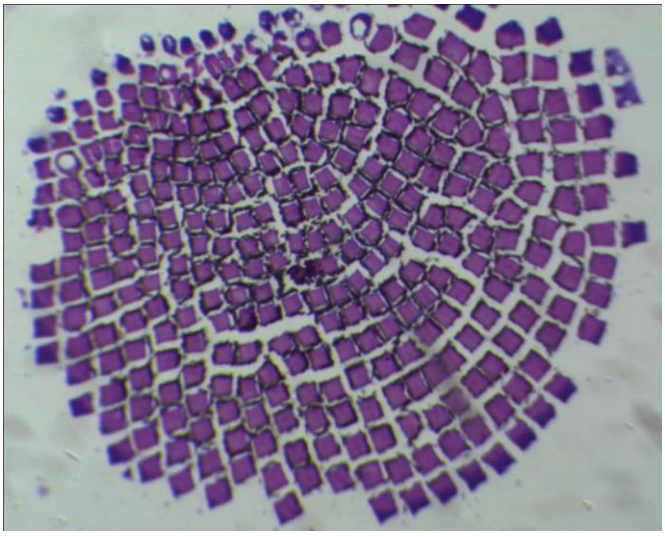

Figure 13. Compound eyes (ob. lens × 8, oc. lens × 10). The eyes are located on the moving outgrowth of the head, called ophthalmite. The eyes of marbled crayfish are complex and called compound eyes. Each eye consists of a large number of small eyes – facets close to each other and separated only by a thin layer of black pigment.

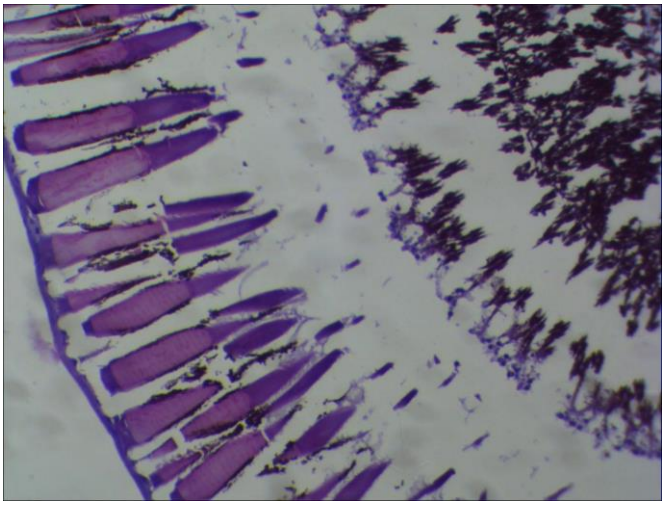

Figure 14. Compound eyes (ob. lens × 40, oc. lens × 10). The compound eye of crayfish consists of a transparent cuticle that covers the crystal cone, constructed of four transparent cells, under each there are 8 photosensitive cells. The nerve endings from these cells give rise to the optic nerve.

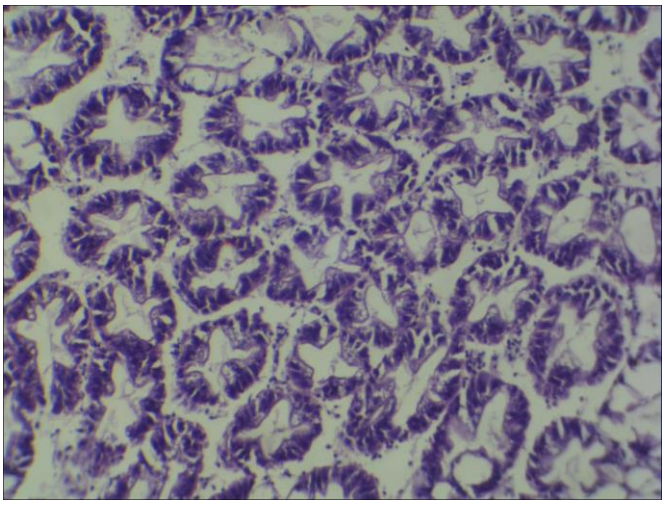

Figure 15. Hepatopancreas (ob. lens × 8, oc. lens × 10). The digestive system of marbled crayfish is represented by a tube, esophagus, stomach, middle intestine, rectum and digestive gland, the hepatopancreas, which consists of small lobes and opens with the ducts into the stomach. The function of this organ was the same as the function of the liver and pancreas of the vertebrates.

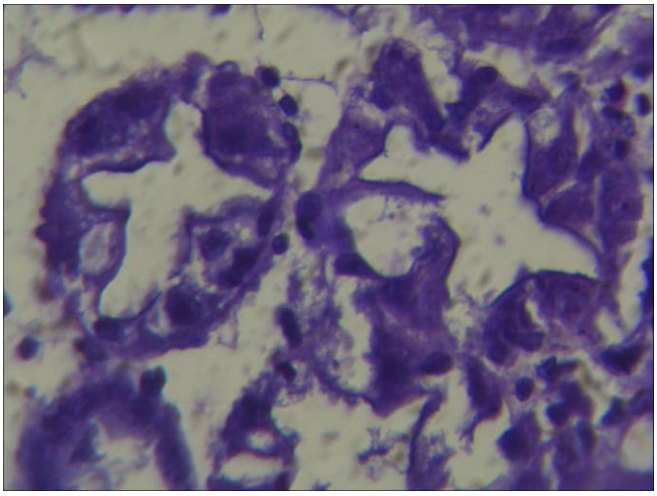

Figure 16. Hepatopancreas (ob. lens × 40, oc. lens × 10). Epithelium of hepatopatopancreactic tube contains 4 types of cells. The tubes bunch into a pair of general ducts that enter the middle gut. The closed end of each tube is composed of embryonic cells (E-cells), which are the precursors of all other types of cells. Proximally from E-cells the accumulating R-cells, vacuolized V-cells and fibrillar F-cells are located.

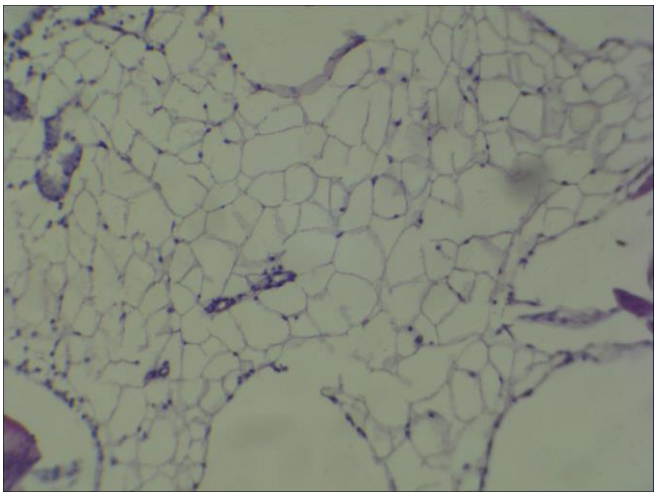

Figure 17. The adipose tissue (ob. lens × 8, oc. lens × 10) is an accumulation of adipocytes that contain a large drop of fat. Between them there are narrow layers of loose connective tissue, which contains fibroblasts, thin collagen fibers, capillaries and hemolymph cells. It participates in water-exchange processes of the body, performs shock-absorption functions.

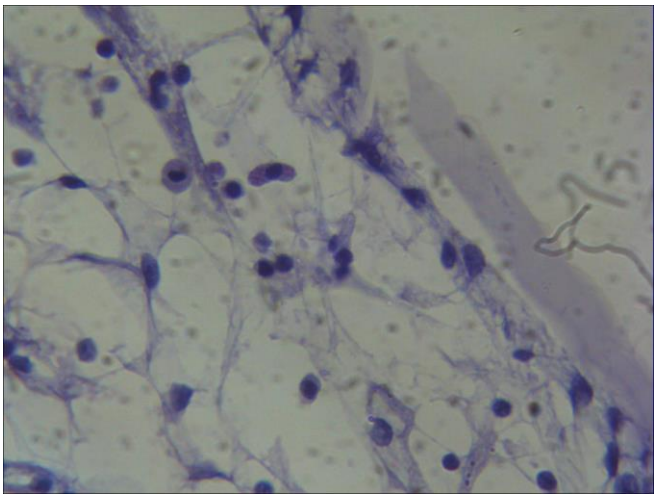

Figure 18. Adipocytes, (ob. lens × 40, oc. lens × 10) Store high energy nutrients, the neutral lipids. The cell nucleus is shifted to the periphery, the main part of the cytoplasm is occupied by the inclusion of lipids. As a result of alcoholic treatment of the histological material, on the preparations the tissue is presented in the form of cavities with a basophilic nucleus.

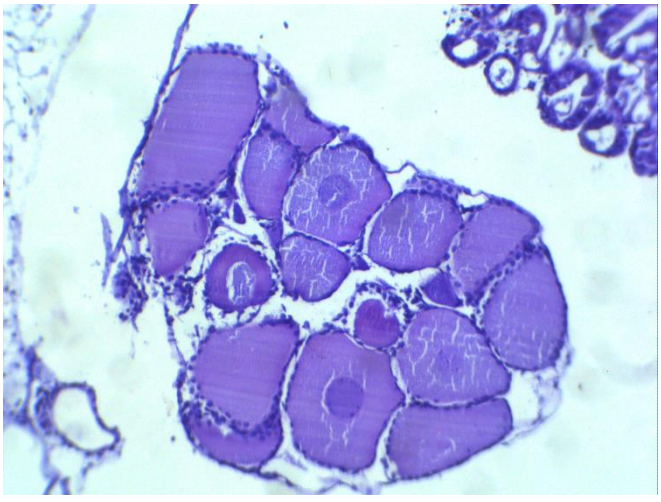

Figure 19. Oocytes in early stages of development (ob. lens × 8, oc. lens × 10). Marbled crayfish are represented only by parthenogenetic triploid females, each lays off unfertilized eggs, and genetically homogeneous individuals develop from them. Small basophilic eggs with a homogeneous cytoplasm. In the middle of the oocyte there is a large cell nucleus.

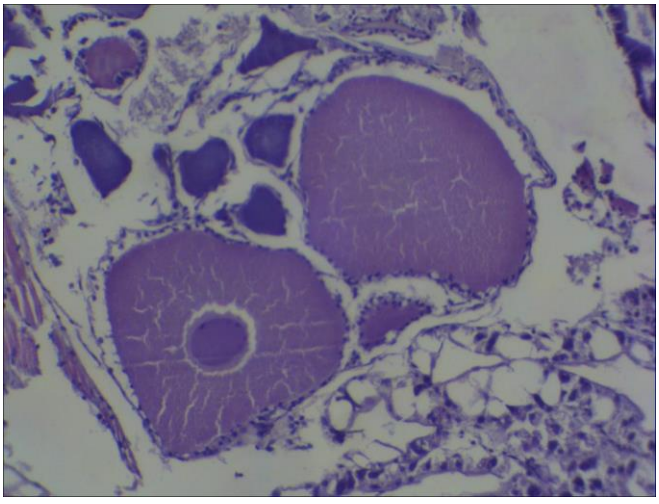

Figure 20. Asynchronous development of oocytes (ob. lens × 8, oc. lens × 10). Due to the continuous process of reproduction of marbled crayfish, asynchronous development of sex cells is observed. The cells of early stages of gametogenesis are presented here – single-layer follicles, and later stages of development.

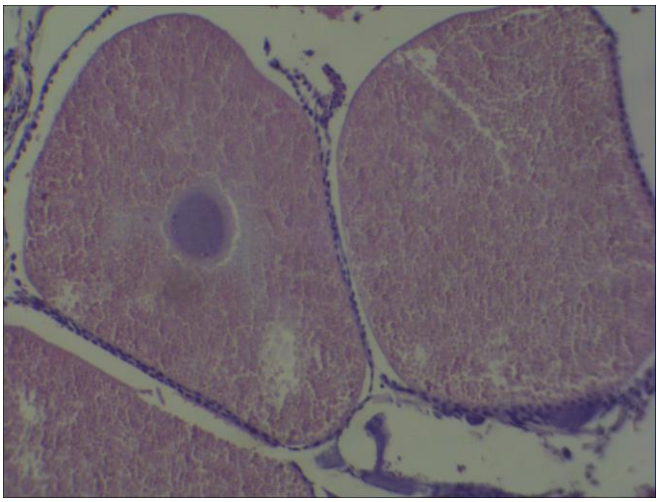

Figure 21. Mature oocytes (ob. lens × 8, oc. lens × 10). These are relatively large cells with a homogeneous cytoplasm and a clear central nucleus. The oocytes are in the follicular membrane. There are small nubbles of the yolk in the cytoplasm, which are located throughout the area of the egg.

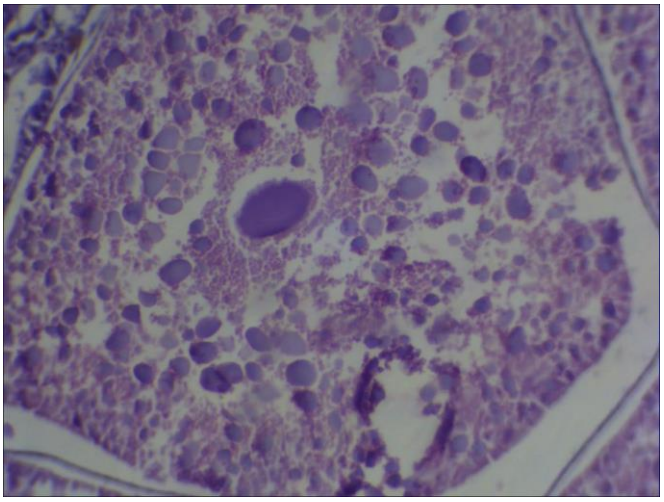

Figure 22. Egg yolk in mature oocyte (ob. lens × 8, oc. lens × 10). Phase of oocyte filling with yolk. Yolk granules are located throughout the area of the oocyte from the membrane to the nucleus. The yolk granules are oval or round.

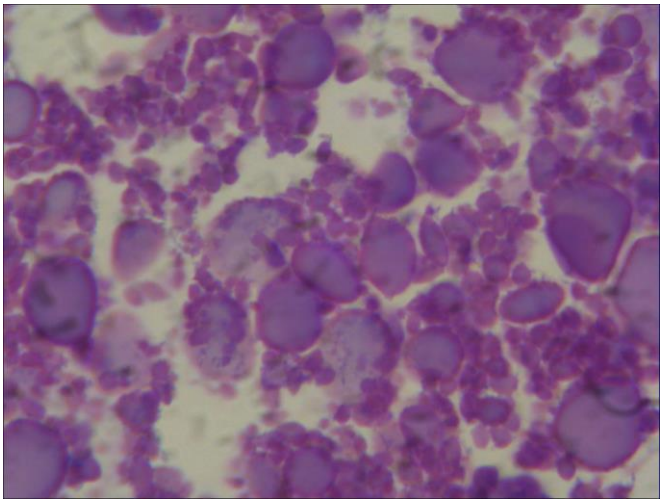

Figure 23. Yolk granules (ob. lens × 90, oc. lens × 10). The yolk plays an important role in the development of the egg. During the maturation of the oocyte, the yolk granules fill the entire cytoplasm of the cell. The yolk granules are rounded and characterized by weak eosinophilia. Upon completion of the oogenesis, the granules of the yolk merge into large drops.

4. CONCLUSION

The educational edition contains the results of own studies on tissues and organs histology of marbled crayfish. All of these preparations are typical of marbled crayfish under normal existence conditions, that is, histological preparations of normal state for marble cancers are given. Due to the influence of certain factors of abiotic and biotic nature, the histological picture of tissues and organs can change significantly, implementing mechanisms of adaptation to new factors of the aquatic environment.

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