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INVERTEBRATE ZOOLOGY

The Morphology and Microscopic Anatomy of the Deep-Sea Echiurid *Protobonellia zenkevitchi* Murina, 1976

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Abstract—Echiurida is a small group of marine benthic invertebrates that burrow in sediments and live a hidden lifestyle. Investigation of the morphology and anatomical features of various organ systems allows better understanding of the biology of these enigmatic animals, many of which are deep-sea species. The morphology and microscopic anatomy of the deep-sea echiurid *Protobonellia zenkevitchi* Murina, 1976 have been studied using the light microscopy and histology methods. The body of *P. zenkevitchi* is divided into a proboscis and trunk. The ciliary grooves and large vacuolated cells in the connective tissue of the distal part of the proboscis suggest a specific position of the proboscis on the sediment surface and a mechanism for sorting food particles. It has been shown for the first time that the coelom is not subdivided into compartments. In the digestive tract, an unusual part of the midgut has been found that was previously unknown in echiurids; it probably performs the function of food storage. This part contributes to thorough food digestion, which is important in the oligotrophic conditions of greater depths. The circulatory system of *P. zenkevitchi* lacks the neuro-intestinal and ring blood vessels. The oocyte storage chamber of the gonoduct has a pore, which apparently connects its cavity with the trunk coelom, but lacks a specialized and well-expressed part, the androecium. A comparative analysis of the microscopic anatomy shows that many organ systems (muscular, coelomic, circulatory, excretory, and reproductive) in *P. zenkevitchi* have a simpler organization compared to those in other echiurids. This can probably be explained by the small body size of *P. zenkevitchi* and the great depths of its habitat. At the same time, *P. zenkevitchi* possesses some unique anatomical characteristics related to the features of the biology of this deep-sea species.

Keywords: Echiurida, Annelida, morphology, anatomy

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INTRODUCTION

Echiurida is a small group of non-segmented marine worms that comprise approximately 250 species that occur at all latitudes of the World Ocean [9]. They were first described in the works of Peter Simon Pallas [17], but even to date the morphology of many of these species remains extremely poorly studied. Echiurids are usually found burrowing in sediments at great depths. Such biological features make them objects of research that are difficult to access; thus, a major part of the works on microscopic anatomy and ultrastructural organization of these animals have been conducted on only a few model species from different families [8]. Many details of the structure of echiurids have not been studied at all [18]. It is not known definitely whether the fine sorting of food particles is related to different structures of the proboscis and, if so, how. The anatomy and physiology of the complexly organized digestive system in echiurids has been extremely insufficiently studied [14, 15]; there is a lack of comprehensive data on the general anatomy of the circulatory system and blood circulation [8]; little is known on the fine structure of blood vessels [3, 4]; the ultrafiltration zones are not identified; the fine organization of the gonads has not been described.

An extension of the number of studied species, a detailed reconstruction of the anatomy of organ systems, and a study of the fine structure of tissues and organs will provide a complete view of the structure of this unusual group of animals, make it possible to understand their biology, and shed light on the stages of echiurid evolution from the initially metameric ancestors to the non-segmented recent forms.

The goal of the present study is to describe the morphology and microscopic anatomy of all the organ system in the deep-sea echiurid *Protobonellia zenkevitchi* Murina, 1976.

MATERIALS AND METHODS

Specimens of *Protobonellia zenkevitchi* from the collection deposited at the Shirshov Institute of Oceanology, Russian Academy of Sciences (Moscow) were the material for the study. Echiurids were collected from station 6138 (depth 5595–5660 m, Sigsbee trawl) during the 45th cruise aboard R/V *Vityaz* on July 11, 1969. The material was fixed with a 4% formaldehyde solution in seawater and stored in 70% alcohol after the fixation.

The external morphology of the worms was examined under a Leica MZ6 binocular microscope. The fine morphology of the proboscis, setae, integuments, and some of the organ systems was studied using the methods of scanning electron microscopy (SEM). Specimens processed by the standard technique were examined under Camscan S2 and JSM 63LA scanning electron microscopes. Three well-preserved specimens of *P. zenkevitchi* were selected to study the microscopic anatomy. The animals, which were processed using the standard technique, were whole embedded in paraplast to obtain a complete series of histological sections. Stained sections were examined under a Zeiss Axioplan 2 light microscope and photographed using a digital camera.

To make the 3-dimensional (3D) reconstructions, the sections were photographed under a LOMO Mikmed-6 microscope equipped with a digital camera, at a magnification of 4×. Every fifth section was photographed; images of a total of 132 sections were taken. Using Infan View 4.28 software, images of a series of sections were transformed into a single format and resized to a uniform size; each image was given a name with a consecutive number, which corresponded to the position of the section in the series. The images were aligned relative to one another and combined into a stack in Amira 5.2.2 software. Imaris 7.0.0 software was used to compose 3D models.

The lettering used in the figures is as follows: *a*, anus; *abv*, axial blood vessel of the proboscis; *al*, anal lobe; *as*, anal sac; *bcp*, blood capillaries of the intestine blood plexus; *cg*, ciliary groove; *cm*, circular musculature; *col*, collateral; *cp*, central prong of seta; *cr*; crop; *csc*, storage chamber of the gonoduct; *ct*, connective tissue; *ctc*, connective tissue cord; *dbv*, dorsal blood vessel; *dep*, dilated distal end of the proboscis; *dev*, diverticulum of the post-siphonal part of the midgut; *dm*, dorsal mesentery; *dp*, distal prong of seta; *en*, envelope of the ventral nervous cord; *eno*, envelope of the oocyte; *f*, ciliary funnel of nephridium; *fg*, funnel of the gonoduct; *fol*, follicle of the nurse cells; *fys*, follicle of the primordial seta; *gc*,glandular cell; *gz*, gizzard; *hng*, hindgut; *il*, intestinal loops; *lbv*, lateral blood vessel of the proboscis; *lc*, lateral coelomic canal of the proboscis; *lf*, lateral folds; *lm*, longitudinal muscles; *lnc*, lateral nerve of the proboscis; *m*, mouth; *men*, muscular envelope of the proximal gonoduct tube; *mh*, muscular hill of the seta; *ml*, muscular lining of the digestive tract; *mpp*, middle part of the proboscis; *n*, nucleus; *nc*, collar of the nephridium funnel; *np*, neuropile; *nu*, nucleolus; *p*, pore of the gonoduct storage chamber; *pc*, cells of the coelom lining; *pec*, cells of the endothelial lining of the blood vessels; *pep*, proximal portion of the proboscis; *ph*, pharynx; *pk*, perikarya; *pos*, post-siphonal part of the midgut; *pp*, proximal prong of the seta; *prb*, proboscis; *prc*, protocuticle; *prs*, pre-siphonal part of the midgut; *rm*, radial muscles; *rms*, radial muscles of the ventral setae; *s*, siphon; *sg*, epithelial septae at the site of opening of the siphon into the post-siphonal part of the midgut; *slp*, sacciform part of the anal sac; *so*, siphonal part of the midgut; *sph*, folds of the sphincter; *ss*, setiferous sac; *stl*, stalk of the nephridium funnel; *tc*, trunk coelom; *tgp*, tubular part of the gonoduct (androecium); *tlp*, tube-like part of the anal sac; *tr*, trunk; *vbv*, ventral blood vessel; *vc*, vacuolated cells of the proboscis connective tissue; *vca*, ventral coelomic chamber; *vep*, ventral epidermis; *vh*, ventral hill; *vl*, ventral lobe of the proboscis; *vm*, ventral mesentery; *vnc*, ventral nerve cord; *vs*, ventral seta; and *ys*, primordial (young) seta.

RESULTS

External Morphology

The body of the echiurid *Protobonellia zenkevitchi* is divided into the proboscis and trunk (Fig. 1a). The total body length of the fixed specimens we examined varies from 8 to 25 mm. The proboscis length is 3–10 mm; the trunk length is 5–14 mm. Individuals with a very short, and presumably regenerating, proboscis were found in the collection (Fig. 1b). Like most other echiurids, *P. zenkevitchi* has an external morphology that is extremely poor in characters. On the ventral side of the trunk, a pair of setae is located under the proboscis, usually on the well-pronounced muscular hill (Fig. 1c). The trunk ends with a small anal lobe that has the anal orifice on the ventral side (Fig. 1d). The trunk integuments are often very thin and transparent and the loops of the intestine filled with detritus are visible through them (Fig. 1e).

The Proboscis

The proboscis in *P. zenkevitchi* is a flattened outgrowth on the dorsal side of the body (Fig. 1a). In the fixed specimens, the proboscis is clearly subdivided into the distal, middle, and proximal parts, which differ in features of morphology and histological organization (Fig. 2a). At the distal end, the margins of the proboscis are thickened and rolled out in such a way as to form an expressed dilatation bearing lobes; the proboscis margin itself has a scalloped form (Figs. 2a, 2b). The rolled-in margins of the distal part of the proboscis form the inner unclosed cavity (Fig. 3a). Ventral ciliary grooves with a diameter of 5–7 μm (Figs. 2c, 3b, 3c) and a depth that varies from 20 μm (Fig. 3b) to 45 μm (Fig. 3c) run along the distal part of the proboscis. A ciliary groove consists of deep invagination of thick epithelium of the ventral side, the thickness of which reaches 20 μm. On the dorsal side of the distal end of the proboscis, the thickness of the epidermal

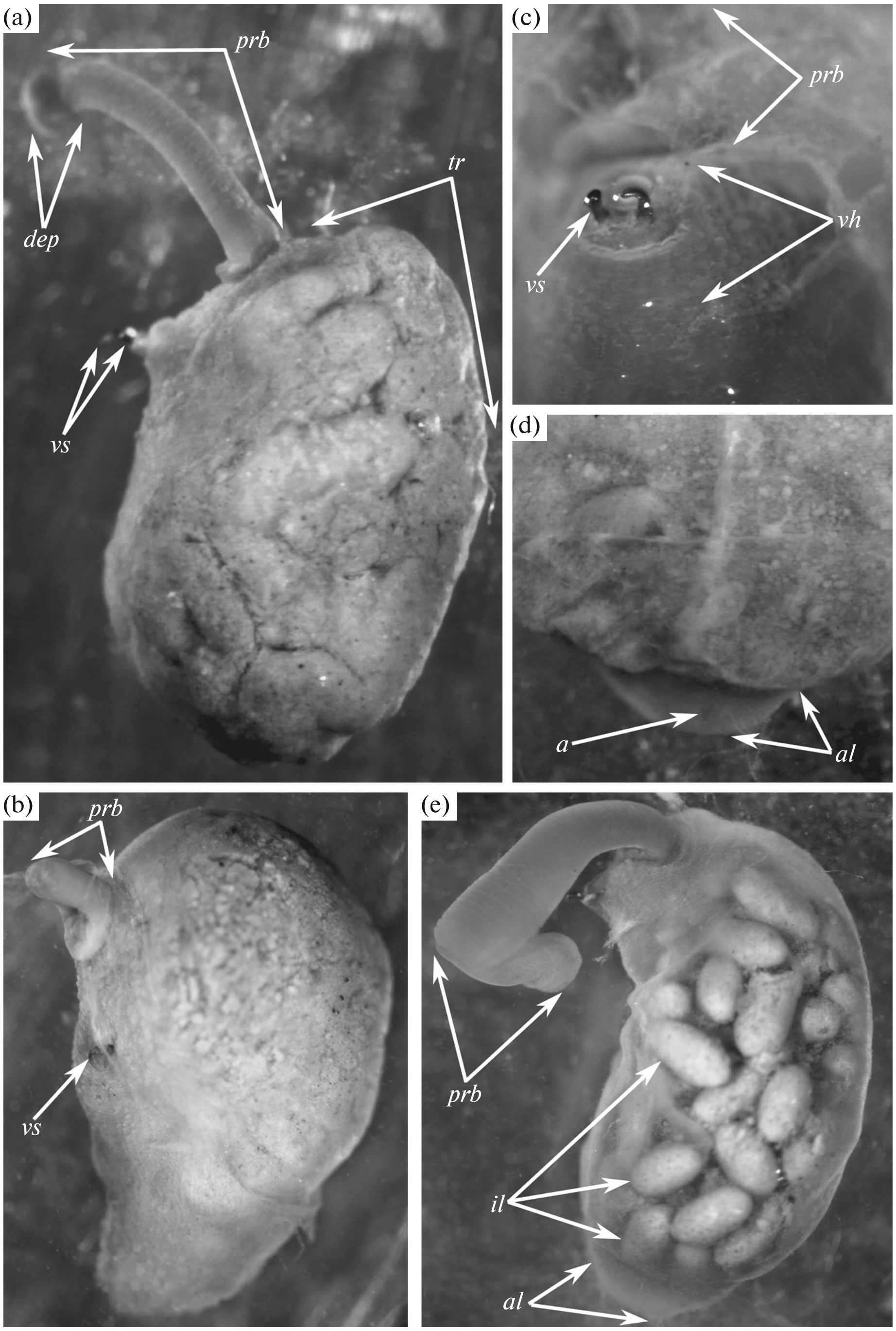

Fig. 1. The features of the external structure of the echiurid *Protobonellia zenkevitchi* (photographs of fixed material): (a) left-side view of the animal; (b) external view of a specimen with a shortened proboscis, ventro-lateral side; (c) ventral setae; (d) posterior end of the body, ventral side; (e) external view of the specimen with thin body walls, through which gut loops are visible. The key to lettering here and in Figs. 2–12 is provided in the Material and Methods section.

layer is 10 μm, which is 2 times lower than that on the ventral side (Fig. 3d). Beneath the epidermis, large and highly vacuolated cells are located on the dorsal and lateral sides of the proboscis. They form a continuous layer with a thickness of 40 μm (Figs. 3a, 3d). The cytoplasm of the cells is filled by large vacuoles with a transparent content. A dense parenchymatous tissue formed of small cells occupies the central part of the distal end of the proboscis. No muscle fibers are found in this part of the proboscis (Figs. 3a, 3d).

The distal end of the proboscis is separated from the remaining part by a pronounced constriction (Figs. 2a–2c). Closer to the mouth, the proboscis margins roll out and their surface acquires a clearly

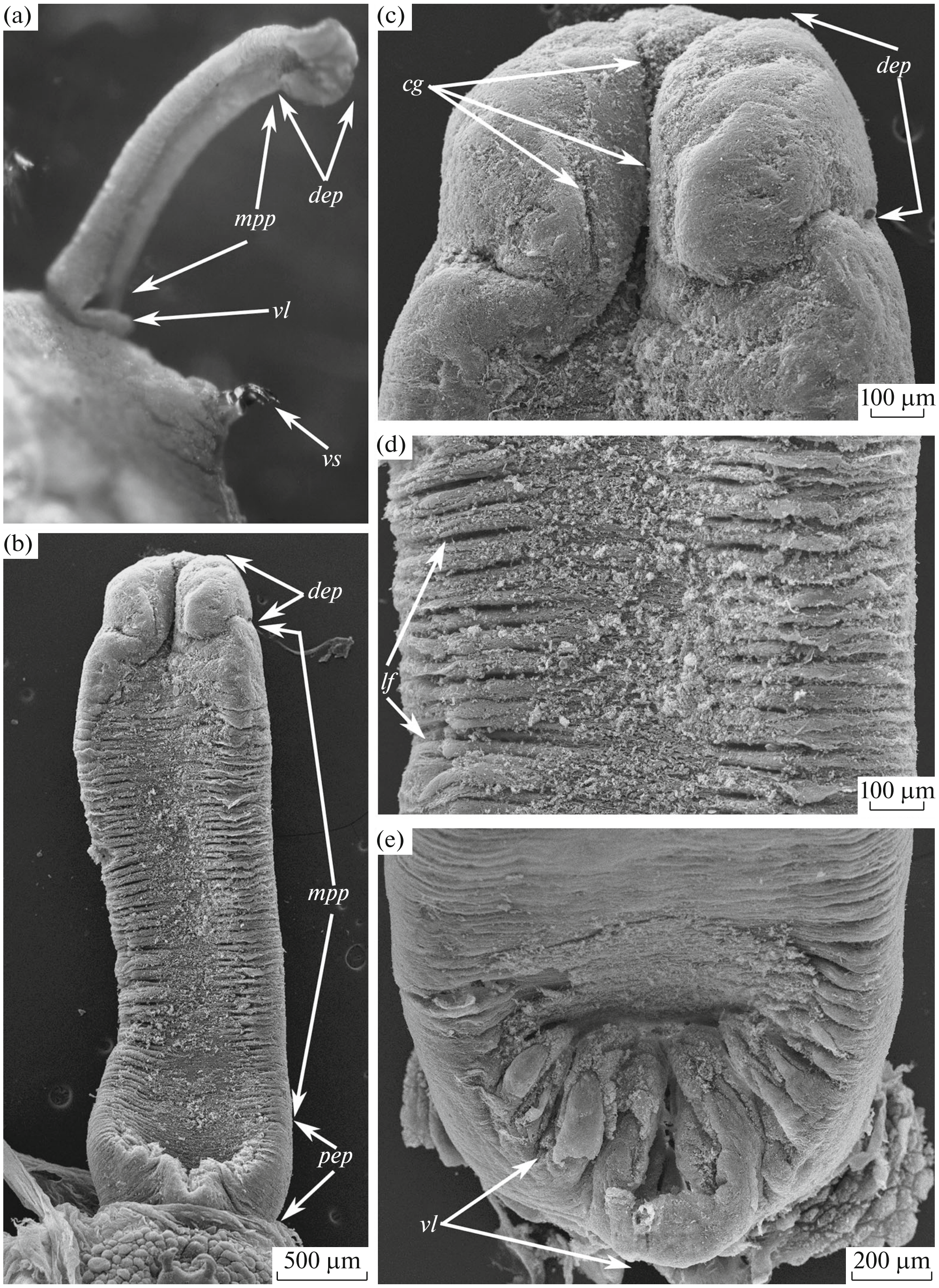

Fig. 2. Details of the proboscis morphology in *Protobonellia zenkevitchi*: (a) general view of the proboscis, ventro-lateral side (photograph of a fixed specimen), which clearly shows the division of the proboscis into the dilated distal part, the elongated middle part, and the proximal part bearing the ventral lobe; (b) general view of the proboscis, ventral side; (c) distal end with deep ciliated grooves visible; (d) ventral surface of the middle part of the proboscis with clearly visible transverse folds of the epidermis; (e) proximal part of the proboscis with ventral lobe ((b–e) SEM data).

expressed cross folding (Fig. 2c). In a cross section the proboscis up to its base has a shape of dorso-ventrally flattened ellipsoid (Figs. 4a, 4c). In the central part of the proboscis, the thickness of the ventral epidermis layer decreases, and ciliary grooves and the layer of vacuolated cells vanish (Figs. 4b, 4d). Beneath the epidermis is a layer of connective tissue that is most pronounced on the dorsal side of the proboscis (Fig. 4b). Beneath the connective tissue, annular fibers form a single layer. The middle part of the proboscis is intersected by cords of connective tissue (which are presumably thin muscle cords) that run in the dorso-ven-

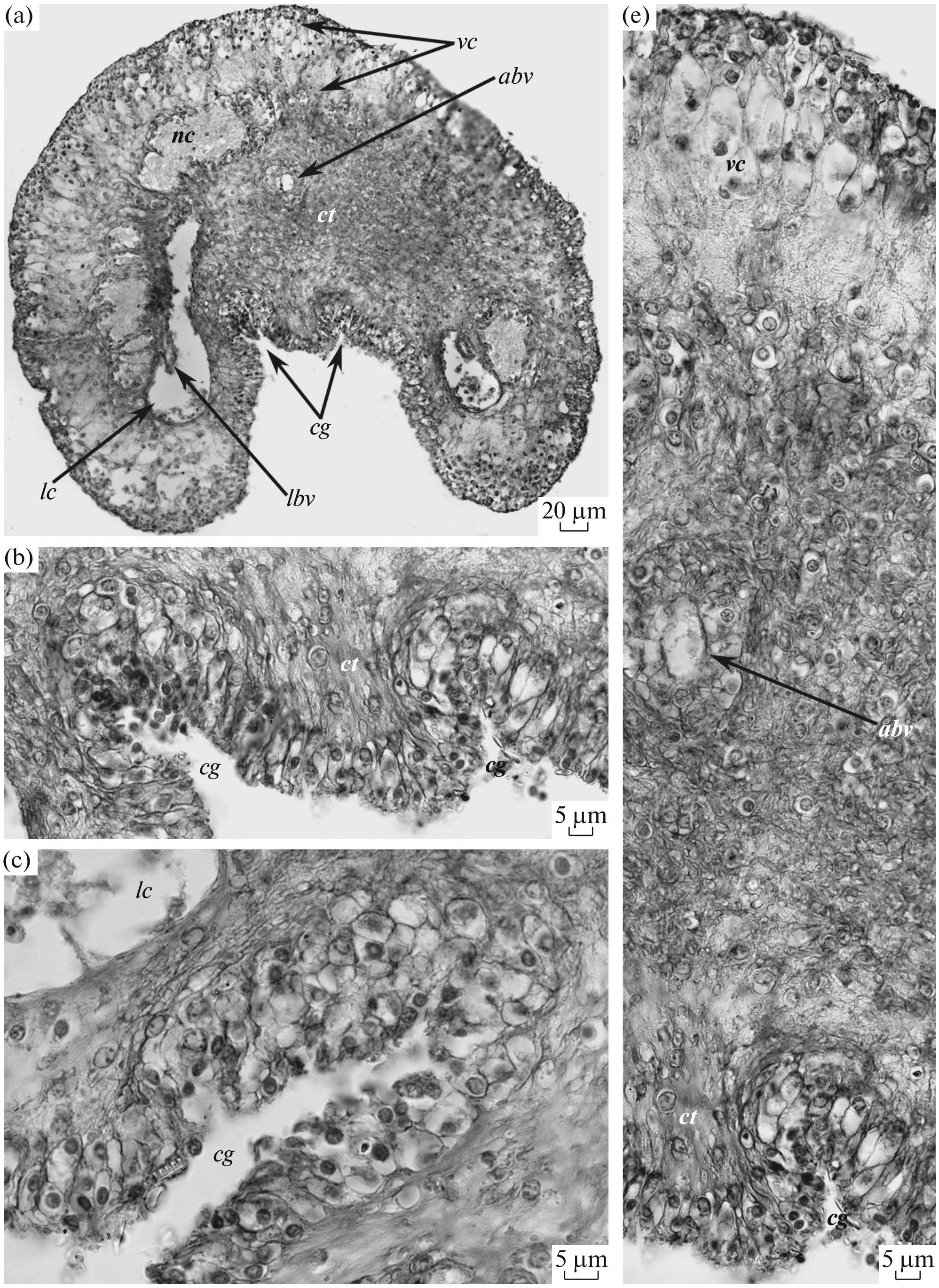

Fig. 3. The organization of the proboscis of *Protobonellia zenkevitchi* in transverse histological sections. In all the sections, ventral side is at the bottom, and the dorsal side is at the top. (a) General view of a cross section through the distal end of the proboscis; (b) ventral ciliary grooves; (c) ventro-lateral ciliary groove; (d) central part of the proboscis from the dorsal to the ventral side.

tral direction (Fig. 4g). The dorso-ventral and transverse connective-tissue cords divide the space of the proboscis into compartments in which longitudinal muscle fibers run, being most numerous in the central part of the proboscis. The musculature is formed by separate muscle cells; the diameter of muscle fibers is 10 μm (Figs. 4a, 4g). At the base of the proboscis, its margins roll up to the ventral side, forming the ventral lip, which is well pronounced in some specimens (Figs. 1a, 1b, 2a). Fusing on the ventral side of the body, the margins of the proboscis form a funnel opening into mouth (Fig. 2e).

The Trunk

In the fixed specimens of the studied species, the shape of the trunk varies from almost rounded to elongated-oval (Figs. 1a, 1b, 1e). On the ventral side of the

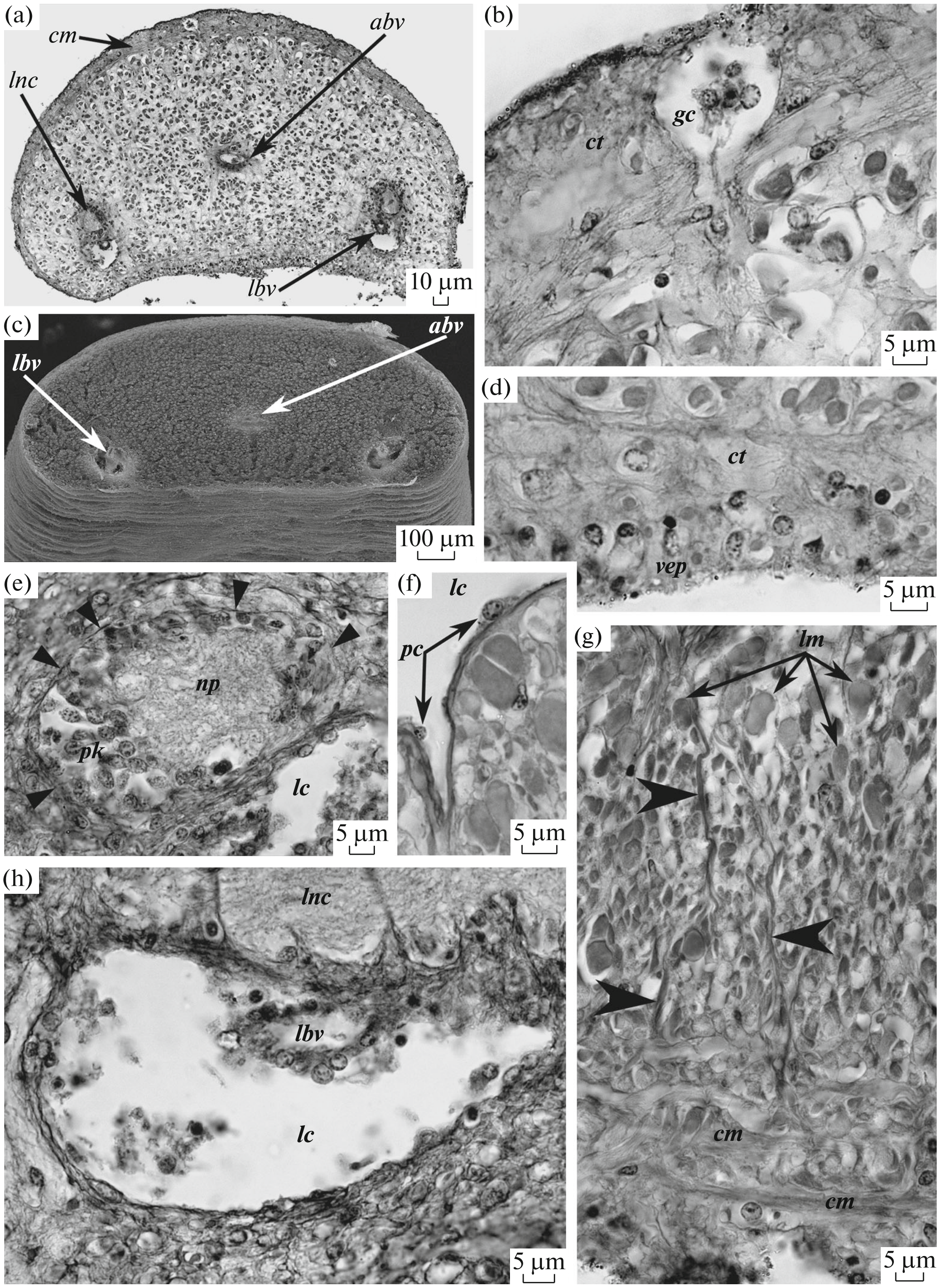

Fig. 4. Details of the histological organization of the proboscis in *Protobonellia zenkevitchi*. In all the sections, the ventral side is at the bottom and dorsal side is at the top. (a) General view of a histological section through the middle part of the proboscis; (b) the wall of the dorsal side of the proboscis, a connective tissue layer, and a large glandular cell; (c) cross section through the middle of the proboscis according to SEM data; (d) ventral epidermis of the proboscis; (e) lateral nerve cord (straight arrows indicate the envelope formed by connective tissue cells around the nerve cord); (f) lining of the lateral coelomic canals of the proboscis; (g) proboscis musculature (dorso-ventral cords are indicated by concave arrowheads); (h) lateral coelomic canals and lateral blood vessels of the proboscis.

trunk, two hook-shaped setae lie in its anterior third (Fig. 1c). Each seta is located at the center of a muscular hill (Fig. 5a) and bears three prongs: claw-shaped distal, leaf-shaped proximal, and rod-shaped central prongs (Fig. 5b). The total length of the working seta is approximately 1 mm, while only one-fifth of its length is visible on the surface. The bases of the setae are located deep in the center of the trunk and are sur-

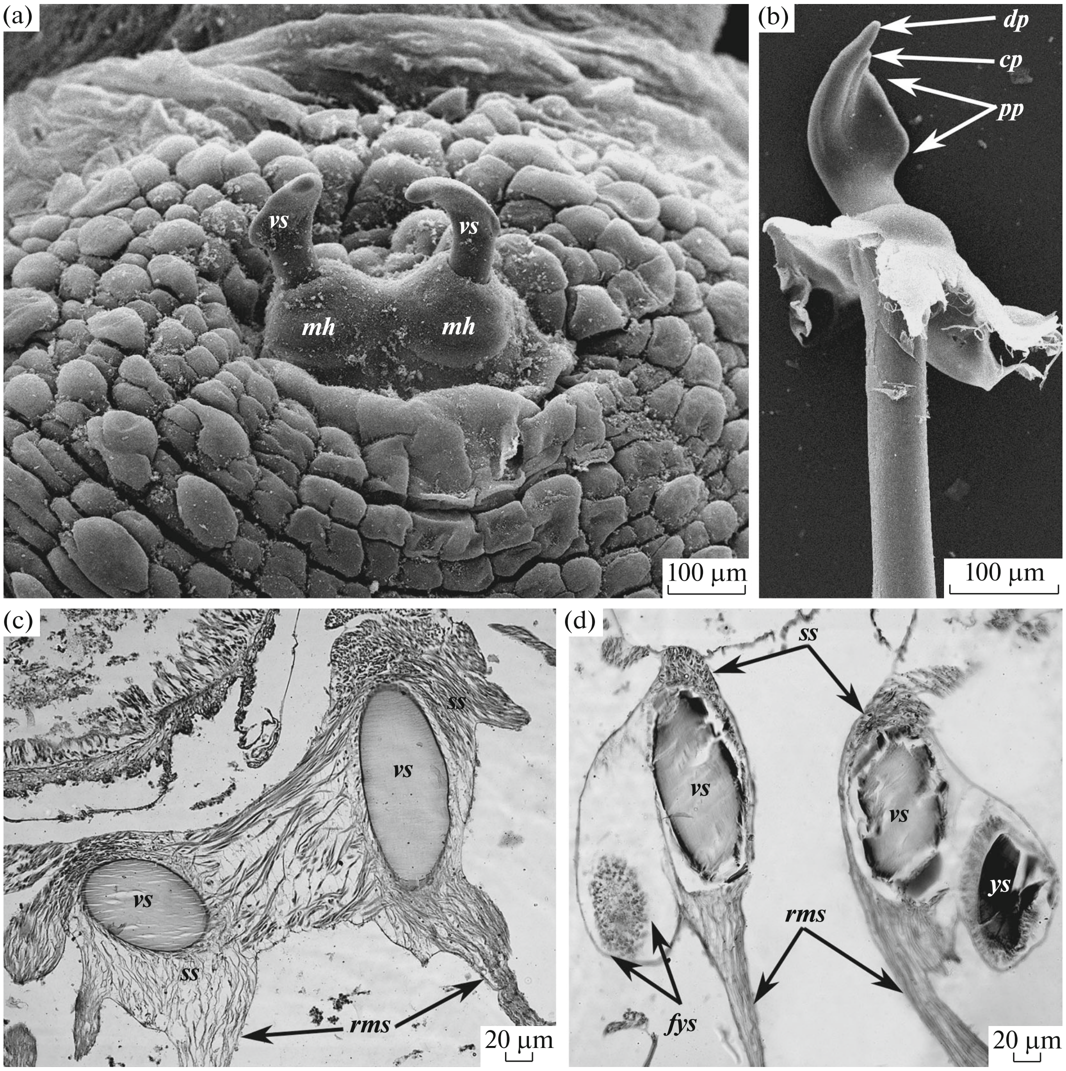

Fig. 5. Some details of the setae structure in *Protobonellia zenkevitchi*: (a) an area of the integument on the ventral side of the body and setae on muscular hills (SEM); (b) morphology of a seta with distal, proximal, and central prongs visible (SEM); (c) transverse histological section of the setiferous sacs and bases of the setae; (d) transverse histological section through the bases of the primordial setae.

rounded by a dense layer of transverse muscle fibers that connect the setae to one another and attach them to the body wall (Fig. 5c). At the base of each long working seta there is one forming seta surrounded by its own setiferous sac (Fig. 5d). The material of forming seta is dense and structurally similar to the peripheral material of the working seta (Fig. 5g).

The Body Wall

The body wall in *P. zenkevitchi* is formed of epithelium, a layer of connective tissue, and several layers of transversal and longitudinal musculature. The greatest thickness of the body wall is observed at the base of the proboscis, around the setae, and in the area of the anal lobe. On the ventral side of the anterior third of the trunk the epidermis forms folds that impart a reticulate structure to the integuments (Fig. 5a). Here, the thickness of the body wall is at a maximum, reaching 360 μm (Fig. 6a). A dense cuticle layer is located above the epidermis layer on the ventral side of the body (Fig. 6b). In this part of the body, the musculature beneath the epidermis is formed by several alternating layers of annular and longitudinal muscle fibers (Fig. 6c). Along the ventral side of the body, muscle bundles of the longitudinal musculature are divided by radial muscular cords into groups (Fig. 6d). The complex pattern of the musculature in this part of the body is supplemented by connective-tissue processes of radial muscular cords, which pass through the layer of longitudinal muscles, cross the trunk coelom, and are attached to the body wall (Figs. 6a, 6d). On the dorsal

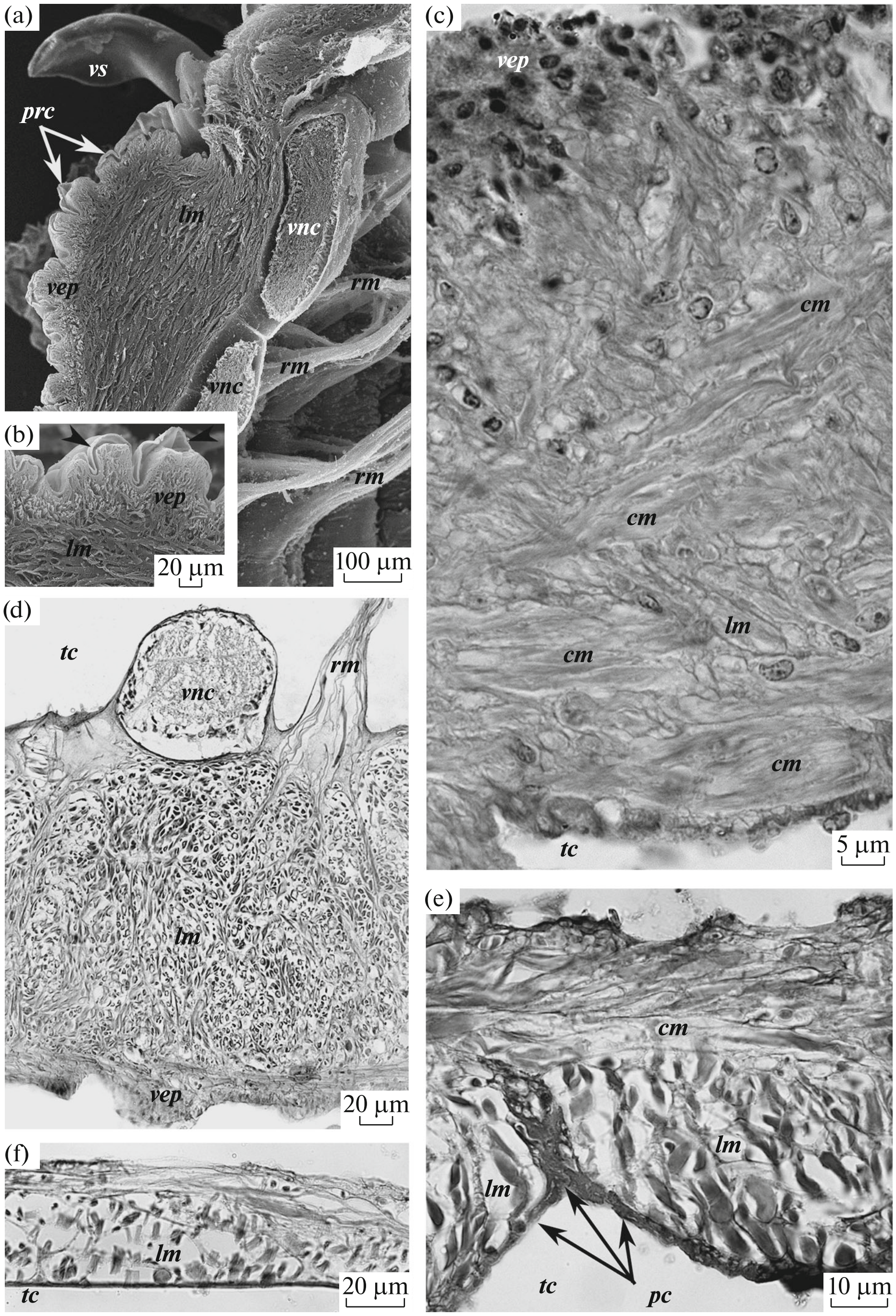

Fig. 6. Details of the body wall structure in *Protobonellia zenkevitchi* ((a, b) scanning electron microscopy, (c–f) transverse histological sections): (a) sagittal section through the bases of the setae; (b) a region of the ventral epidermis (the thick layer of the protocuticle is indicated by concave arrowheads); (c) wall of the ventral side of the body in the anterior third of the trunk; (d) wall of the ventral side of the body in the middle of the trunk; (e) wall of the dorsal side of the upper third of the trunk; (f) wall of the lateral sides of the trunk.

side of the anterior third of the trunk, the body wall is formed by a thin epidermis, a thick layer of transversal muscles, and separate bundles of longitudinal muscles, which are separated by cords of coelothelial cells (Fig. 6e). On the major part of the trunk, the body wall of the dorsal and lateral sides of the body consists of a very thin layer of epidermis, connective tissue, and longitudinal musculature (Fig. 6f). The thickness of the body wall here is not larger than 40 μm.

The Coelom

The coelom in this species is whole, without partitions. Two parts of the coelom can be differentiated:

the proboscis coelom and trunk coelom (Fig. 7a). The proboscis coelom consists of lateral perivascular canals that run along the ventro-lateral sides of the proboscis (Fig. 7b). These lie deep in the connective tissue and are separated from it by only a thin coelomic epithelium (Figs. 4f, 4h). Lateral blood vessels run in the lateral coelomic cavities of the proboscis (Fig. 4h). At the distal end of the proboscis, the perivascular coeloms merge with one another, forming a single, wide transverse cavity. At the base of the proboscis, its lateral sides join with one another forming the mouth orifice and the perivascular coelomic cavities shift to the ventral side and enter the trunk (Fig. 7d). They extend farther, remaining isolated and connected with pharynx, and merge together to form a single canal, within which the lateral blood vessels of the proboscis, also merging, form the ventral blood vessel. This short, single coelomic canal runs along the pharynx on the ventral side (Fig. 7c). Near the setiferous sheath, the canal merges with the trunk coelom and ceases to be an independent structure.

The trunk coelom occupies the entire space among the internal organs (Fig. 7e). Near the setiferous sheath, the trunk coelom is generally subdivided by radial muscles of setae and pharynx (Fig. 7c). In the anterior quarter of the trunk, *P. zenkevitchi* has a dorsal mesentery related with the dorsal blood vessel (Fig. 7d). However, the dorsal mesentery is very short: it is absent already in the anterior third of the trunk (Fig. 7c). The ventral mesentery, which is located directly under proboscis, has the form of a thick muscular cord (Fig. 7d), which, lower, originates two connective-tissue cords surrounding the ventral nerve cord (Fig. 7c). In the middle of the trunk, the ventral mesentery has the form of a short cord containing the ventral blood vessel (Fig. 7e). No other mesenteries are found in the middle of the trunk (Fig. 7e). In the posterior part of the trunk a visible group of radial cords occur that extend from the ventral body wall to the wall of the hindgut (Fig. 7f). The coelothelium has a similar histological organization in different parts of the trunk: it is formed by flattened cells with a rounded nucleus bearing the nucleolus (Figs. 6c, 6f, 6e).

The Digestive System

In *P. zenkevitchi*, the intestine occupies a considerable volume of the body and exceeds the length of the trunk, forming numerous bends and folds (Fig. 8a). The mouth orifice is located at the anterior end of the body, where the proboscis margins are bent onto the ventral side, forming deep folds (Fig. 8c). The mouth passes into the pharynx, which is attached to the body wall by several radial muscle cords, as well as by the ventral and dorsal mesenteries (Fig. 8d). The pharynx is followed by a sacciform expansion, the esophagus (Fig. 8f), passing into the gizzard, which forms a small loop and ends with a short and voluminous crop (Fig. 8e). The pharynx, esophagus, gizzard, and crop are parts of the ectodermal foregut, which passes into the endodermal midgut. The midgut is the longest part of the digestive tract. It is subdivided into the pre-siphonal (Fig. 8h), siphonal (Fig. 8g), and post-siphonal (Fig. 8k) parts. The pre-siphonal part is a long tube with a small diameter, forming three U-shaped bends (Fig. 8a). The pre-siphonal part passes into the siphonal part of the midgut, which is characterized by the presence of the diverticulum, a siphon (Fig. 8i) that runs along the dorsal side of the intestinal tube. The post-siphonal part is the longest one in the midgut. It forms two ascending and two descending branches (Fig. 8b). This part of the digestive tube forms two sacciform expansions: one is located at the level of the ventral setae, while the other is located ventrally at the level of the middle body (Fig. 8b). The ventral sacciform expansion extends to the posterior third of the trunk and grows narrower, passing into a short tube of the post-siphonal part, which, in turn, passes into the hindgut. The sacciform expansions have very thin walls and a large diameter; food residues in the lumen of these expansions were observed in all the specimens we examined (Fig. 8j). The hindgut (Fig. 7f) forms a short ascending branch that opens as the anal orifice on the ventral side of the body (Figs. 8a, 8b).

The Circulatory System

In the circulatory system of the studied echiurids ascending and descending blood vessels can be differentiated (Fig. 9a). The ascending blood vessel in the proboscis consists of the axial vessel that passes in the middle of the proboscis deep in the connective tissue (Figs. 3d, 4a). After reaching the distal margin of the proboscis, the axial vessel is T-shaped branched, forming the transverse blood vessel, and passes into lateral blood vessels that run along the lateral sides of the proboscis in the perivascular coelomic cavities (Figs. 4a, 4h). The lateral blood vessels of the proboscis, descending downwards, extend to the base of the proboscis, shift to the ventral side, and thus occupy the latero-ventral position (Fig. 7d).

In the anterior third of the body, the lateral blood vessels merge together, forming a single ventral blood vessel (Figs. 7c, 9c). The ventral blood vessel extends along the digestive tract to the level of the setiferous sacs. Here, the thickness of the walls of the ventral blood vessel is greatest and its internal walls are formed by complete endothelial lining (Fig. 9c). After the merging of the perivascular and trunk coeloms, the ventral blood vessel shifts to the ventral side. Further, it extends along the entire ventral side of the body, being connected with the coelomic envelope of the ventral nerve cord. In the posterior third of the trunk the ventral blood vessel is associated with the gonad; it originates thin capillaries, on which germ cells develop; the lumen of the blood vessel is small (Fig. 9e).

The reverse blood flow, from the posterior end of the body to the anterior end, apparently runs through

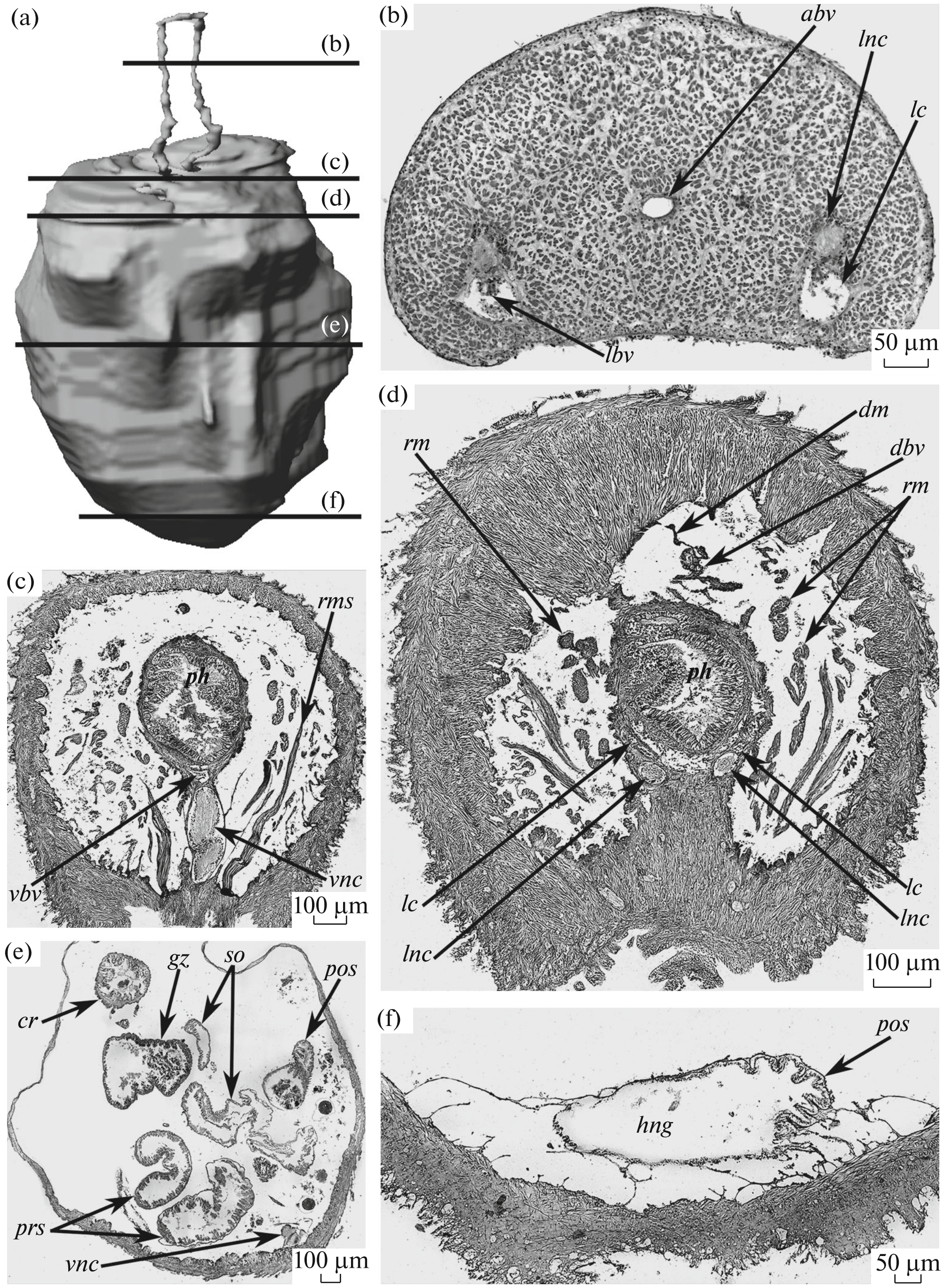

Fig. 7. Coelom organization in *Protobonellia zenkevitchi*. (a) 3D-reconstruction based on a series of histological sections and (b–f) transverse histological sections: (b) through the middle of the proboscis, (c) above the level of the ventral setae (with radial muscles of the setiferous sacs visible), (d) at the base of the proboscis (with numerous radial muscles of the pharynx), (e) through the middle of the trunk (the ventral and dorsal mesenteries are absent), and (f) through the anal lobe at the site of the opening of the post-siphonal part of the midgut into the hindgut.

a branched system of the intestine blood plexus, developed between the intestinal and coelomic epithelia (Figs. 9a, 9f). In the anterior third of the body, at the level of the setiferous sheaths, the dorsal blood vessel originates from the blood plexus. It immediately loses its connection with the gut and lies freely in the trunk coelom (Fig. 9d); in the upper third of the trunk, it is connected with the dorsal body wall via several connective-tissue cords (Fig. 9b). The wall of the dorsal blood vessel is formed by cells of the coelomic epithelium of the trunk coelom and the continuous endothelial lining, which is separated with a layer of dense non-cellular

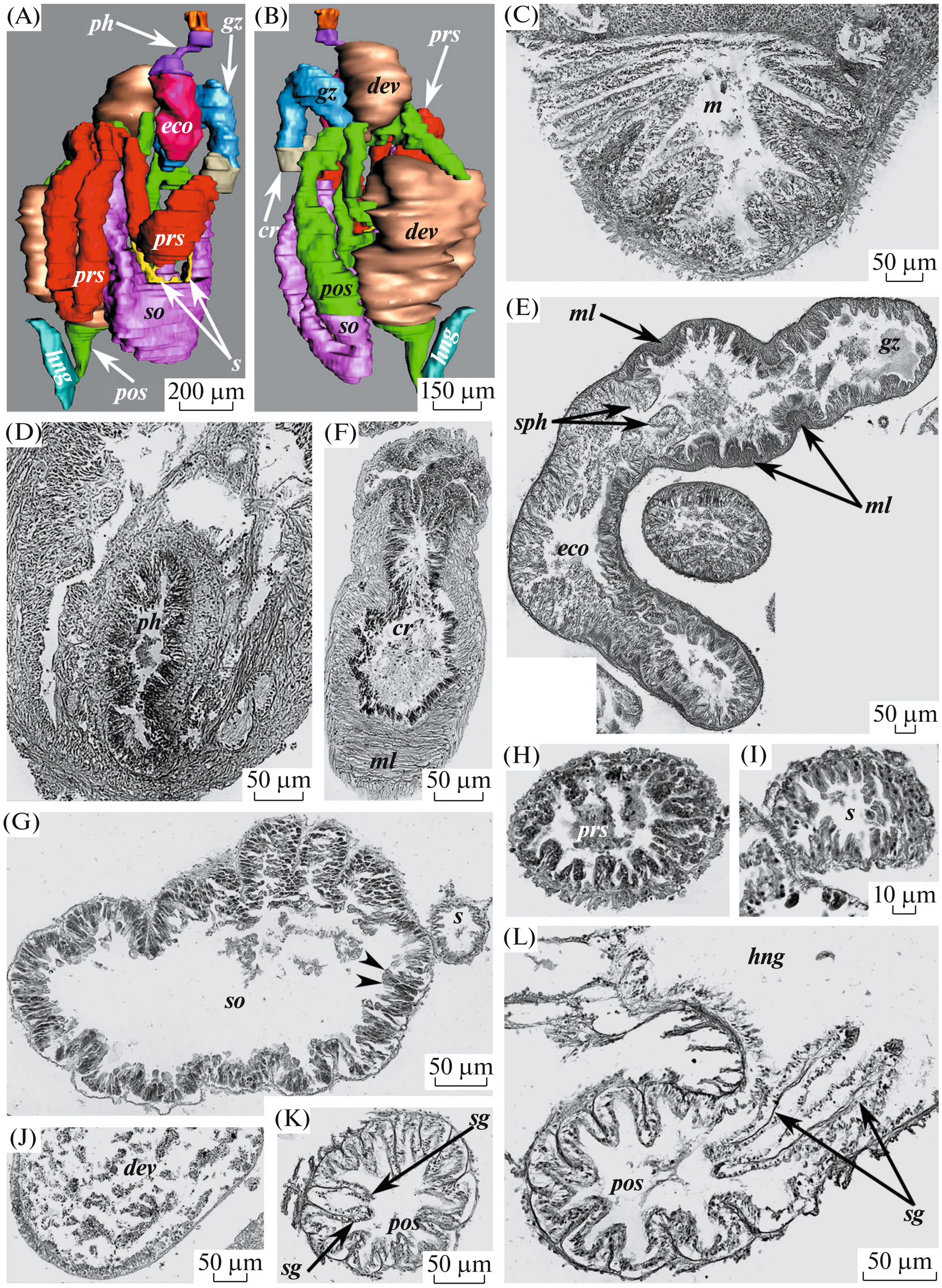

Fig. 8. The organization of the digestive system in *Protobonellia zenkevitchi*. (a, b) 3D reconstruction based on a series of histological sections and (c–l) transverse histological sections: (a) general view of the digestive tract, left side; (b) general view of the digestive tract, right side; (c) base of the proboscis and mouth orifice; (d) pharynx; (e) crop; (f) esophagus and gizzard; (g) siphonal part of the midgut; (h) pre-siphonal part of the midgut; (i) siphon; (j) large thin-walled diverticulum of the post-siphonal part of the midgut; (k) post-siphonal part of the midgut; (l) site of the transition of the midgut (post-siphonal part) into the hindgut.

matter. The dorsal blood vessel runs along the dorsal side of the body to the base of the proboscis, where it passes into the axial blood vessel of the proboscis.

The Nervous System

The nervous system in *P. zenkevitchi* consists of the ventral nerve cord and the nerve loop in the proboscis (Fig. 10a). Two nerve cords run along the ventro-lateral sides of the proboscis. The nerve cords of the proboscis are located deep in the connective tissue, but are separated from it by a thin layer of cells (Fig. 4e). At the distal end of the proboscis, the lateral nerve cords merge, forming a transverse nerve constriction. Descending down to the base of the proboscis, the lat-

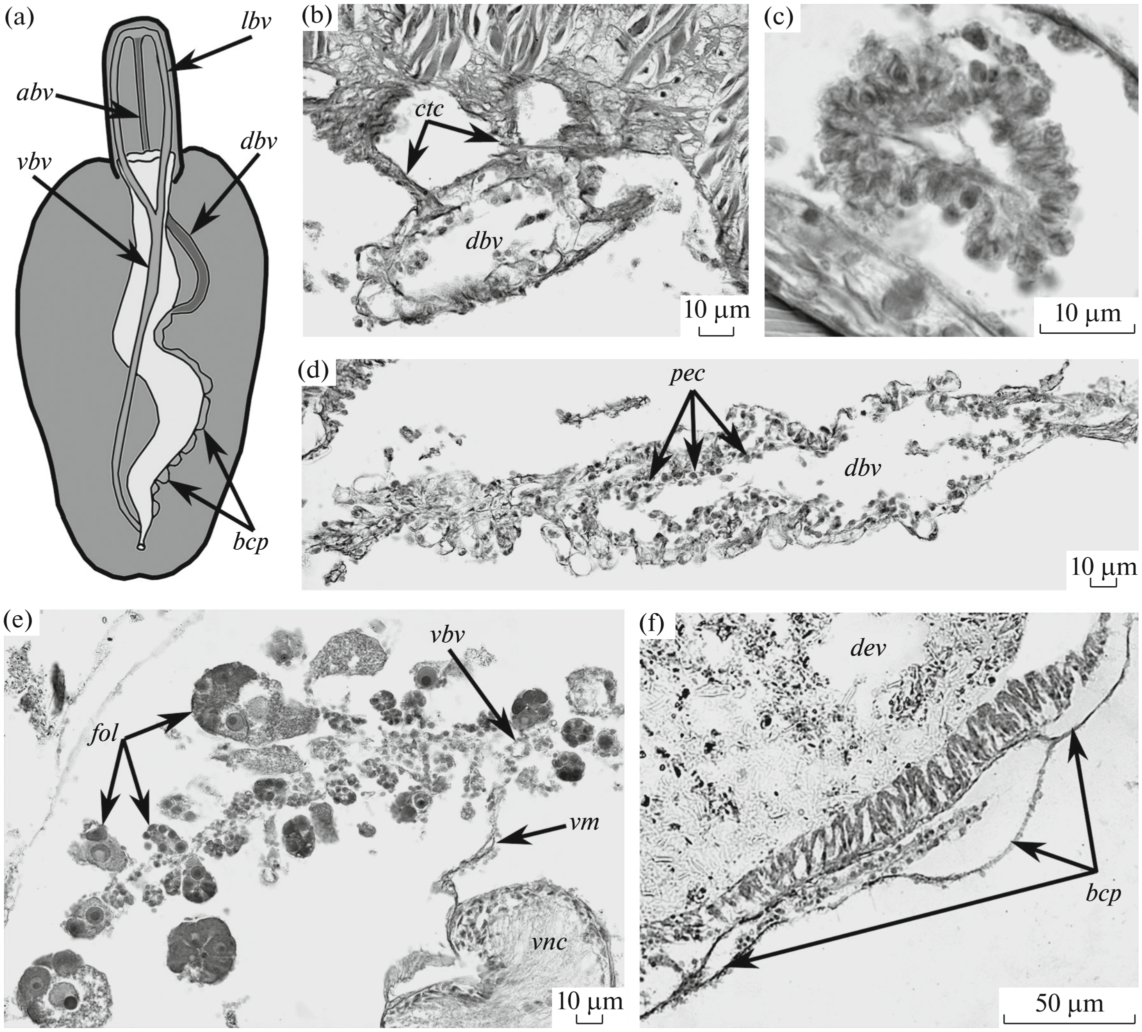

Fig. 9. Organization of the circulatory system in *Protobonellia zenkevitchi*. (a) General pattern of the circulatory system structure (intestine anatomy is simplified) and (b–f) transverse histological sections: (b) dorsal blood vessel immediately under the proboscis, with connective-tissue cords visible between the vessel and the body wall; (c) ventral blood vessel in the anterior third of the trunk; (d) dorsal blood vessel in the anterior third of the trunk; (e) ventral blood vessel and associated gonad follicles in the posterior half of the trunk; (f) capillaries of the blood plexus developed around the midgut.

eral nerve cords shift to the ventral side (Fig. 7d) and merge with one another, forming the ventral nerve cord. In the anterior third of the trunk, the nerve cord runs in the ventral chamber between two connective-tissue cords, which delimit the ventral chamber between the body wall and pharynx (Figs. 7c, 10c). This part of the ventral nerve cord has the largest diameter and is characterized by the presence of transverse connective-tissue cords that separate the nerve cord (Fig. 10f). Descending down along the ventral side of the body, the ventral nerve cord adjoins the body wall and is surrounded throughout its length by a thin layer of connective tissue, which is lined by coelothelium on the side of the body cavity (Fig. 10b). At the posterior end of the trunk, the ventral nerve cord passes for some distance along the dorsal wall of the hindgut and terminates on it (Fig. 10e). Throughout its length, the ventral nerve cord branches into collaterals with a diameter of 15 μm (Fig. 10e) that extend into the tissue of the body wall (Fig. 10d). The collaterals are composed of only processes of nerve cells, to which processes of muscle cells of the body wall approach (Fig. 10d).

The nerve cords have a similar histological structure: the central part of the cord is occupied by a large neuropile, while perikarya are arranged along the perimeter (Figs. 4e, 10b, 10c, 10f).

The Excretory System

The excretory system in *P. zenkevitchi* consists of one pair of anal sacs located on the lateral sides of the gut in the lower half of the trunk. Each anal sac is subdivided into two parts: sacciform (distal) and tubular

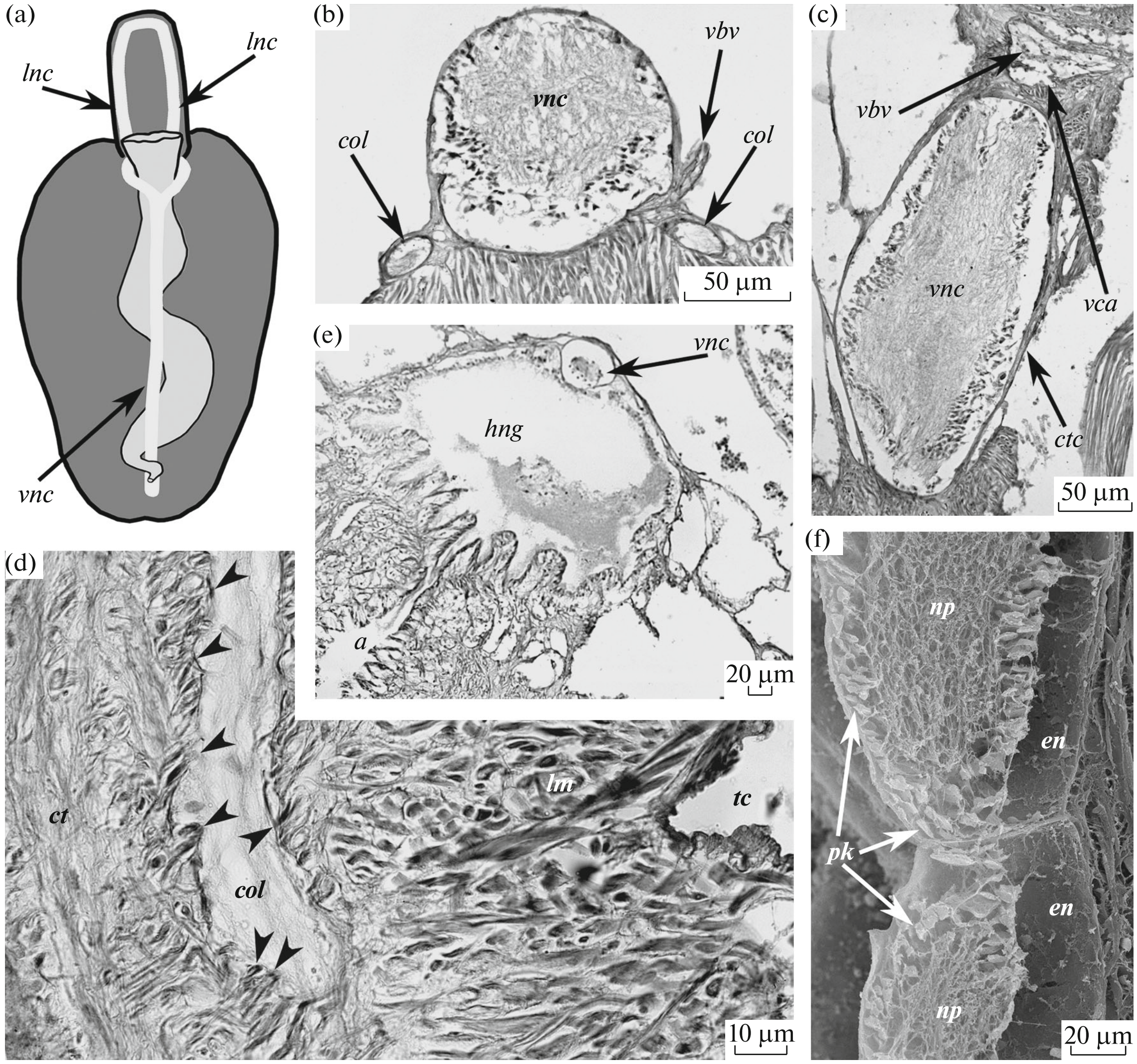

Fig. 10. The organization of the nervous system in *Protobonellia zenkevitchi*. (a) General pattern of the nervous system structure (intestine anatomy is simplified) and (b–e) transverse histological sections: (b) ventral nerve cord in the posterior half of the trunk; (c) ventral nerve cord in the anterior third of the trunk; (d) collaterals that run deep in the body wall of the trunk and contact (points of contacts are indicated by arrowheads) with muscle cells; (e) ventral nerve cord in the posterior third of the trunk, where the ventral position of the hindgut and anus and a more dorsal position of the nerve cord are observed. (f) Longitudinal section through the ventral nerve cord and its envelope according to SEM data.

(proximal) (Fig. 11a). Both parts are separated by an indistinct sphincter and differ in the histological structure of the wall (Fig. 11c). The cavity of the anal sacs communicates with the body cavity via ciliary metanephridial funnels located on long cylindrical or barrel-shaped stalks (Figs. 11a, 11b). The funnels consist of scalloped folds of epithelium separated from the stalks by a narrow constriction, or collar (Figs. 11b, 11g). In the posterior part of the trunk, anal sacs open into the hindgut (Fig. 11d). The wall of the sacciform part of the anal sacs is formed by a high columnar epithelium. The epithelium consists of epithelial-muscular cells, the basal parts of which form long processes containing myofilaments (Fig. 11f). The processes are oriented at an angle to the longitudinal axis of the anal sac, forming a kind of muscle lattice. On the side of the body cavity the anal sacs are lined by a layer of coelomic epithelium.

The Reproductive System

The reproductive system in the female *P. zenkevitchi* consists of an unpaired gonad located in the posterior part of the body. It is associated with the ventral blood vessel and capillaries diverging from it (Fig. 9e). The gonad is an aggregation of follicles, each of which consist of a developing oocyte with a diameter of 10–15 μm and associated nurse cells (Fig. 9e). In the cavity of the trunk coelom of *P. zenkevitchi* are follicles that have disconnected from a blood capillary.

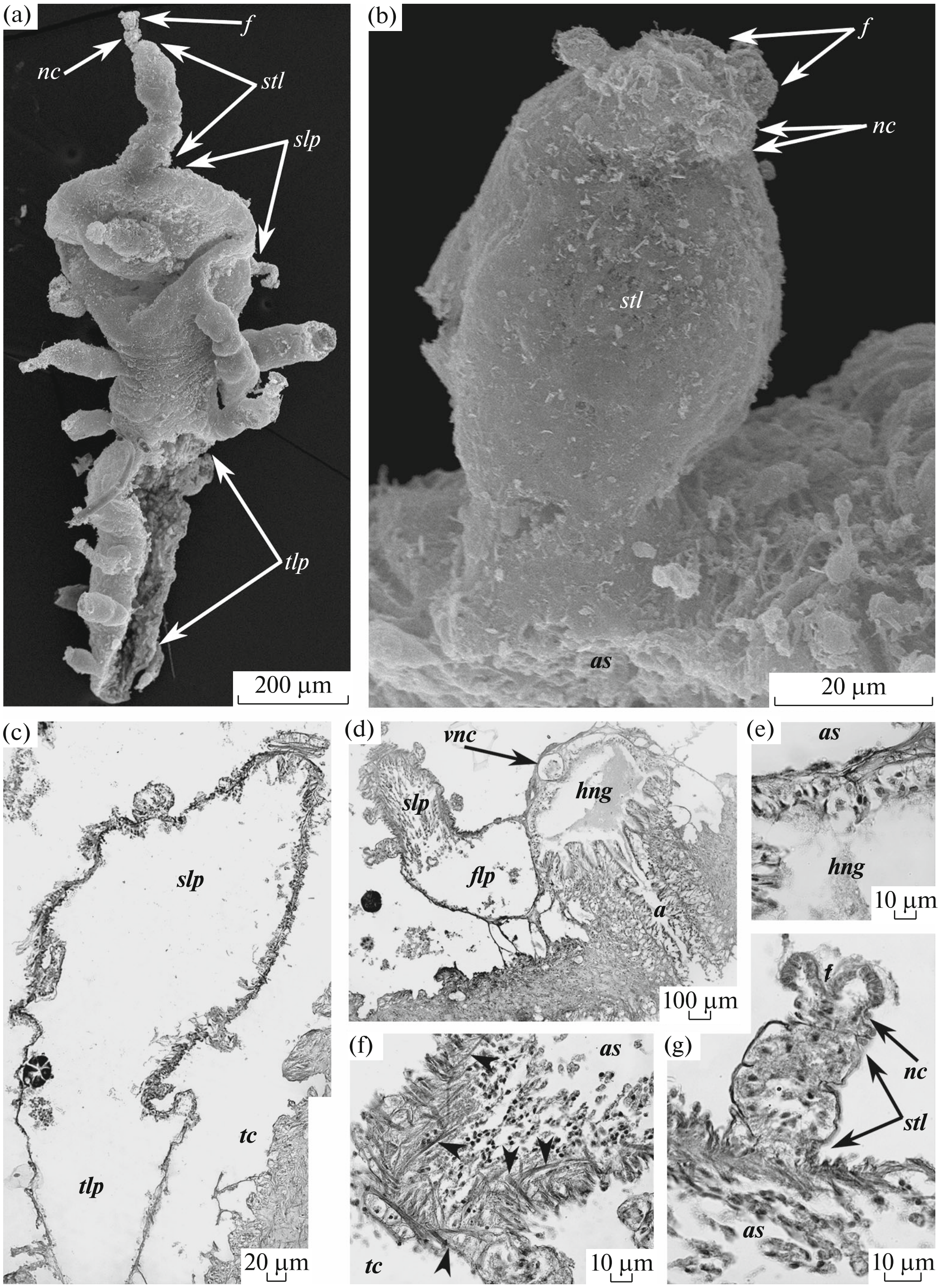

Fig. 11. Some details of the excretory system structure in *Protobonellia zenkevitchi*. (a, b) SEM and (c–g) histological sections: (a) external view of the anal sac with clearly discernible subdivision into the sacciform and tubular parts; (b) separate funnel of the nephridium; (c) longitudinal section through anal sac showing the subdivision into the sacciform and tubular parts with the indistinct sphincter between them; (d) transverse section through anal lobe, the site of the connection of the hindgut and anal sac; (e) diaphragm between the anal sac cavity and the hindgut; (f) tangential section through the anal sac with muscle processes of the cells of the lining of the anal sac (indicated by arrowheads); (g) longitudinal section through a nephridium funnel.

Oocytes in these follicles reach a size of 40 μm; sparse nurse cells form a compact “cap” at one of the poles of oocyte (Fig. 12a). Oocytes that have left the follicle and whose diameter reaches 100 μm are found in the body cavity. They are surrounded by a non-cellular membrane; their nucleus lacks nucleolus (Fig. 12b).

P. zenkevitchi possesses a specialized unpaired gonoduct. It is located on the ventral side, in the anterior third of the body at the level of the base of the setiferous sheath, on the right of the ventral nerve cord. The gonoduct consists of the sacciform (distal) and tubular (proximal) parts (Fig. 12c). The distal part

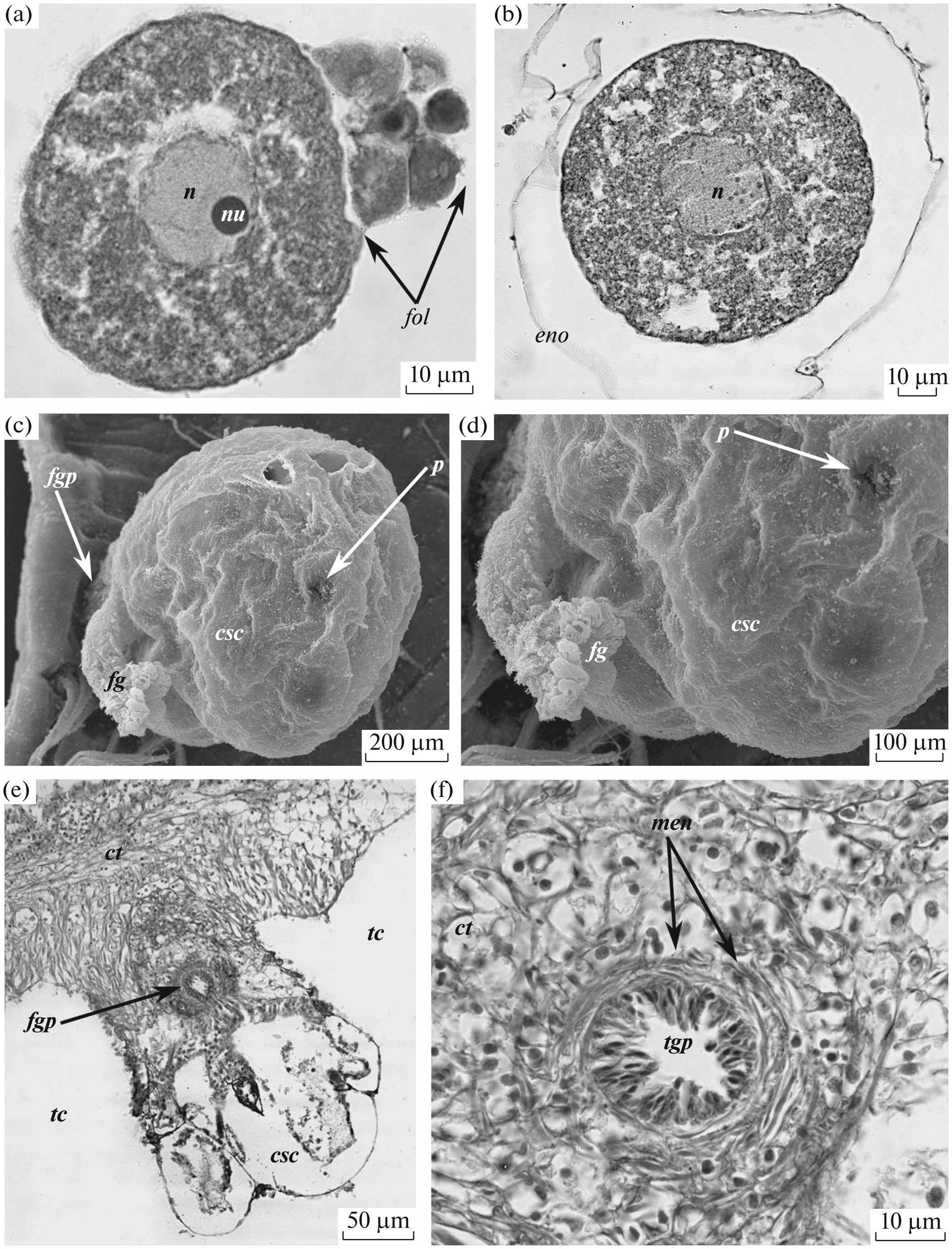

Fig. 12. Details of oogenesis and organization of the gonoduct in *Protobonellia zenkevitchi*. (a, b, e, f) Histological sections and (c, d) SEM: (a) oocyte disconnected from blood capillaries, freely floating in the cavity of the trunk coelom and having a "cap" of nurse cells; (b) oocyte surrounded by a membrane in the body cavity; (c) general view of the gonoduct; (d) storage sac and the pore of the storage sac; (e) oblique section through the storage sac and the tubular proximal part of the gonoduct; (f) cross section through the proximal tube of the gonoduct.

(storage sac) has scalloped walls and internal chambers (Figs. 12c, 12e). At the base of the storage sac is a ciliary funnel (Fig. 12c). On the dorsal side of storage sac is a pore presumably connecting the cavity of storage sac with the cavity of the trunk coelom (Fig. 12d). The proximal part of the gonoduct has the shape of a narrow short tube that passes deep in the tissue of the body wall and connects the cavity of the storage sac with the environment (Figs. 12c, 12e, 12f). The wall of the tubular part of the gonoduct is composed by the columnar epithelium, forming low folds (Fig. 12f). The tube of the gonoduct has an envelope of annular muscle fibers that run in the connective tissue layer.

DISCUSSION

The external morphology of the echiurid *Protobonellia zenkevitchi* is typical of all echiurids, whose bodies are divided into an unsegmented sacciform trunk and a proboscis that can be stretched out significantly [1, 11]. The anal lobe found in *P. zenkevitchi*, which bears the ventrally located anus, was not previously observed in echiurids. The location of the anus on the ventral side of the trunk was described from *Bonellia viridis*; however, the anus in living echiurids is located terminally and the anal lobe is absent. It is probable that the ventral position of the anus and the formation of anal lobe result from fixation and strong contraction of the musculature in the posterior end of the body.

The proboscis structure varies between echiurids and is often used as one of the distinguishing characters. The proboscis in *P. zenkevitchi* shows some morphological features that are probably related with feeding habits. Most echiurids are sediment feeders that collect detritus particles from the sediment surface using the proboscis. As a rule, the proboscis is positioned on the surface in such a way that its ventral side faces the water column, the dorsal side faces the substrate, and the terminal and lateral margins of the proboscis are slightly embedded in the sediment [8]. Food particles are collected using cilia and mucus secreted by cells of the proboscis, as well as due to contractions of the proboscis muscles [10]. The diameter of captured particles can vary greatly: from 90 μm to 0.5–2 mm [5, 10]. Segregation of food particles, as described from some echiurids, occurs at the base of the proboscis, where protrusions are formed that allow the animal to discard the largest food particles that are unfit for eating [5]. Fine segregation of food into small and large particles has not been described from echiurids.

The morphological features of the proboscis in *P. zenkevitchi*, such as the presence of the dilated distal end, the epidermis of which forms ciliated grooves, and connective tissue that contains large vacuolated cells, suggest a special way of positioning the proboscis on the sediment surface and consuming food particles. Thickened and reinforced with vacuolated cells, the distal margin of the proboscis rests against the bottom surface; the more proximal parts are raised above the bottom and slightly bent to the ventral side from the flanks. Due to the elevated position of the proximal part of the proboscis above the bottom only the distal margin of the proboscis is involved in collection of food particles. Feeding grooves extend along the distal margin; sorting out of food particles is performed here. Small food particles trapped in the lumen of a feeding groove move to the mouth due to the work of the ciliary epithelium of the proboscis. Large particles cannot enter the feeding groove and remain on the bottom. The diameter of the feeding groove is 5–7 μm, which means that food of *P. zenkevitchi* is comprised of detritus particles and organisms with a size no larger than 5–7 μm.

In the vast majority of echiurids, a pair of hook-shaped setae is located above the gonopores on the ventral side of the body [8, 18]. The results of our studies show that *P. zenkevitchi* also has a pair of ventral setae with a complex morphology. In addition to large working setae, *P. zenkevitchi* has primordial setae located dorsally relative to the working ones. The presence of primordial setae has also been described from other echiurid species [2, 21].

In most echiurids, the coelom usually consists of two non-communicating parts: trunk coelom and proboscis coelom [18]. In most of the studied species, proboscis coelom has a complex organization, including lateral perivascular cavities and a network of coelomic canals developed beneath the ventral epidermis. It is believed that the proboscis coelom in echiurids is isolated from the trunk coelom and consists of a separate coelomic compartment [1, 7, 20]. As our studies have shown, the coelomic system of the proboscis in *P. zenkevitchi* is organized more simply than that in other echiurid species. The species we studied lacks the ventral network of coelomic canals in proboscis. This is probably due to the relatively small size of the proboscis and the exceptionally developed muscular, connective, and supporting tissues, which in this species form a very dense layer devoid of cavities and gaps. The distinguishing feature of the coelomic system of the proboscis in *P. zenkevitchi* is that it does not form a separate coelomic compartment and is connected with the trunk coelom.

The digestive system of all echiurids is subdivided into several parts, which differ anatomically and histologically [18]. The differences in the structure of different parts of the digestive tract are caused by the functions performed by various parts [14, 15]. The digestive system in *P. zenkevitchi* is distinguished by an extremely long midgut, which forms several ascending and descending branches. *P. zenkevitchi* has a special thin-walled diverticulum related with the post-siphonal part of the midgut, which is characterized by a large volume and very thin walls. In all the examined specimens, this diverticulum was filled with detritus. This formation was not observed in the intestine of other members of the echiurids and is probably a unique anatomical feature of *P. zenkevitchi*. This specialized part of the intestine apparently performs the function of food storage, which can explain the presence of detritus in its lumen. On the other hand, the presence of an additional part of the gut in *P. zenkevitchi* results in a significant extension of the digestive tract, which provides more thorough processing of food. Formation of the specialized part of the intestine is probably related to the great depths of the species habitat: the specimens we studied were collected from a depth of more than 5000 m. The deep-sea zone of the World Ocean is characterized by exceptional oligotrophy: very small amounts of food reach these depths; thus, thorough food processing and storage are of great importance here.

The circulatory system of echiurids is usually closed, with the exception of the species *Urechis caupo*, which lacks a circulatory system [18]. The circulatory system includes two major vessels (dorsal and ventral), as well as the supplementary neuro-intestinal and ring vessels [6, 8, 13, 16, 18]. Compared to the classic pattern, the blood system in *P. zenkevitchi* is organized more simply. This species lacks the neuro-intestinal and ring blood vessels, the dorsal blood vessel is very short, and the network of capillaries of the intestine blood plexus is poorly developed. These anatomical features of the circulatory system in *P. zenkevitchi* are apparently related to the small size of its body and occurred as a result of reduction. Another unusual trait in *P. zenkevitchi* is the complete endothelial lining developed in the dorsal blood vessel, which was not previously reported from echiurids.

The structure of the nervous system is similar between all the studied echiurid species within the group [18]. Unlike the typical members of the echiurids in which the nerve cord at the posterior end of the body adjoins the body wall and innervates the musculature of this region of the trunk [19], in *P. zenkevitchi* the ventral nerve cord in the posterior third of the trunk runs along the dorsal wall of the hindgut and is not connected with the body wall.

The excretory system of the metanephridial type, which consists of anal sacs opening into the hindgut, is characteristic of all echiurids. The anatomical structure of anal sacs in echiurids is very diverse [12]. Simple anal sacs, each consisting of an unbranched tubular outgrowth bearing ciliated funnels, are typical of members of Thalassematidae. In species of the Urechidae group, the tubular anal sac bears small swellings; each has a ciliate funnel. Anal sacs of bonellids show the most complex organization: sacciform anal sacs can be highly branched, forming large outgrowths of the second and third orders. On these outgrowths, numerous ciliary funnels are located, each on a long stalk. Compared to other members of Bonellidae, the excretory system in *P. zenkevitchi* is organized more simply: the anal sacs do not form large outgrowths and their general anatomy is similar to that in members of Thalassematidae. At the same time, in *P. zenkevitchi*, each ciliated funnel is located on a long stalk, whose presence is an anatomical feature of all bonellids [12]. The small size of excretory trees in *P. zenkevitchi* can apparently be explained by the small size of the body and the small volume of the trunk coelom.

In the structure of reproductive system, echiurids demonstrate a significant diversity of gonoduct anatomy [12]. Gonoducts in members of Bonellidae have a specific structure [6]. In females of Bonellidae, gonoduct consists of the storage sac, opening into the coelom via a ciliary funnel, and the proximal dilated part, the androecium, inhabited by dwarf males and opening outwards via a gonopore [6, 18]. Fertilization of oocytes occurs in the cavity of the storage sac, where they enter from the body cavity through the funnel, while the seminal fluid enters from the androecium. The reproductive system in the *P. zenkevitchi* specimens we studied differs from the typical one. The differences consist in the structure of the gonoduct, which in *P. zenkevitchi* has a shortened globe-shaped storage sac with a pore, probably connecting the cavity of sac and the trunk coelom. This pore has not been previously described from other members of Bonellidae. It is probable that spermatozoa are released through it into the cavity of the trunk coelom, where fertilization occurs. The presence of oocytes surrounded by a membrane (possibly the fertilization membrane) in the trunk coelom can serve as indirect evidence for this idea. Another interesting feature of the gonoduct anatomy in *P. zenkevitchi* is the lack of a specialized androecium.

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