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Detection of bacteria associated with the tegument of caryophyllidean cestodes

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Summary

The association of bacteria with the surface of the tegument in two species of caryophyllidean cestodes (*Caryophyllaeus laticeps* and *Khawia armeniaca*) inhabiting the intestines of various cyprinid fishes was studied for the first time using transmission and scanning electron microscopy. The attachment sites of the bacteria were closely connected with the apical surface of the tegument by means of special holdfast structures, such as tufts and two types of colonic crypts. In all cases of firm attachment, the holdfasts (tufts and filaments) were generated by the cellular wall structures of the bacteria. The higher density of bacteria in the middle and posterior parts of the tapeworm's body is probably due to the structural and functional differences between various sections of the caryophyllidean tegument. The calculation of morphological parameters of microtriches shows a more important role of filamentous microtriches in the amplification of the cestode surface for digestive-transport processes. It can be assumed that the association of intestinal microflora with the tegument may be beneficial to tapeworms. In particular, the tegument-associated bacteria may participate in the metabolism of cestodes and be involved in the absorption of nutrients by the parasite.

Key words: cestodes; bacteria; ultrastructure; microtriches; symbiotic relationship

Introduction

As it is known, the intestine of fish contains rather dense bacterial populations. In general, the range of bacterial taxa in the gastrointestinal tract of freshwater fishes is similar to that found in the fish environment, and the number of bacteria fluctuates seasonally and in the different regions of the gastrointestinal tract (Lee, 1980; Austin & Al-Zahrani, 1988). The number of microorganisms within the digestive tract of fish is much greater than in the surrounding water, indicating that the intestine provides favorable ecological niches for these organisms (Cahill, 1990). Some microbial types live freely in the lumen, some colonize the intestinal crypts and others interact intimately with the gut surface (Savage, 1980; Beveridge, 1980; Berg, 1996). There are very few articles that specifically consider the mechanisms of association between bacteria and the fish intestine (Corpe, 1980; Lee, 1980). According to the published literature, it appears that the intestinal microflora supplies the fish organism with various nutrients. Also, bacteria synthesize many vitamins, essential amino acids and other substances necessary for the vitality of fish (Ugolev, 1985; Tannock, 1999; Kuz'mina *et al.*, 2000). Many features of the biochemistry, physiology and immunology of the animal intestine are, in fact, microflora-associated characterristics (Tannock, 1999).

The fish intestinal tract provides an environment rich in nutrients for many helminthes (cestodes, trematodes). Little is known about associations between these worms and bacteria. Morris (1973), Aho *et al.* (1991), Hughes – Stamm *et al.* (1999) described the presence of bacteria on the surface of trematodes. The investigation of the mucosa-associated microbial populations of the cestode tegumental surfaces is still in its infancy. The complex physiological mechanisms of interaction between the microflora and the cestodes are gradually becoming clear (Izvekova & Lapteva, 2002; Izvekova, 2003). The ultrastructural studies of sym-biotic microflora associated with cestode tegumental sur-face are absent.

This report is devoted the occurrence of bacteria in caryophyllidean cestodes, *Caryophyllaeus laticeps* and *Khawia armeniaca* that inhabit the intestine of freshwater cyprinid fishes.

Material and Methods

Cestodes *Caryophyllaeus laticeps* were extracted from the intestine of bream (*Abramis brama*) cought in the Rybinsk reservoir (Russia). Specimens of *Khawia armeniaca* were removed from the intestine of khramulya (*Varicorinus capoeta sevangi*) taken from Lake Sevan (Armenia). Live tapeworms were washed in Hank's physiological solution, and then small fragments of each specimen were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for electron microscopy. After rinses in cacodylate buffer, the specimens were post-fixed in 1 % aqueous osmium tetroxide. During the preparation for transmission electron microscopy, the specimens were dehydrated in ethanol, transferred to anhydrous acetone, and embedded in Epon-Araldite. Ultrathin sections were stained with uranylacetate and Reynolds lead citrate, and examined under a JEM-100C transmission electron microscope. For scanning electron microscope observations, the parasites were dehydrated in a graded ethanol series with a final change in absolute ethanol. Then the animals were critical-point-dried with liquid CO_2. The specimens were mounted on the stubs, sputter-coated with gold-palladium and examined on a JEOL-25 S and LEO-1420.

A morphometric analysis of the length, diameter and density of microtriches was done using the method of Graeber & Storch (1979).

Results

Before considering the symbiotic microflora, it is necessary to show the structure of the tegumental surface of caryophyllidean cestodes that are lined externally by a syncytial tegument. Two types of microtriches cover the surface of *K. armeniaca* and *C. laticeps* with the regional variations in size, shape and number of microtriches per unit area of the tegument. The transmission electron microscope examination of the tegument in both worms revealed cone-shape microtriches covering the surface of the anterior portion of these cestodes (Fig. 1a). The tegument of the middle and posterior parts of the body is lined by filamentous microtriches (Figs. 1b, 5a, b). The microtriches in each region of the caryophyllidean tegument are relatively uniform in size. The morphometrical parameters of microtriches from various parts of the caryophyllidean cestodes are presented in Table 1. Assuming the data given in Table 1, the amplification of the surface in cestodes due to their microtriches is calculated excluding the dense distal shaft of microtriches as a functional amplification factor according to Threadgold & Robinsson (1984).

The scanning electron microscopy of the *K. armeniaca* surface demonstrates that the bacteria are not just randomly distributed throughout the tegument of caryophyllidean cestodes, but are mostly concentrated in the middle and posterior parts of the tapeworm body (Fig. 2a). Scanning microscopy provides limited evidence of intimate association between the bacteria and the cestode tegument. Most of the bacteria come in close proximity to the microtrichial brush border layer of the cestode surface, and are placed in parallel with the surface tegument. The longitudinal axis of some bacteria is perpendicular to the surface, and micro-

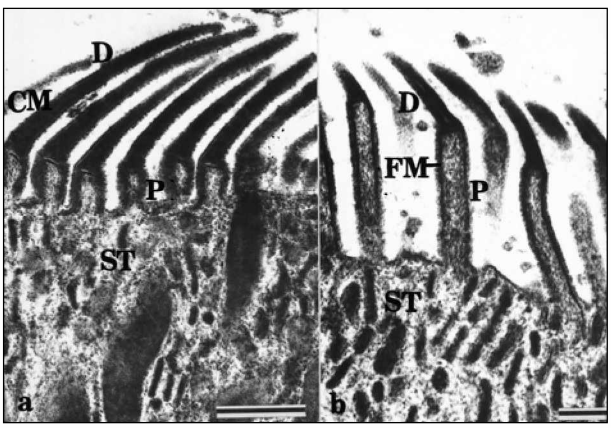

Fig. 1. *Caryophyllaeus laticeps* tegument with a) cone-shape microtriches (CM) on the anterior part of the body and b) filamentous microtriches (FM) on the middle and posterior parts. Scale bars – 0.2 µm. D – distal shaft of microtriches; P – proximal shaft of microtriches; ST – syncitial tegument

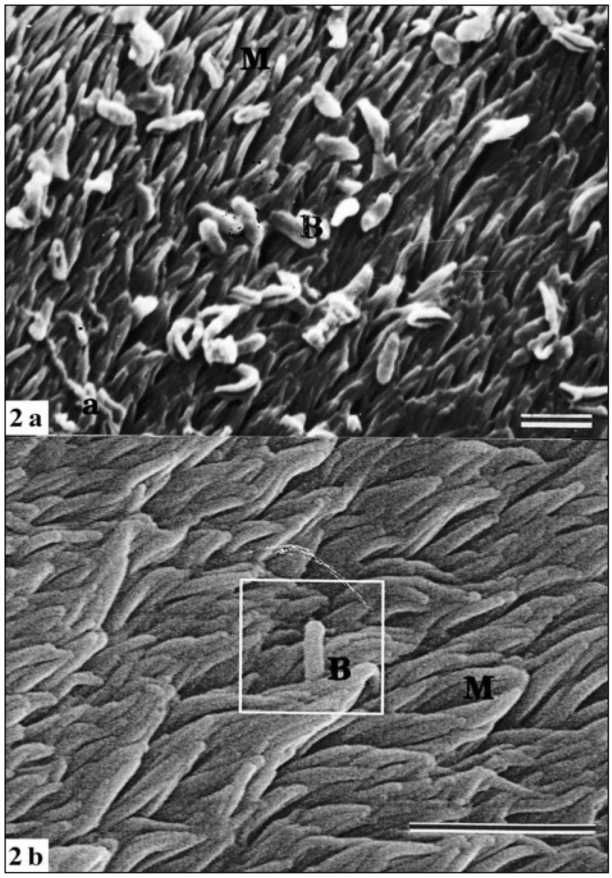

Fig. 2. Scanning electron micrographs showing bacterial cells (B) associated with *Khawia armeniaca* surface a) on the middle and posterior parts of the cestode body, scale bar – 10 µm, and b) on the anterior part. Scale bar – 1 µm. M – microtriches

Table 1. Morphometrical parameters of microtriches in caryophyllidean tapeworms

Morphometrical parameters	*Caryophyllaeus laticeps*		*Khawia armeniaca*	
	Anterior portion	Middle and posterior portions	Anterior portion	Middle and posterior portions
Length of proximal shaft (μm)	0.22 ± 0.02	0.57 ± 0.03	0.56 ± 0.03	0.82 ± 0.01
Width of proximal shaft (μm)	0.16 ± 0.01	0.12 ± 0.003	0.17 ± 0.007	0.12 ± 0.003
Length of distal shafts (μm)	1.08 ± 0.04	0.43 ± 0.02	1.11 ± 0.05	0.63 ± 0.02
Number of microtriches per square unit area (μm^2)	31	32	28	42
Functional amplification factor	3.8	8.7	7.3	14.5

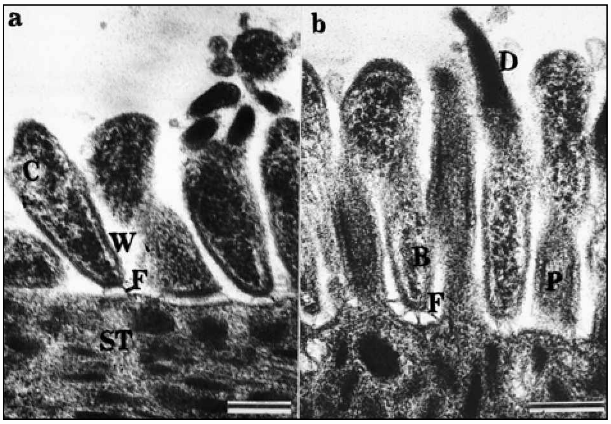

Fig. 3. Sagittal section through the portions of microtriches and bacteria associated with tegument of *Caryophyllaeus laticeps* via a) two and b) four stalk-like tufts (F). Scale bars – 0.2 μm. C – cytoplasm of microbial cell; W – wall of microbial cell

bial cells are located in the intermicrotrichial space. Finally, only solitary bacteria are present on the anterior part of the *K. armeniaca* surface (Fig. 2b).

The transmission electron microscopy analysis has revealed the tegument of *C. laticeps* and *K. armeniaca* to be colonised with different types of bacteria that are firmly attached to the tegument at the bottom of the intermicrotrichial space (Figs. 3b, 4, 5a, b, 6). The attachment of the bacteria is perpendicular to the surface. There are no ultrastructural differences between the parts of the tegument carrying microorganisms and lacking them (Figs. 1b, 5a, b). All types of the observed bacteria are characterised by a dense cytoplasm that contains numerous ribosomes and dense granules (Figs. 3a, 4, 5a, b). All bacteria are bounded by an inner plasma membrane and the outer cell wall (Figs. 3 – 5). A few morphologically distinct groups of bacteria were found.

One of the individual morphotypes was revealed on the tegument of *C. laticeps* (Figs. 3a, b, 6a). In the sections of worms, the bacteria are elongate in profile, and measure approximately 0.15 μm in diameter and 0.6 μm in length. These organisms have dense cell walls (Fig. 3a, b). Electron micrographs of the sections of caryophyllidean surfaces clearly show the special structures between the bacteria and the tegument. The bacteria are characterised by a

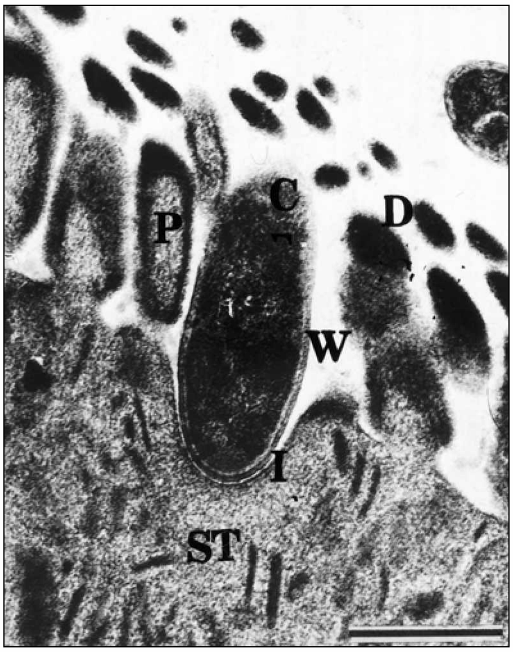

Fig. 4. Non segmented bacterium attached to the surface of *Khawia armeniaca* into apical surface invagination (I). Scale bar – 0.5 μm

distinct attachment site with special holdfast structures, from two (Fig. 3a) up to four stalk-like tufts (Figs. 3b, 6a). Each tuft is represented by closely connected filaments derived from the outer cell wall of the bacterium. The tufts enable the bacteria to stick to the cestode tegument.

At least two morphotypes of bacteria were detected on the tegument of *K. armeniaca* (Figs. 4, 5a, b, 6b, c). In the first morphotype, the ends of non-segmented bacteria are inserted into the invaginations of the tegumental surface (Figs. 4, 6b). The gap between the bacterium and the tegumental surface is very thin, this space is electron-lucent (Figs. 4, 6b). This morphotype is characterised by dense cytoplasm and a thick but moderately electron-lucent cell wall. These organisms measure proximately 0.3 μm in diameter and the 0.8 μm in length.

The second type, segmented bacteria, is presented by the chains of rod-shaped organisms (Figs. 5a, b, 6c). The individual cells of the chain are separated from each other by a plasma membrane and connected by a common bacterial cell wall (Figs. 5a, b). The attachment sites of such bacteria form small invaginations in the apical cytoplasm of the tegument. The area of immediate contact between the bacterial cell and the tegumental membrane appears striated due to the presence of thin filaments (Figs. 5a, b, 6c).

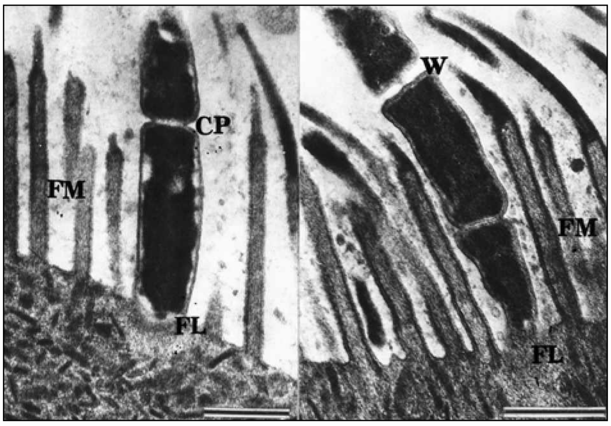

Fig. 5. Colonial bacteria (a, b) attached to the *Khawia armeniaca* tegument via the base of colonic crypt. The area of immediate contact appears striated due to the presence of thin filaments (FL). Scale bars – 0.5 μm. CP – place of connection of individual cells

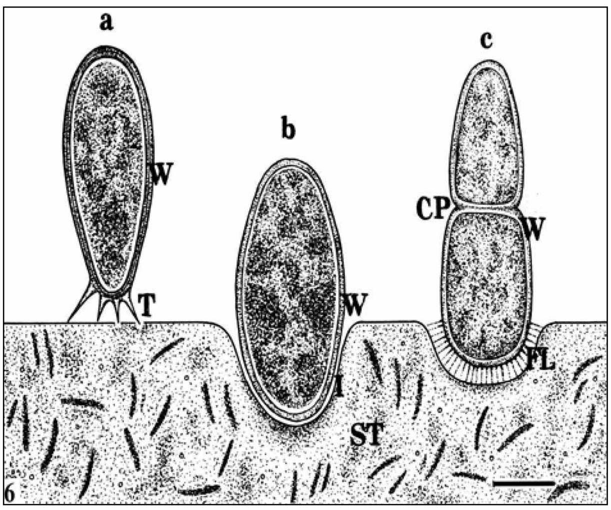

Fig. 6. The attachment sites of the bacterium holdfast regions: a) four stalk-like tufts; b) and c) two types of colonic crypts with (c) and without (b) filaments into the space of the gap between bacterium and tegument. Scale bar – 0.2 μm

Discussion

This study represents the first ultrastructural research of microbial flora associated with the apical tegumental surface in two caryophyllidean species of tapeworms. Using transmission electron microscopy investigations, we found one morphological form of bacteria attached to the surface of *C. laticeps*, and two morphotypes of bacteria attached to the surface of *K. armeniaca*. The difference between the morphological microbial forms on the surface of two caryophyllidean species is probably the result of the different conditions inside the bream and khramulya intestines as well as in the environmental conditions in the Rybinsk Reservoir and Lake Sevan. It is also important to note that the bream and the khramulya are characterised by the different types of feeding. The bream is a typical benthophage, while the khramulya is a typical detrithophage.

According to transmission electron microscopy observations, the bacteria that have been found on the tegumental surface are associated with the cestode tegument by means of special holdfast structures, such as tufts and two types of colonic crypts. Similar adhering of microbial cells to the tegument via the colonic crypts was observed in trematodes (Morris, 1973; Aho *et al*., 1991; Hughes-Stamm *et al*., 1999). The adhesive property of *C. laticeps* bacteria by means of stalk-like tufts is a unique characteristic of the specialised adhesion never described before. In all cases of firm attachment, the cellular wall structures of the bacteria produce the holdfasts (tufts and filaments). The attachment sites of the bacteria are closely associated with the tegument of the caryophyllidean cestodes.

Scanning electron microscopy investigations of the *K. armeniaca* surface have also shown the presence of bacteria associated with the cestode tegument, but without apparent attachment to the surface. In this case, according to Beveridge (1980), other forces, such as surface tension and Van der Waals forces, may also help to overcome the mutual charge repulsion between the tapeworm and the bacteria.

None of the observed microbial forms appear to harm the tapeworms. We have not found any ultrastructural evidence of damage to the cestode tegument caused by the microbial cells.

The distribution of these bacteria along the caryophyllidean tegument is constant, and most of the microorganisms occur in the middle and posterior parts of the tapeworm's body. Microbial cells are also present in minute quantities in the anterior part of the helminth's body. This distribution pattern is probably related to the structure and function of the different sections of cestode tegument. There are two types of microtriches on the apical surface of the caryophyllidean tegument. Cone-shaped microtriches cover the surface of the anterior portion of the worm. The microtriches on the middle and posterior surfaces are filamentous ones. Cestodes lack a digestive tract, and they have to obtain all of their nutrients through the tegument. There are extensive electron microscope studies that have shown the universal amplification of the cestode surface by microtriches. It is also proved by numerous investigations that the proximal shaft of the microtriches is primarily involved in the absorption of nutrients, whereas the spine assists in anchoring the parasite, and absorption of nutrients is restricted to the middle and posterior parts of the tapeworms covered by filamentous microtriches. Consequently, their degree of amplification may have functional significance (Graeber & Storch, 1979; Threadgold & Robinson, 1984; Kuperman, 1988). It is a well known fact that the ultrastructure of the proximal part of the cestode micro-triches and fish enterocyte microvilli, the basis of membra-ne-linked digestion, is quite similar. These organelles in-crease the digestive-transport surfaces both in helminthes and fishes (Kuperman, 1988; Kuperman & Kuz'mina, 1994; Izvekova *et al.*, 1997). The delimiting plasma membranes of both structures are rich in carrier molecules that provide a transport facility to sugars, amino acids, fatty acids and nucleosides (Pappas, 1983; Pappas & Uglem, 1990; Halton, 1997). As it was already mentioned in Table 1, the higher values of the functional amplification factor (calculated excluding the dense distal spine) were found for the middle and posterior sections of *C. laticeps* and *K. armeniaca*. Calculations show the functional amplification factor of 3.8 and 8.7 for the anterior and 7.3 and 14.5 for the middle and posterior parts of *C. laticeps* and *K. armeniaca* respectively. This, in turn, implies the more important role of filamentous microtriches for the amplification of the cestode surface for digestive-transport processes in the Caryophyllidea. The investigation on the lack of phosphatase activity on the caryophyllid scolex surface (Hayunga, 1979) is also in agreement with the notion that the absorption of nutrients is restricted to the middle and posterior parts of the caryophyllid tapeworms. It may also be connected with the fact that the scolex of caryophyllidean cestodes is buried in the mucosa of the fish intestine. Other suggestions have included the possibility that the filamentous microtriches appear to be flexible and may act to agitate the adjacent microhabitat (Rothman, 1963; Thomson *et al.*, 1980).

It is known that the end-products of the carbohydrate catabolism in cestodes are organic acids (lactate, succinate, acetate). The production of these incompletely oxidized end-products persists even under aerobic conditions (Barrett, 1984). It is possible to assume that, being attached to the worm tegument, the bacteria use the end-products of its carbohydrate catabolism as a source of carbon. The distribution of bacteria mainly in the middle and posterior parts of the tegument participating in digestive-transport processes in caryophyllidean cestodes allows us to suggest that the association of intestinal microflora with the cestode tegument may also be beneficial to tapeworms. Microbial cells in close proximity to the tegument may participate in cestode nutrition. Cestodes lacking lumenal digestion possess both the mechanisms of membrane digestion and active transport. The parasite uses host enzymes, adsorbing them onto the body surface, as well as the hydrolysis products of the intestinal microflora enzymes (Ugolev, 1985; Kuz'mina *et al.*, 2000; Izvekova, Lapteva, 2002; Izvekova, 2003). Physiological studies of the microflora associated with the digestive-transport surfaces of the pike intestine and its parasite *Triaenophorus nodulosus* (Izvekova & Lapteva, 2002; Izvekova, 2003) have confirmed the presence of the same bacteria in the fish intestine and on the cestode tegument. The cultivation of these bacteria in the media rich in proteins increased the amino acid content of the media testifying to proteolysis by the microflora produced enzymes. It enables us to suppose that the microorganisms revealed in our studies can secrete enzymes hydrolysing the high-molecular substrates – proteins and carbohydrates, with hydrolysis products being utilized by the bacteria and the caryophyllidean cestodes as well.

Thus, the results of the performed investigations may be reduced to the following basic statements:

The association of bacteria with the tegument in two species of caryophyllidean cestodes inhabiting the intestines of cyprinid fishes was shown for the first time using transmission and scanning electron microscopy.

Special structures responsible for the attachment of bacteria to the tegument were found. These holdfasts derived from the microbial cell wall are represented by stalk-like tufts and crypts with and without filamentous material.

The absence of apparent damage to the cestode tegument by the microbial cells along with the presence of some special holdfast structures makes it possible to suppose that the detected bacteria are rather symbiotic than accidentally found.

Perhaps, bacteria associated with the tegumental surfaces may be involved in the metabolism of cestodes.

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References

AHO, J. H., UGLEM, G. L., MOORE, J. P., LARSON, O. R. (1991): Bacteria associated with the tegument of *Clinostomum marginatus* (Digenea). *J. Parasitol.*, 77: 784 – 786

AUSTIN, B., AL-ZAHRANI, A. M. J. (1988): The effect of antimicrobial compounds on the gastrointestinal microflora of rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.*, 33: 1 – 14

BARETT, J. (1984): The anaerobic end-products of helminthes. *Parasitol.*, 88: 179 – 198

BERG, R. D. (1996): The indigenous gastrointestinal microflora. *Trends in Microbiol.*, 4: 430 – 435

BEVERIDGE, T. J. (1980): Bacterial structure and its implications in the mechanisms of infection: a short review. *Can. J. Microbiol.*, 26: 643 – 653

CAHILL, M. M. (1990): Bacterial flora of fishes: A review. *Microb. Ecol.*, 19: 21 – 41

CORPE, W. A. (1980): Microbial surface components involved in adsorption of microorganisms onto surfaces. Charper 4. In BITTON, G., MARSHALL, K.C. (Eds.): *Adsorption of microorganisms to surface*. John Wiley & Sons, New-York Chichester-Brisbane, Toronto: 105 – 144

GRAEBER, K., STORCH, V. (1979): Elektronenmikroskopische und morphometrische Untersuchungen am Integument von Cestoda and Trematoda (Plathelminthes). *Zool. Anz.*, 202: 331 – 347

HALTON, D. W. (1997): Nutritional adaptations to pa-

rasitism within the Platyhelminthes. *Int. J. Parasitol.*, 27: 693 – 704
HAUYNGA, E. G. (1979): The structure and function of the scolex glands of three caryophyllid tapeworms. *Proc. Helminthol. Soc. Wash.*, 46: 171 – 179
HUGHES-STAMM, S. R., CRIBB, T. H., JONES, M. K. (1999): Structure of the tegument and ectocommensal microorganisms of *Gyliauchen nahaensis* (Digenea: Gyliauchenidae), an inhabitant of herbivorous fish of the great barrier reef, Australia. *J. Parasitol.*, 85: 1047 – 1052
IZVEKOVA, G. I. (2003): Proteolytic activity of microflora of digestive-transport surfaces in the pike intestine and its parasite *Triaenophorus nodulosus* (Pallas, 1781) (Cestoda, Pseudophyllidea). *Biol. Vnut. Vod.*, 3: 82 – 87
IZVEKOVA, G. I., KUPERMAN, B. I., KUZ'MINA, V. V. (1997): Digestion and digestive transport surfaces in cestodes and their fish hosts. *Comp. Biochem. Physiol.*, 118 A: 1165 – 1171
IZVEKOVA, G. I., LAPTEVA, N. A. (2002): Microflora of digestive-transport surfaces of the pike intestine and its parasite *Triaenophorus nodulosus* (Cestoda, Pseudophyllidea). *Biol. Vnut. Vod.*, 4: 75 – 79
KUPERMAN, B. I. (1988): *Functional morphology of lower cestodes. Ontogenetic and evolutionary aspects.* Nauka, Leningrad
KUPERMAN, B. I., KUZ'MINA,V. V. (1994): The structure of intestinal epithelium in fishes with different types of feeding. *J. Fish Biol.*, 44: 181 – 193
KUZ'MINA, V.V., IZVEKOVA, G.I., KUPERMAN, B.I. (2000): Peculiarities of nutrition physiology in cestodes and their host-fish. *Usp. Sovremen. Biol.*, 120: 384 – 394
LEE, A. (1980): Normal flora of animal intestinal surfaces. Chapter 5. In BITTON, G. and MARSHALL, K.C. (Eds): *Adsorption of microorganisms to surface*. John Wiley & Sons, New-York, Chichester-Brisbane, Toronto: 146 – 173
MORRIS, G. P. (1973): The morphology of associations between a trematode (*Megalodiscus temperatus*) and bacteria. *Can. J. Zool.*, 51: 1313-1314
PAPPAS, P. W. (1983): Host-Parasite Interface. In ARME, C and PAPPAS, P.W. (Eds): *Biology of the Eucestoda*. Academic Press. 2: 297 – 334
PAPPAS, P. W., UGLEM G. L. (1990): *Hymenolepis diminuta* (Cestoda) liberatesan inhibitor of proteolytic enzymes during in vitro incubation. *Parasitology*. 101: 455 – 464
ROTHMAN, A. N. (1963): Electron microscope studies of tapeworms: The surface structures of *Hymenolepis diminuta* (Rudolphi, 1819) Blanchard, 1892. *Trans. Am. Microscop. Soc.*, 82: 22 – 30
SAVAGE, D. C. (1980): Colonization by and Survival of Pathogenic Bacteria on Intestinal Mucosal Surfaces. Chapter 6. In BITTON, G. and MARSHALL, K.C. (Eds): *Adsorption of microorganisms to surface*. Publication John Wiley & Sons. New-York Chichester-Brisbane Toronto. 175 – 206
TANNOCK, G. W. (1999): Analysis of the intestinal microflora: a renaissance. *Antonie van Leewenhoek*, 76: 265 – 278
THOMPSON, R.C.A., HAYTON, A.R., JUE SUE, L. P. (1980): An ultrastructural study of the microtriches of adult *Proteocephalus tidswelli* (Cestoda: Proteocephalidea). *Z. Parasitenkd.*, 64: 95 – 111
THREADGOLD, L. T., ROBINSSON, A. (1984): Amplification of the cestode surface: a stereological analysis. *Parasitology*, 89: 523 – 535
UGOLEV, A. M. (1985): *Digestion evolution and principles of an evolution of the function*. Nauka, Leningrad

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