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ZOOLOGY

The Structure of the Epithelial Surface of the Gastrointestinal Tract of Pikas (*Ochotona pallasi* and *O. dauurica*, Lagomorpha, Ochotonidae): Functional and Species Specificity

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Abstract—The macro- and microrelief of the surface of the digestive tract mucosa of two pika species—Pallas's (*Ochotona pallasi*) and Daurian (*O. dauurica*)—were studied in detail using whole-mount preparations and scanning electron microscopy. The structural features of the intestinal mucosal surface specific of mammals, such as the formation of projections on the crest of the cecal spiral fold and microcells in the colonic ampulla, were studied. It was found that the colonic mucosa forms sparse large conical villi in pocket cavities and on the surface of muscle bands. Significant differences in the cecal mucosal relief were found between the species studied. The possible functional significance of the identified morphological features is discussed.

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The structure of the digestive tract of pikas (Ochotonidae) has all the features characteristic of the members of the order Lagomorpha: a single-chamber
1 glandular stomach with a well-developed fornix, a long cecum with lymphoid formations and a deep spiral fold, and a colon forming a number of muscular pockets. Unlike the large intestine of other members of the order (hares and rabbits), in pikas it is even more complicated and differentiated. Although the anatomical features of the digestive tract of pikas were first described in the late nineteenth century (Tullberg, 1899), the fine structure, which is required for the functional interpretation of the morphological speci-
2 ficity, has been studied only fragmentarily in several species (Naumova, 1981).

The purpose of this study was to consider the structure of the epithelial surface of different intestinal regions of two previously unstudied pika species (Pallas's pika *Ochotona pallasi pallasi* and Daurian pika *O. dauurica dauurica*, belonging to the subgenus *Pika* and *Ochotona*, respectively) at the macro- and microscopic levels and give a functional interpretation of the identified features.

MATERIALS AND METHODS

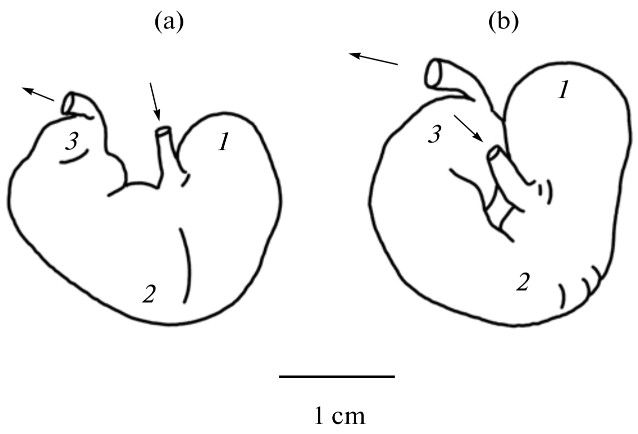
This study was performed with five Pallas's pikas caught in the Agadyr district of Karaganda oblast (Kazakhstan) and six Daurian pikas caught in the Trans-Baikal region. The digestive tract of the hunted animals was immediately fixed in 4% formalin. The stomach and intestines and then the mucosa washed clean of the contents were photographed with a Nicon Coolpix 4500 digital camera (Nicon Corporation, Japan), with special attention given to the ileocecal 3
junction. Drawings were made from photographs using the Gimp 2.6 software and a Wacom Bamboo Fun graphic tablet (Wacom, Japan).

The architectonics of mucosal surfaces of the different gastrointestinal tract regions examined for total preparations were stained with hematoxylin Karrachchi and using a scanning electron microscope CamScan MV 2300 (Electron Optic Services Inc., Canada). To study tissue samples by scanning electron microscopy, they were dehydrated in alcohols and acetone and then air-dried.

RESULTS

Macromorphological features of the digestive tract. Similarly to the previously studied pika species (Naumova, 1981), the glandular single-chamber stomach of the Pallas's and Daurian pikas differs from that of rodents by a well-developed fornix and a strongly 1
curved horseshoe shape. Due to these features, it resembles a two-chamber stomach, in which the right and left halves are separated by a deep angular notch and an angular fold projecting into the gastric lumen.

The relative length of the intestine in these species exceeds more than 11 times the length of the body, which does not differ from this parameter in the previously studied species. This is reached mainly due to the large relative length of the small intestine, which accounts for up to 65% of the total length of the intes-



1 1 **Fig. 1.** Pallas's pika stomach shape at (a) uniform distribution of contents and (b) substantial filling of the stomach fornix. Designations: *1*, fornix; *2*, body; and *3*, pyloric part of the stomach.

tine in both species. The length of the cecum (together with the appendix) is also significant and reaches 13.5% of the total length of the intestine.

Despite the great length of the cecum, its weight in pikas is extremely small (less than 5% of the body weight). This is significantly smaller than in small herbivorous rodents, in which it accounts for 7–10% of the body weight (Naumova, 1999). The spiral fold in the examined Pallas's and Daurian pika specimens contained 32–36 and 50–52 turns, respectively. The inconsistent number of turns in each species may be due to individual and age variability or gender. The maximum diameter of the cecum, barely reaching 1.3 cm, was found only in some animals in two small areas each 2 cm long, located in the middle of the cecum body. These areas were separated by a narrow interception 0.6 cm in diameter; the diameter of the major part of the cecum was only 0.5–0.8 cm.

4 Interspecific differences in the macrostructure of the digestive tract of pikas have not been identified. On the outer surface of the cecum, the line of the base of the spiral fold was clearly seen in all pikas; the lymphoid formations on the top of the cecum and at the base of the ileum were developed approximately to the same extent. At the beginning of the colon, a unique region for mammals, the ampulla, is located, which drastically differs in appearance from both the cecum and the colon and looks like a thick-walled tube with a reticular pattern on the smooth outer surface. The colonic ampulla is dilated in the initial portion and then gradually narrows in the distal direction. Similarly to all lagomorphs, the walls of the major and minor colons form three and one, respectively, rows of muscular pockets.

Functional variability of the digestive tract. Despite the very similar general structure of the digestive tract of pikas, the pattern of filling with contents of its different parts may vary considerably in different individuals of the same species. Individual variability in the configuration of the large intestine largely depends on the phase of feeding activity—alternation of consumption of plant food and cecotrophs (evacuated cecal contents). Since precise data on the feeding activity of pika species are still missing, we evaluated the digestive phase by the consistency of the contents of the colon. The presence of an amorphous mass in the rectum and distal part of the colon was regarded as the cecotroph evacuation phase.

The configuration of the stomach and intestines in the five Pallas's pikas caught at different times was different (Fig. 1). The fornix of the stomach of the animal 1
caught at 7:06 a.m. was filled with cecotrophs and separated from the other part of the stomach by an intersection. Of the three pikas caught in the morning (at 5:50, 7:06, and 9:00 a.m.), the intestine of only the animal caught at 9:00 was in the cecotroph evacuation phase: the rectum and colon were filled with liquid contents. The colonic ampulla and one of the pockets of the major colon were severely swollen (Fig. 2). In the pikas caught early in the morning, the distal part of the colon contained solid pellets of excrements, and the pockets in the colon were filled relatively evenly. Approximately the same intestinal configuration was observed in the pikas caught in the evening.

All Daurian pikas were dissected in the evening (17:00 to 21:00). The examined animals had the same differences in the large intestine configuration as the Pallas's pikas. The distal part of the colon and the rectum of all animals contained several small pellets of solid excrements.

Macrostructure of the epithelial surface. Among other phytophagous mammals, including lagomorphs, pikas are distinguished not only by anatomical differentiation of the large intestine but also by the extremely complex structure of the cecal and colonic mucosal surface. In pikas, unlike hares, the crest of the spiral fold of the cecum is equipped with so-called projections—outgrowths protruding into the intestinal cavity. The shape of these projections significantly differed in the species studied (Fig. 3). In Daurian pikas, the spiral fold has a strongly dissected free edge carrying closely spaced filamentous projections. The length of the projections varies over the cecum. In the area of the apex and body of the cecum, the projections are shorter, their length is 0.5–1 cm; however, in the cecal ampulla, they are substantially elongated and become 2 cm long. The Pallas's pika differs from the Daurian pika primarily in the shape of the free edge of the spiral fold. In this species, projections in the body of the cecum have the shape of closely spaced short broad petals, sometimes overlapping each other, as a result of which the free edge of the spiral fold looks scalloped. However, in the cecal ampulla, projections are modified and take, as in the Daurian pika, a fila-

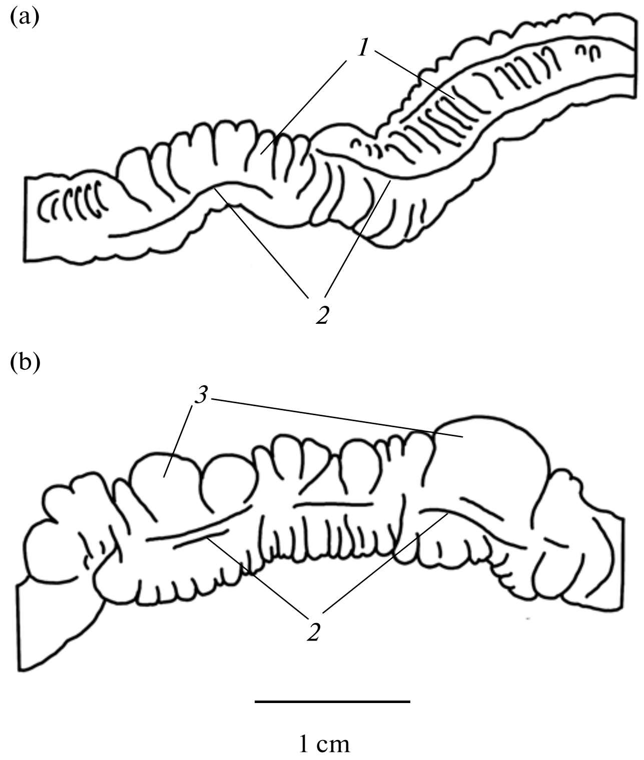


Fig. 2. Colon shape (a) at relaxed muscular pockets and (b) in the contraction phase. Designations: *1*, empty muscular pockets; *2*, muscular bands; *3*, muscular pockets filled with chyme.

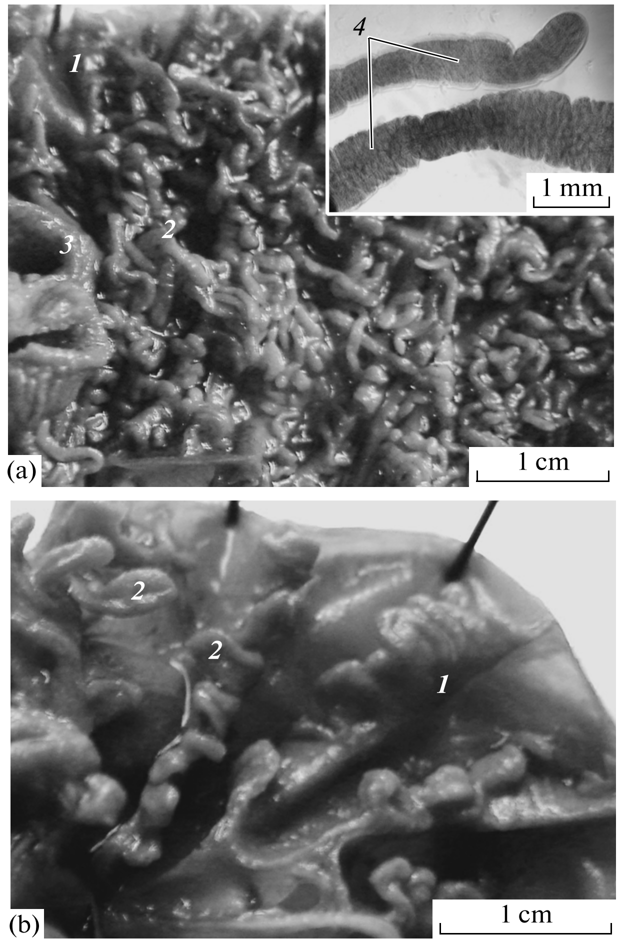


Fig. 3. Cecal mucosal surface relief in (a) Daurian and (b) Pallas's pikas. Designations: *1*, spiral fold base; *2*, spiral fold projections; *3*, ileal sphincter; *4*, fragments of spiral fold projections (whole-mount preparation).

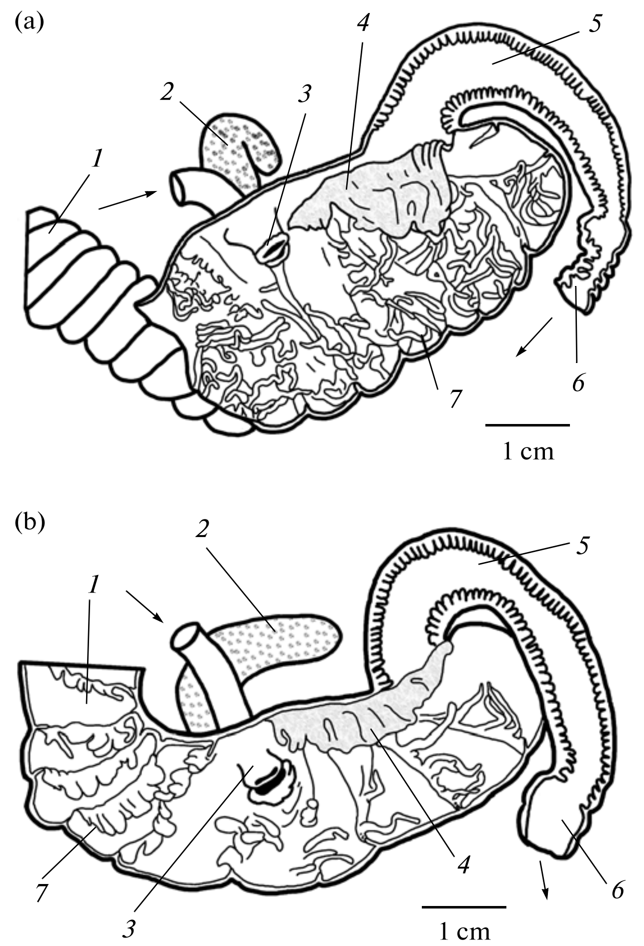
mentous shape, although they do not reach such a great length and density.

3 The ileocecal valve also has an unusual structure (Fig. 4). The ileal sphincter from the mesenteric side protrudes into the cecal cavity, forming the upper valve cusp. The opening of the lymphoid formation is located next to it. The lower valve cusp represents a wide blade deeply protruding into the lumen of the cecum, which extends from the sphincter muscle of the ileum to the beginning of the colon, separating the cecum and colon cavities. In the Daurian pika, this
3 part of the ileocecal valve is about two times higher than that in Pallas's pika.

The colonic ampulla mucosa forms deep cells that give the surface a reticular appearance. In the proximal part of the ampulla, cells are extended along the intestine perimeter, as a result of which their mouths look fissured. In the colon itself, the mucosa forms large widely spaced villi, which are found both in the cavities of muscular pockets and on the surface of muscular bands separating the pockets.

Ultrastructure of the intestinal mucosa surface. The shape of small intestine villi in pikas and the pattern of their variability in the cranio-caudal direction are generally typical of mammals. Wide spade-like villi, formed in the initial part of the duodenum, gradually turn into conical and fingerlike villi, decreasing in height in the caudal direction. In the jejunum of pikas, the width of villi decreases, but they remain closely spaced and flattened and, when examined from the surface, have a diamond shape. Such villi help to retain finely dispersed contents and bacteria between them; however, no fusion of their bases, similar to that found in hares (Naumova et al., 2013), was observed in pikas. The microstructure of the cecal mucosal surface is also typical for mammals: numerous crypt mouths are seen on the epithelial surface that forms microfolds.

An unusual structure of the mucosal relief is characteristic of the colonic ampulla, which is distinctly separated morphologically (Fig. 5). The crests of cells are expanded and covered with a dense layer of epithelial cells that can completely close the mouths of the cells, which open into the ampullar cavity. In some animals, open mouths of cells were seen, which con-



3 **Fig. 4.** Macrorelief of the mucosal surface of the ileocecal junction of (a) Daurian and (b) Pallas's pikas. Designations: *1*, cecum body; *2*, additional appendix; *3*, ileal sphincter (ileal and auxiliary appendix mouths are seen);
3 *4*, lower cusp of the ileocecal valve; *5*, colonic ampulla; *6*, colon; *7*, cecal spiral fold projections.

tained a secretion with numerous bacteria. The wall of the ampulla is thickened at the expense of the mucosa, which contains deep cells. In the cavities of mucous cells, the mucosa forms short villi, which can be seen on the cross section of the intestinal wall.

In the colon of pikas, the mucosa forms widely spaced large conical villi up to 0.5 mm high, which bear second-order villi (Fig. 6).

RESULTS AND DISCUSSION

Food specialization. Pikas are strictly herbivorous animals feeding in winter almost exclusively on hay (Sokolov et al., 1994). For mammals with intestinal fermentation with a small and medium body size, the use of poor food resources in the course of evolution was made possible due to emergence of a physiologically normal cecotrophy—repeated passage of food through the digestive tract (Naumova, 1999; Hirakawa, 2001; Naumova et al., 2013).

The general similarity of the structure of the large intestine in pikas and hares suggests that the revealed features of their specialization are associated with coprophagia. The ability of pikas to produce two types of excrement was found for the first time in the northern pika *Ochotona hyperborea* (Haga, 1960). It was noticed that, in addition to the conventional solid pellets that are produced by the northern pikas primarily during the day, at night they produce soft dark feces, which are dried and then eaten. Later, more detailed studies of cecotrophy in the northern pikas showed that pikas eat some of the soft feces by removing them from the anus during the periods of rest and that the rhythmicity of these acts is associated with the polyphase activity, which is not synchronized among different individuals (Pshennikov et al., 1990). This fact was confirmed by our data. Although Pallas's pikas were caught at different times, cecotrophs in the distal parts of the colon were found only in one animal that was caught at 9:00 a.m. A clear dynamics of filling different parts of the intestine with food, similar to that observed in rabbits (Naumova et al., 2013), was not observed either. Fluoroscopic and radiographic observation of the colon motility in rabbits revealed three types of intestinal muscle contractions, which are characterized by a high frequency and differ in the periods of evacuation of solid excrements and cecotrophs (Ehrlein et al., 1983). Apparently, the frequent change of periods of rest and feeding activity in pikas masks the differences in the pattern of the digestive tract filling.

The small volume of the cecum in the consumers of cellulose feed indicates the impossibility of long-term fermentation of structural carbohydrates in this organ. In Pallas's pikas, which consume up to 130 g of green grass or 40 g of hay per day when kept in cages (Derevschikov, 1971; Sludsky et al., 1980), a filled cecum weighs less than 10 g. This fact indirectly indicates a high rate of food passage through the digestive tract in pikas. Even in a larger representative of lagomorphs, *Lepus brachyurus* (Hirakawa and Okada, 1995), the average time of food delay in the digestive tract is 3–4 h.

Functional significance of specific structures. The structure of the cecum and colon in pikas, similarly to hares, provides separation of the contents in order to save the bacterial mass and ensure the possibility of repeated food passage through the digestive tract. The large lower cusp of the ileocecal valve prevents the 3
mixing of the enriched contents returned to the cecum from the colon with the chyme of the cecum. The reticular structure of the mucosa in the colonic ampulla may serve to collect and accumulate the fine chyme fraction enriched in bacterial protein for its subsequent introduction into the soft feces. The easily digestible components of feed and cecotrophs are

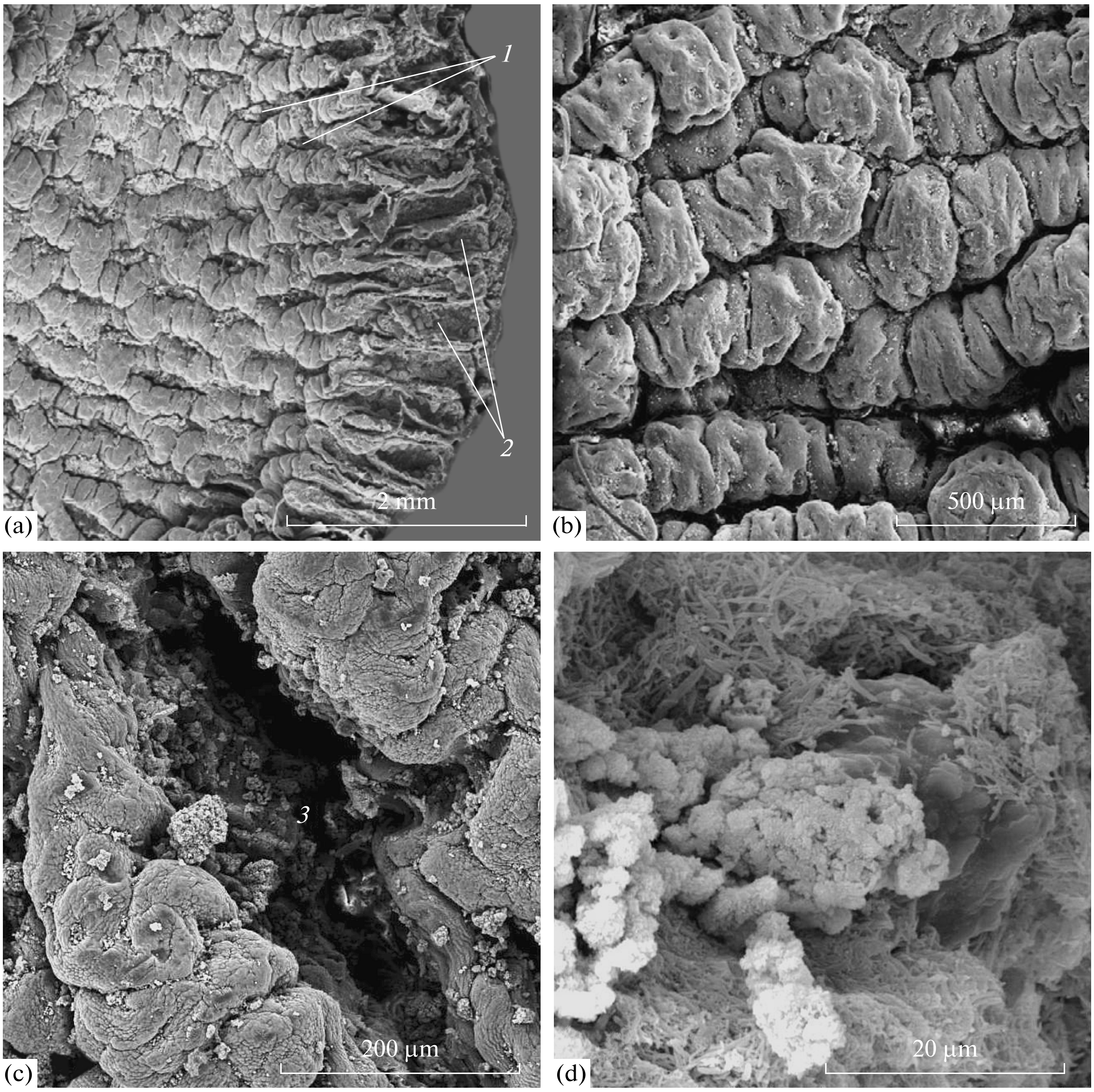


Fig. 5. Colonic ampulla. Pallas's pika: (a) mucosal cells and (b) epithelial surface with closed mouths of cells. Daurian pika: (c) open cell mouth and (d) bacterial associations on the epithelial surface. Designations: *1*, cell mouths; *2*, cell cavities lined with villous mucosa; *3*, open cell mouth.

assimilated in the long small intestine with a well-developed villous mucosa.

The extremely intricate structure of the mucosal surface of the large intestine definitely accelerates the assimilation of food. Cecal spiral fold projection and colonic villi facilitate the mixing of the contents and the outflow of metabolites, the accumulation of which inhibits microbial fermentation.

Microbial nitrogen fixation. The isolation of the *nifH* gene from the bacterial community of the cecum and colon of the northern pika demonstrated the existence of microbial nitrogen fixation in the gastrointestinal tract of pikas (Formozov et al., 2012). This highly important mechanism, which is realized due to cecotrophy, is probably the main component in the complex of adaptations of pikas to consuming poor feed. The functional significance of nitrogen fixation is confirmed by the fact that, in the northern pika, the copromass manifold surpasses the consumed feed in the content of essential amino acids, which are supplied by the microorganisms (Pshennikov et al., 1990).

Specific features. In the pika species considered in this study, two essential characteristic features in the structure of the cecal mucosal surface were distinguished: the specific shape of spiral fold projections and the lower cusp of the ileocecal valve. In the Dau- 3
rian pikas, belonging to the subgenus *Ochotona*, the

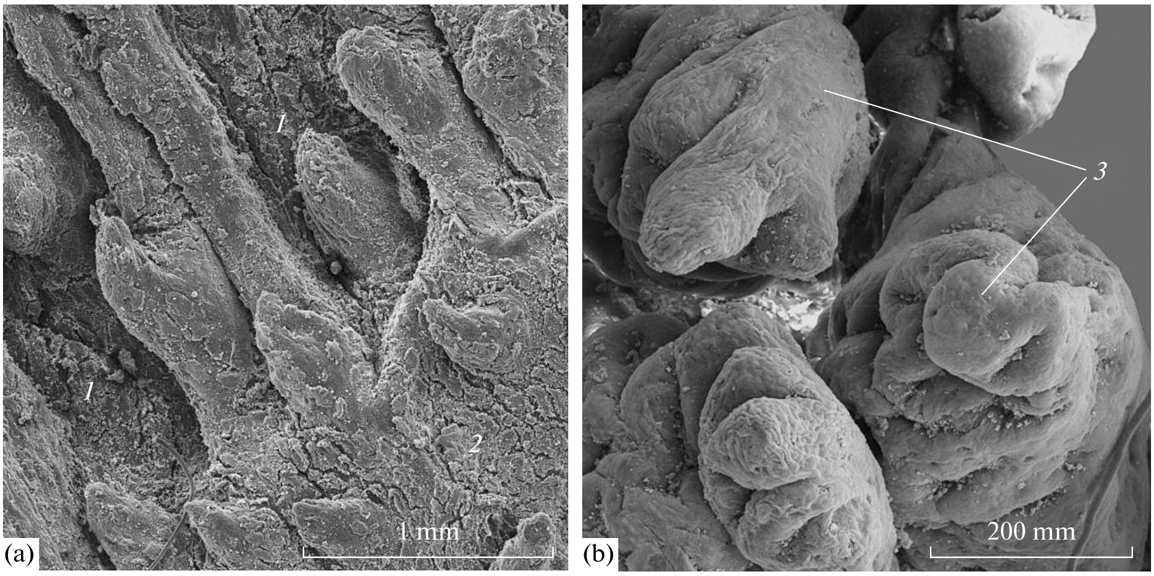


Fig. 6. Colonic mucosal surface of Pallas's pika: (a) general view and (b) individual villi. Designations: *1*, muscular pocket cavities; *2*, muscular band surface; *3*, surface relief of individual villi.

projections have a filamentous shape and are so long that they leave almost no free space in the cecum cavity. This is due to the fact that the number of turns formed by the spiral fold in this species is 1.5 times greater. In Pallas's pikas, belonging to the subgenus *Pika*, the cecal spiral fold projections are characterized by a scalloped shape and a significantly shorter length. According to preliminary data, the alpine pika (*Ochotona alpina*, subgenus *Pika*) is very close to Pallas's pikas in the shape of the free edge of the spiral fold, and the steppe pika (*Ochotona pusilla*, not identified to the subgenus level1) is characterized by fingerlike projections (outgrowths are elongated but significantly shorter than the filamentous formations in the Daurian pika).[1] Daurian and Pallas's pikas also markedly differ in the size and shape of the lower cusp of the
3 ileocecal valve. These features may also have a functional reason associated with food consumption and characteristic features of their assimilation in the digestive tract.

ACKNOWLEDGMENTS

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[1] Erbaeva (1988) classified the steppe pika with the subgenus *Lagotona*; Hoffmann and Smith (2005), with the subgenus *Ochotona*. According to our molecular genetic data (Grigorieva et al., 2011), the steppe pika forms the basal branch of the subgenus *Pika*, which agrees well with the standpoint of Ivanitskaya and Vorontsov (1973), based on karyological data.

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Translated by M. Batrukova

SPELL: 1. fornix, 2. fragmentarily, 3. ileocecal, 4. Interspecific