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Nodular coccidiosis of the pikeperch *Stizostedion lucioperca* and Volga perch *Stizostedion volgensis*

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ABSTRACT: In early spring, coccidiosis often develops in the gut of pikeperch *Stizostedion lucioperca* and Volga perch *S. volgensis* living in Lake Balaton, Hungary. Gamonts and oocysts cause nodular infection of enterocytes in specific portions of the gut. Besides those present in the nodules, merozoites and gamonts occur diffusely in the epithelium of the intestine and the pyloric sacs. The different developmental stages are located in the apical cytoplasm of enterocytes and are released from the cells in unsporulated condition. The sporulation of oocysts takes 48 h in tap-water at 20°C. The oocysts, which are 13–13.5 µm in diameter in an unsporulated state, grow to 17–20 µm by the end of the sporulation process, and ellipsoidal sporocysts composed of 2 valves and containing banana-shaped sporozoites develop in them. On the basis of oocysts derived from pikeperch, the parasite is described as *Goussia desseri* n. sp.

KEY WORDS: *Goussia desseri* · Apicomplexa · New species · Pikeperch · Volga perch · Nodular coccidiosis · Histology

INTRODUCTION

The number of known fish coccidia has doubled in the last 2 decades. The number of species assigned to the genera *Eimeria*, *Goussia*, *Crystallospora* and *Calyptospora* now exceeds 200. Although the majority of the known species are still intestinal coccidia, coccidian parasites which develop in extraintestinal locations (liver, spleen, kidney, swimbladder, serous membranes) are also numerous. In addition, there are marked differences in the location of gut-parasitic coccidia. The majority of *Eimeria* and *Goussia* species parasitizing the intestine of fish cause a so-called diffuse coccidiosis, in which the oocysts are distributed diffusely in a large segment of the gut and can be detected in most cases from the apical part of the epithelial cell cytoplasm. The number of species characterised by an extracytoplasmic location in the epithelial cells is also high. For species developing in an extracytoplasmic location but having a Stieda body, Dyková & Lom (1981) proposed the establishment of a new genus, *Epieimeria*. This genus was, however, made synonymous with the genus *Eimeria* by Benajiba et al. (1994). The nodular type of development has been known since the studies of Moroff & Fiebiger (1905) and Schäperclaus (1943); however, until the investigations of Molnár (1982) this form, previously reported only from the common carp, was considered an oddity. First from the tench, and later from other cyprinids as well, Molnár (1982, 1989) described species which developed in nodules concentrated in specific areas of the gut epithelium. According to Marinček (1973a) and Molnár (1989), in addition to their unique location, the species of nodular development differ from the majority of gut parasites in that their development is seasonal and follows an annual cycle.

This paper describes a *Goussia* species causing nodular coccidiosis in the gut of the pikeperch *Stizostedion luciperca* and Volga perch *S. volgensis* under the name of *Goussia desseri* n. sp.

MATERIALS AND METHODS

In a survey conducted in 1994 and 1995 to study the parasite fauna of the pikeperch and Volga perch, observations were made on coccidian infections of these

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fish species. In 1994 a total of 55 pikeperch and 30 Volga perch, and in 1995 a total of 53 pikeperch and 4 Volga perch, were examined. The fish were collected from different areas of Lake Balaton, Hungary. The majority of the specimens were caught by fishermen with dragnets, and the remaining specimens were caught in the littoral zone with a net of our own. The fish were transported to the laboratory in plastic bags filled with oxygen and kept in aquaria until examined 1 or 2 d later. The majority of the fish examined were 3-summer specimens; however, in our own catch fry and 2-summer specimens also occurred. The fish were killed by decapitation. Their gut, which no longer contained food, was opened longitudinally, and the intestinal mucosa was examined under a stereomicroscope. Mucus samples were drawn from different locations of the gut and pyloric sacs, and examined for the presence of oocysts under 400-fold magnification. If a mucus sample contained oocysts, a portion of the mucus was placed in tap-water in a Petri dish and the oocysts allowed to sporulate at 20 to 22°C. Attempts to induce sporulation of oocysts in epithelial cells which had accumulated in affected areas of the gut were made in a similar manner. To prevent excessive bacterial growth, some loopfuls of penicillin and streptomycin were added to the water. Oocyst sporulation was examined daily by microscopy, and the image projected with the help of a video recorder was used to prepare drawings.

Small pieces from the intestine of fish that proved to be infected were fixed with Bouin's solution, and embedded in paraffin wax. Sections 3–5 µm in thickness were made and stained with haematoxylin and eosin solution. The histological sections were photographed with a camera in a Zeiss Jenaval microscope. For description and illustration of the new species, oocysts obtained and histological slides prepared from pikeperch were used.

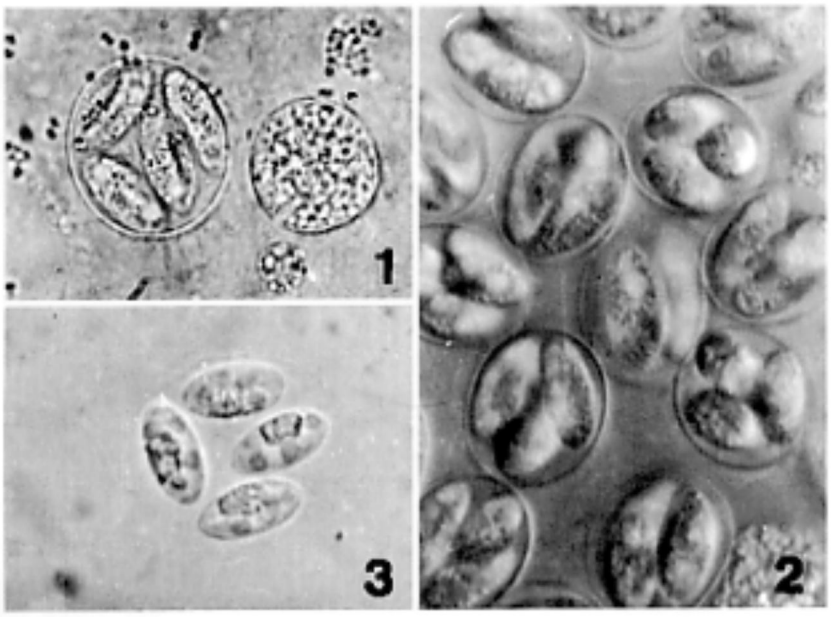
The dimensions of the new *Goussia* species were determined by measuring 50 oocysts, and the mean values and standard deviations are expressed in µm.

RESULTS

Of the 6 Volga perch specimens examined in April 1994, unsporulated oocysts were found in the gut of a 2-summer and a 3-summer fish. In 1 of these 2 fish, 4 whitish areas the size of a pinhead but well visible to the unaided eye were discovered in the intestinal mucosa. In April 1994 no pikeperch were subjected to examination. In later periods of the same year, no coccidia were detected in the gut of the 55 pikeperch and 26 Volga perch specimens examined. In 1995, we examined 4 pikeperches and 1 Volga perch in February, and found oocysts in the intestinal mucosa of 1 of the 4 pikeperches but observed no nodules. The Volga perch proved to be free of coccidia. In March, oocysts were found in the intestinal mucus of 1 Volga perch and 4 out of the 8 pikeperch specimens examined. Nodules were seen in 2 infected fish. In April oocysts were not found in the 4 pikeperches dissected; however, in the gut of a 2-summer Volga perch a nodular coccidiosis was observed. In later months of the year no infections were recorded.

Both the oocysts found in the intestinal mucus and those scraped out from the nodules were recovered in unsporulated state (Fig. 1) and proved to be spherical or short-ellipsoidal homogeneous formations 13–13.5 µm in diameter. The oocysts had a coarsely granular structure and the oocyst wall was not discernible by light microscopy. Oocysts collected from the pikeperch did not differ from those derived from the Volga perch in structure or size. Similarly, no differences were found between oocysts isolated from nodules or from nodule-free cases. After 20 to 24 h of sporulation in tap-water at 20°C the oocyst wall separated from the sporont and fluid accumulated between the 2 membranes. The sporont, still measuring 13–13.5 µm in diameter, was located in an oocyst 17.2 (15–18) µm in diameter. In the oocyst wall 2 layers were discernible. The inner, thicker layer (0.3 µm) was covered by a very thin membrane. In the coarsely granular sporont 2 to 4 refractile bodies 1.5–2 µm in diameter were discernible. After 42 h the oocysts sporulated, their diameter increased to 18–20 µm, and sporocysts exhibiting typical *Goussia* species structure were visible in them (Fig. 2). At 48 h the coarsely granular residuum still completely filled the sporocysts, but sporozoites containing 2 bright formations each were already discernible through it. Following shrinkage of the residuum, sporocyst structure became clearly apparent by the 72nd hour of sporulation (Fig. 3). Therefore, the new species is described on the basis of oocysts found in pikeperch and sporulated for 72 h.

In histological sections, meronts 9–12 µm in size proved to be the earliest developmental stages. Each of these contained 8 banana-shaped merozoites measuring 8.5–9 × 1.5–1.7 µm (Fig. 4). In the majority of cases, the merozoites were arranged in the same direction within the meront. No data were obtained on the number of merogony generations. Guts which also appeared nodular from the outside were found to harbour intensive infection by macrogamonts, microgamonts and young oocysts in the affected segment (Figs. 5 & 6). Infection was so intensive in these areas that parasite-free epithelial cells were seen only exceptionally. The diameter of the mostly spherical young oocysts (Fig. 7) reached 12–14 µm, while that of the slightly larger, spherical or short-



Figs. 1 to 3. *Goussia desseri* n. sp. oocysts from the gut of pikeperch and Volga perch. Fig. 1. Strongly compressed unsporulated and sporulated oocysts from the gut of Volga perch. ×1000. Fig. 2. Oocysts in the intestinal mucus of the pikeperch after 42 h of sporulation. ×1000. Fig. 3. Free sporocysts of *G. desseri* n. sp. from pikeperch after 72 h of sporulation. ×1000

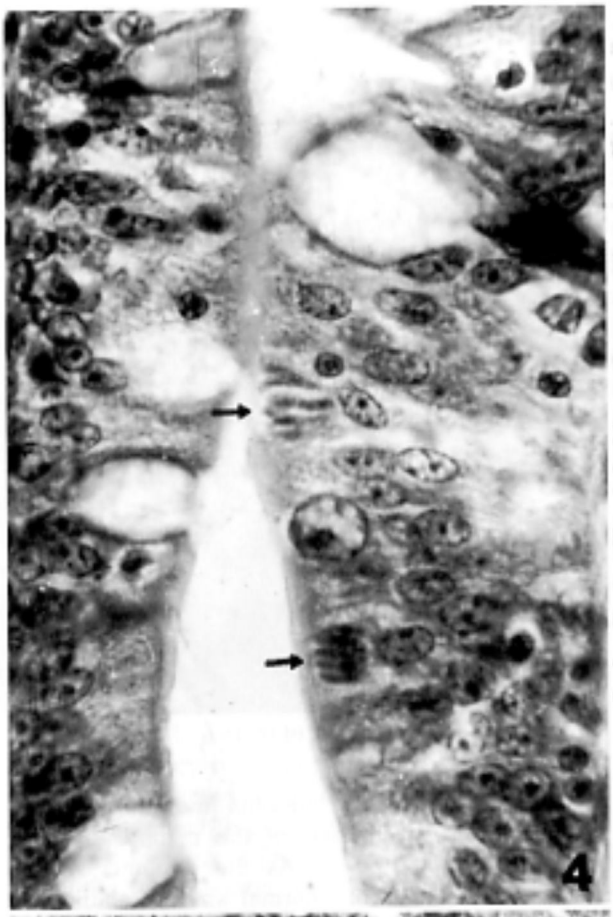
ellipsoidal microgamonts (Figs. 6, 8 & 9) was 11–20 µm. The macrogamonts and the young oocysts stained pale with haematoxylin and eosin. Young microgamonts 11–12 µm in diameter (Fig. 8) could be distinguished by their darker colour which was due to the intensive haematoxylin staining of dot-like microgametes located in them which measured approx. 1 µm in diameter. Older microgamonts, which were 17–20 µm in diameter, contained a few dozen filiform microgametes 8–9 × 0.3–0.5 µm in size. These microgametes also stained intensely with haematoxylin; however, this was less apparent because of the loose structure of the gamont (Fig. 9). In pikeperch and Volga perch specimens excreting oocysts but not showing the nodular form of coccidiosis, the trophozoites (Fig. 10), macrogamonts and microgamonts (Fig. 11) were diffusely distributed in the epithelium. Infections could be found not only in the gut epithelium but also in the epithelium of the pyloric sacs. Such diffusely distributed formations were seen also in the nodule-free parts of guts affected with nodular coccidiosis. Merozoites and gamonts showing diffuse distribution in the gut epithelium were found mostly in the apical part of the mucosal folds, and only exceptionally occurred in deeper parts of the folds. The developmental stages located in the nodules caused a lentil-shaped infection in the mucosa: in the marginal parts of the nodule only the enterocytes located close to the tip of the folds were infected, while in central parts of the nodule enterocytes were infected throughout the entire height of the folds (Fig. 5). Outside the nodules mainly merogonic and gamogonic stages and some young oocysts were found. Both the meronts and the gamonts occurred in the apical part of the cytoplasm of enterocytes (Figs. 4, 10, 11). As the gamonts were markedly larger than the normal size of epithelial cells, the latter appeared to be arranged in several rows; however, infected enterocytes located in either the superficial or the deeper regions adhered to the basal membrane with a narrow band of cytoplasm, and the nuclei of infected enterocytes were pressed into this basal region (Figs. 7 & 9).

Description of the species on the basis of 50 sporulated oocysts

Goussia desseri n. sp.

Type host:	*Stizostedion lucioperca* (L.)
Additional host:	*Stizostedion volgensis* (Gmelin)
Locality:	Lake Balaton, close to the cities Keszthely, Fonyód and Balatonszemes, Hungary
Site of infection:	Epithelium of the gut and pyloric sacs
Type material:	Histological slides deposited in the protozoological collection of the Zoological Department, Hungarian Natural History Museum, Budapest. Coll. no.: Pcr.1, 2, 3. Photo negatives and video recordings in the author's possession.

Sporulated oocysts (Fig. 12) 18.3 (17–20) µm in diameter. Some oocysts, compressed under the weight of the coverslip, 20.4–23 µm in diameter. Oocyst wall



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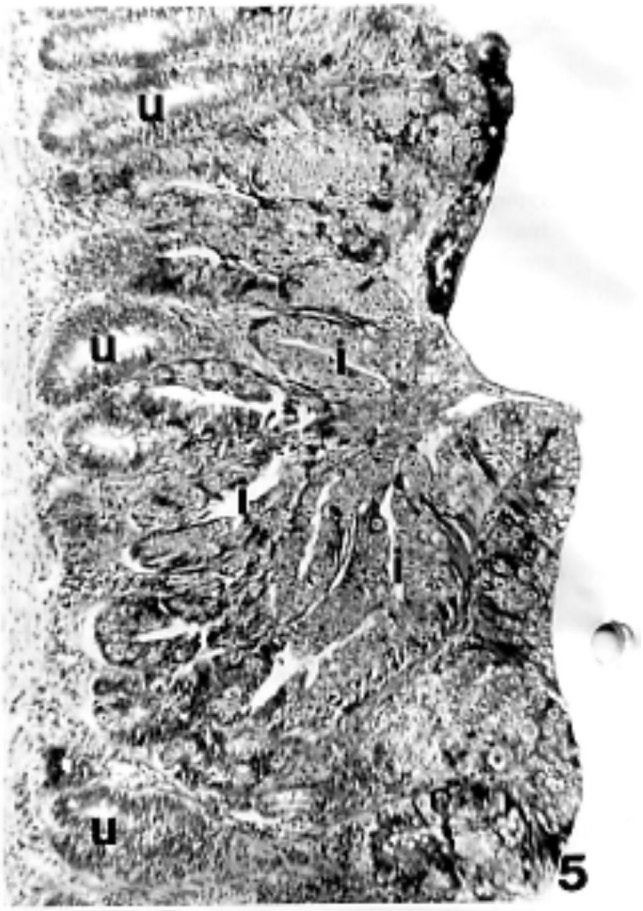

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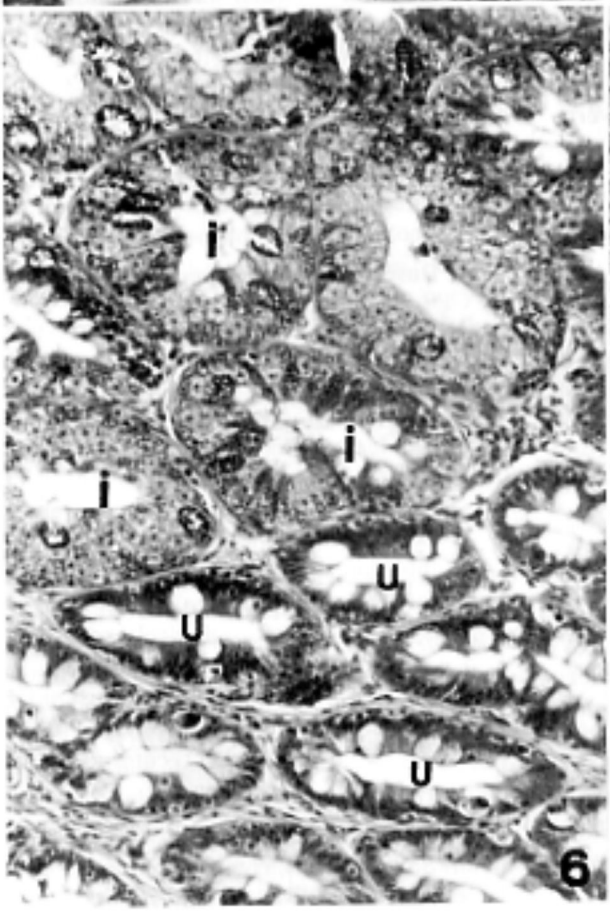

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Figs. 4 to 11. Various developmental stages of *Goussia desseri* n. sp. in the enterocytes of the pikeperch *Stizostedion lucioperca*. Histological sections stained with haematoxylin and eosin

Fig. 4. Meronts with merozoites (arrows) in the apical cytoplasm of the enterocytes. ×1000. Fig. 5. Lentil-shaped infection of the mucosal epithelium in the gut with *G. desseri* n. sp. (i) Regions infected by gamonts, (u) uninfected folds. ×150. Fig. 6. Infected (i) and uninfected region of intestinal folds (u). In the infected region each enterocyte harbours macro- or microgamonts of *G. desseri* n. sp. ×250. Fig. 7. Young oocysts in the apical parts of enterocytes. Nuclei of enterocytes (arrows) are pressed to the basal region of the damaged cells. ×500

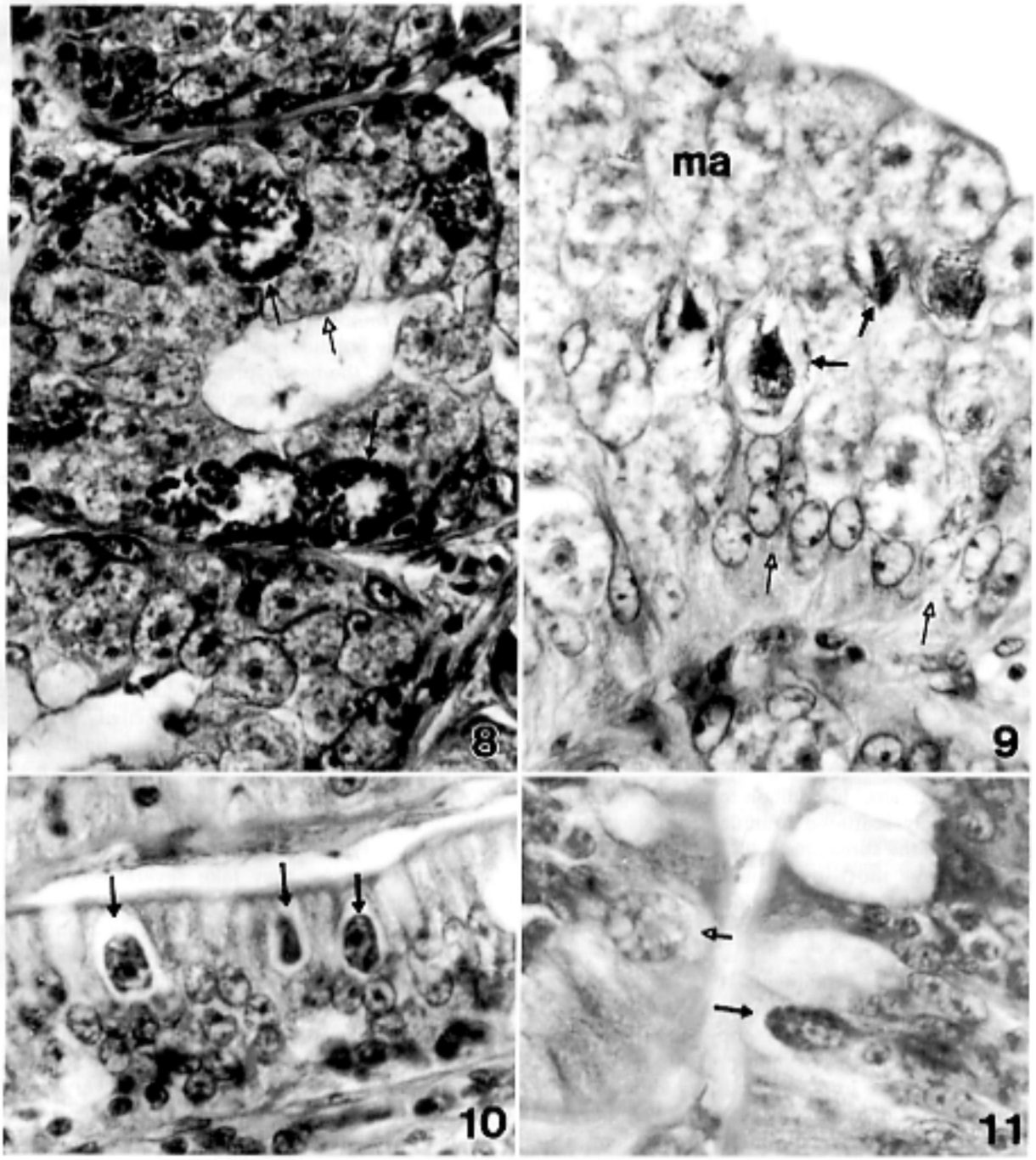

Fig. 8. Macrogamonts (empty arrow) and young microgamonts (solid arrows) with dot-like microgametes in the epithelium of the infected intestinal folds. ×1000. Fig. 9. Macrogamonts (ma) and matured microgamonts with elongated microgametes (solid arrows) in the intestinal epithelium. The nuclei of the infected cells (open arrows) are restricted to the basal part of the cytoplasm. ×1000. Fig. 10. Young trophozoites (arrows) in the apical cytoplasm of enterocytes outside the nodules. ×800. Fig. 11. A young microgamont (solid arrow) and a developing macrogamont (open arrow) in the non-nodular part of the intestinal epithelium. ×800

Fig. 12. Schematic illustration of a sporulated oocyst of *Goussia desseri* n. sp.

smooth, colourless, composed of 2 very thin layers. Oocyst contains no micropyle, polar granule or oocyst residuum. Oocyst contains 4 ellipsoidal sporocysts which loosely fill the oocyst space and are usually arranged on the same plane and sometimes in an irregular shape. Sporocysts 13.8 (13.5–14.5) µm long and 6.8 (6.5–7) µm wide. Sporocyst wall thin, single-layered. Longitudinally running suture connecting the 2 sporocyst valves well visible on sporocysts. Stieda body or Stieda body-like formation not seen on sporocysts. Each sporocyst contains 2 banana-shaped sporozoites 10.6 (10.3–11.1) × 3.5 (3.3–3.7) µm in size, in head to tail arrangement. Sporozoites contain a larger and a smaller refractile body which corresponds to a formation already seen in the sporont. After sporulation for 48 h, the sporocyst contains an oval, coarsely granular residuum 7 × 4 × 4 µm in size. After 72 h sporulation this shrinks to a finely granular residuum 6 × 4 × 3 µm in size. In addition to this compact residuum, diffusely scattered granules can also be observed among the sprozoites.

Oocysts leave the fish in unsporulated state and complete sporulation in the outside world.

Comments

In its nodular appearance and oocyst structure, *Goussia desseri* n. sp. resembles all species causing nodular coccidiosis. In oocyst size it is identical with the species *G. subepithelialis* Moroff & Fiebiger and *G. balatonica* Molnár, but differs from them with respect to the bilayered oocyst wall. The parasite has been named in honour of S. S. Desser, a renowned specialist in the area of Apicomplexa parasitizing cold-blooded vertebrates.

DISCUSSION

Until this study, the occurrence of coccidia in *Stizostedion* species had not been reported, despite the fact that coccidia are known to be very common parasites of other percid fishes including *Perca fluviatilis*, *P. flavescens*, *Gymnocephalus cernuus*, etc. (Pellérdy & Molnár 1971, Molnár & Fernando 1974, Desser & Li 1984, Jastrzebski 1984, Shulman 1984). The literature contains only a single reference to coccidian infection of the pikeperch: Shulman (1984) gave the pikeperch as host to the species *Goussia schulmani* (Kulemina, 1969). This is obviously an erroneous reference, as Kulemina (1969) described the species in question from ide (*Leuciscus idus*).

The number of species causing nodular coccidiosis in fish is relatively low. As pointed out by Marinček (1973a) using the example of *Goussia subepithelialis*, these species develop according to an annual life cycle, and their oocyst shedding is limited to a short period early in the year. A more thorough survey performed in the early spring period could probably demonstrate the occurrence of numerous other species of nodular development in different fish hosts. Based upon the morphology of their oocysts, some histologically less studied species, including *Goussia aurati* (Hoffman), *Goussia schulmani* (Kulemina) and *Goussia molnari* (Jastrzebski), are likely to have a nodular type of development.

The data reported here indicate that *Goussia desseri* n. sp. is a parasite which develops according to an annual cycle and is expected to occur in March and April. This parasite seems to form oocysts slightly earlier than the species known from cyprinids; however, in view of the activity of the host fish species in the winter months, this finding is not surprising. According to the present results, oocysts leaving the fish in an unsporulated state undergo sporulation in 2 to 3 d at 20°C; at the 4 to 10°C water temperature typical in the given period, however, the sporulation process may take several weeks under natural conditions.

Goussia desseri n. sp. seems to be a common parasite of the 2 very closely related *Stizostedion* species, and causes similar infection in both fish hosts. In contrast to earlier observations, besides the nodular forms, solitary merogonic and gamogonic stages can also be found in different segments of the gut. Combined infection caused by different species is a very common finding in the case of coccidia. The occurrence of nodular coccidiosis caused by *G. subepithelialis* in common carp (*Cyprinus carpio*) together with infection caused by *G. carpelli*, a parasite commonly occurring throughout the year, is a natural phenomenon (Schäperclaus 1954). These 2 species sharply differ from

each other both in morphology and size. There are, however, examples of the simultaneous occurrence of seasonally developing species which show only slight differences in morphology and size. A good example of the latter is the similarity between the nodular species *G. balatonica* parasitizing the gut of *Blicca bjoerkna* and *G. pannonica*, a species characterised by epicellular development (Molnár 1989). While, however, these species differ both in morphology and in location, in the case reported here the solitary gamonts and those forming nodules produce oocysts of identical structure.

The pathogenic role of the parasite is not known. At the given intensity of infection it does not have expressed pathogenicity even if nodules visible to the unaided eye have already appeared. At the same time, intensive infection produces pathological lesions similar to those reported by Schäperclaus (1954) and Marincek (1973b) for *Goussia subepithelialis* coccidiosis, especially in host fish cultured in fish farms (Horváth et al. 1984). The unsporulated oocysts of *G. desseri* are released from the epithelial cells directly into the gut lumen. In this regard, the parasite resembles the species described by Molnár (1982) from tench, and sharply differs from *G. subepithelialis*. In particular, the majority of oocysts of the latter species become stuck in the gut epithelium and are driven by the host reaction into a subepithelial location from where they will be released only later, due to a secondary expulsion process.

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