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Known and Unknown Eimerian Parasites of Fishes in Hungary

L. PELLÉRDY, K. MOLNÁR

Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest

Abstract. In the course of studies on coccidian infestation of fishes in Hungary, 52 *Cyprinus carpio*, 6 *Carassius carassius*, 60 *Rutilus rutilus*, 32 *Scardinius erythrophthalmus*, 28 *Blicca björkna*, 24 *Chondrostoma nasus*, 137 *Gobio gobio* and 22 *Gobio albipinnatus Belingi* were examined. Negative findings were obtained with 40 *Alburnus alburnus*, 5 *Alburnoides bipunctatus*, 22 *Tinca tinca* and 6 *Rhodeus sericeus amarus*. A detailed redescription is presented of *Eimeria subepithelialis* Moroff and Fiebiger, 1905 from the intestine of the carp, and *Eimeria metschnikovi* (Laveran, 1897) Reichenow, 1921 from the spleen of *Gobio gobio*, and a new species, *Eimeria scardinii*, is being described from the kidney of *Scardinius erythrophthalmus*. Reference is made to three not yet nearer defined *Eimerian* ssp. residing in the kidneys of the fish species *Rutilus rutilus*, *Blicca björkna* and *Chondrostoma nasus*. Description of the latter three coccidia will be the subject of a further study.

Occurrence of coccidia in fishes has been known since the past century, yet adequate studies on their morphology and life cycles are, unfortunately, still lacking. More detailed information has been available exclusively on coccidia of some economically important fish species, while scanty descriptions have been published on the fairly large numbers of parasites found in other fish hosts. These data, namely the earlier ones, do not serve as a firm enough basis for confirming or excluding the validity of the parasite species in question.

In this country, as yet, no papers have been published dealing with fish coccidia. This report has been written partly with the aim to fill in this gap, partly to outline the aspects of possible control measures.

Coccidia parasitic in fishes belong to the suborder Eimeriidea (Poche, 1913), family Eimeriidae (Minchin, 1912) and most of them to the genus *Eimeria* Schneider. Within this genus Labbé created the subgenus *Goussia* for coccidia whose sporocysts show two lobules fused by a suture. Since, however, the bilobular structure as well as the suture are apparent only exceptionally and, on the other hand, the species classified into the subgenus *Goussia* do not differ from *Eimeriae* in any other respect, in the meantime this subgenus has been disregarded and the type *Goussia* is now used only to denote a group of features in sporocyst morphology.

In the course of our relevant examinations, parasitological examinations have been carried out on the following river and pond fish species (figures in brackets indicate the numbers of individuals examined): *Cyprinus carpio* (52), *Carassius carassius* (6), *Rutilus rutilus* (60), *Scardinius erythrophthalmus* (32), *Blicca björkna* (28), *Chondrostoma nasus* (24), *Gobio gobio* (137), *Gobio albipinnatus Belingi* (22). All of these species were found to harbour coccidia. The following host species were examined with negative results: *Alburnus alburnus* (40), *Alburnoides bipunctatus* (5), *Tinca tinca* (22) and *Rhodeus sericeus amarus* (6). Coccidia parasitizing the parenchymal organs and intestines were identified in impression smears and intestinal scraps and contents, respectively. In positive cases the given organ was examined also by histological staining methods (hematoxylin-eosin, Mallory and Schiff technique). Detailed redescriptions of the coccidian species found by us are presented also with the aim to complete the scanty data available.

Eimeria subepithelialis Moroff et Fiebiger, 1905

This parasite has been found in the intestinal wall of carps (*Cyprinus carpio*) maintained in pond farms. A notable intensity of infestation occurred only among the 1—2 months old carp brood, where it reached 80—90 %. In these pond farms regular losses have been observed but. since *Eimeriae* have invariably occurred simultaneously with other parasites, e.g. *Dactylogyrus* (Trematode: Monogenea) and sanguinicoles (Trematode: Digenea), no reliable estimation of losses from coccidiosis has been possible.

Among the more than 1 year old carps, coccidiosis due to *Eimeria supepithelialis* was found only in a single specimen, in contrast to MARINCEK (1965) who reported to have encountered it in large numbers in 2-year old carps. In the single case observed by us, white foci of pin-head size were seen with the naked eye to cover an about 4 cm long section of intestine. Histological examinations have shown that masses of oocysts encased by a thin connective tissue capsule were located beneath the epithelium down to the muscular mucosa (Plate I. Fig. 1). The crypts of Lieberkühn became inapparent or, occasionally, a completely lost definition. Agglomerations (nests) of oocysts were frequently surrounded by an inflammatory cell infiltration. In some epithelial cells adjacent to the oocyst nests, endogenous stages (trophozoites) were seen to locate, surrounded by a pale cytoplasmic halo.

The oocysts are thin, smooth, round bodies, 18—22 μ in diameter. They are subject to endogenous sporulation and accordingly most of them have 4 sporocysts. The latter are elliptic bodies, 14—16 μ in length and about 8 μ in width. The sporocysts are tapering towards one end, with nuclei situated proximal to the tapering pole at about one quarter of their full lengths. Among the sporozoites a granular inner residual body is seen. No external residual body is present in the oocysts (Plate I. Fig. 2).

MARINCEK (1965) described some more endogenous stages. The mature schizonts contained 5—17 merozoites. The schizonts measured 9 — 12.5 × 7.8 — — 9.3 μ, the merozoites 4.5 — 6.8 × 0.7 — 0.1 μ. Macrogametes were 14 — 17.1 μ in size. Microgamete dimensions were 12.5 — 46.8 × 6.2 — 23.4 μ (!). MARINCEK gives oocyst diameters as 15.6 — 18.75 μ, noting that also diameters of 20—25 μ were found. Sporocyst sizes were 9.3 — 12.5 × 4.6 — 6.2 μ.

In addition to *Eimeria subepithelialis*, three further *Eimerian* species have been described from the carp. In 1921, LÉGER and STANKOVITCH described *Eimeria carpelli* which, according to SCHÄPERCLAUS (1954), is identical with *Eimeria cyprini*, described by PLEHN in 1924. We, too, have repeatedly encountered this parasite in the intestinal contents of the carp and refer in this context to STANKOVITCH (1921), who has described in detail its endogenous cycle and morphological features. Mass invasion of *Eimeria carpelli* results in extensive intestinal inflammation to be distinguished from the focal nature of *subepithelialis*-coccidiosis. The two species can be differentiated also by the dimensions of oocysts, *subepithelialis* oocysts being 20 μ in diameter, whereas *carpelli* oocysts hardly more than half of it.

According to the original description, the oocysts of *Eimeria wierzejski* Hofer, 1904 are 11—12 μ in diameter, also being much smaller than those of *Eimeria subepithelialis*. Occasionally, such oocysts are seen in large numbers in intestinal scraps yet, there is now reason to doubt the correctness of this description, presented more than 50 years ago, and simultaneously also the validity of the species.

Eimeria metschnikovi (Laveran, 1897) Reichenow, 1921

Synonym: *Coccidium metschnikovi* Laveran, 1897

In this laboratory, regular seasonal examinations of *Gobio gobio* were conducted over the whole year, including post mortem parasitological examinations of 137 *Gobio gobio* and 22 *Gobio albipinnatus Belingi* Slastenenko. The examined fishes were derived from two faunistic areas: a mountain brook (Magyarkut, Börzsöny), and a streamlet (brook Tápió), meeting the river Tisza in the Great Hungarian Plane. In the latter, the extensity of infestation was lower as compared to the former. Out of the fishes examined, 16.5 % of *Gogio gobio* and 9.4 % of *Gobio albipinnatus* have been shown to be infected with *Eimeria metschnikovi*.

In the course of faunistic studies performed in late summer on the fish *Gobio gobio*, we found in its spleen large numbers of yellowish-white nodules of pin-head size, encased by a thick connective tissue capsule (Plate II. Fig. 1) including 30—50 or more Eimerian oocysts (Plate II. Fig. 2). In addition, some oocysts were encountered also in the liver. Based on the characters of sporulated oocysts encased in the capsule, the species was identified as *Eimeria metschnikovi*.

Later SCHULMAN and ZAIKA (1962) reported to have found this species in the intestine, spleen, liver and kidney of *Gobio albipinnatus tenuicorpus* Mori, whose habitat is the river Amur. As shown by the attached photos, obviously the same parasite has been described by ARVY and MOREAU (1966) from the spleen and liver of *Gobio gobio* without, however, determining its systematic position. Confusion has presumably arisen owing to the simultaneous presence of *Myxosporidia* which occur in the spleen of nearly all fishes and are easily mistaken for certain stages of coccidia.

During the autumn, examinations conducted on 12 and 8 fishes in September and November, respectively, revealed infection with *Eimeria metschnikovi* in only 3 specimens in the September series. These fishes harboured coccidia exclusively in their spleen, showing macroscopic oocyst nodules, lesser in number, but identical in nature with those encountered during the initial studies.

In the late autumn and winter (February), fishes harboured no coccidia yet; during sectioning in the early spring, islets containing reddish tissue debris, destroyed deformed *Myxobolus* spores and supposedly non-viable oocysts were frequently encountered in their spleen.

A few oocysts were first found during March in the splenic parenchyma. At the beginning of April, a *Gobio* fish showed large numbers of macrogametes with granular cytoplasm scattered throughout its spleen (Plate III. Fig. 1). By the end of April, 3 fishes were found to harbour, besides stages resembling the above macrogametes but exhibiting a coarser granulation, also oocysts in the initial phase of sporulation (Plate III. Figs. 1—2). These developmental stages occurred less sporadically, being arranged in more or less adjacent groups surrounded by inflammatory tissue reaction.

During May, besides a few early (non-sporulated) oocysts, large numbers of sporulated oocysts were present, arranging in groups surrounded by connective tissue capsules. In this phase, the spleen of infected hosts did not show any gross lesions, being seemingly identical to those of non-infected fishes. Oocysts were present infrequently in the liver together with very massive infestation of the spleen, but never in the kidney. In an overwhelming majority of cases besides *Eimeriae*, also *Myxobolus* spores were found in the spleen.

By the end of June, splenic nodules could be viewed under a hand magnifying lens and by the end of the summer season they became still more conspicuous.

Our observations suggest that of the possible localizations described by SCHULMAN and ZAIKA, the spleen is the primary site of coccidian parasites of *Gobio* fishes in Hungary.

Summarizing the above observations, a redescription of *Eimeria metschnikovi* is presented below:

Oocysts are round, smooth bodies with a thin wall, attaining 20—27 μ in diameter. They occur in sporulated form in the host organism. The 4 sporocysts are bluntly elliptic in shape, with a thin wall and without a Stieda body. No external residual body is apparent; internal residual bodies are conspicuously bulky, refractive, with diameters up to 2 μ. Several oocysts show a signet-ring like thickening of the wall which corresponds to a nuclear remnant of the host cell.

Released sporocysts are 15×9 μ in size. Owing to the rich inner residual body, the outlines of the sporozoites are hardly apparent. *E. metschnikovi* oocysts localize in the spleen, less frequently in the liver and—according to Laveran—sometimes also in the kidney and intestine. In the spleen they arrange in smaller or larger groups or nests. In the initial phase the connective tissue capsule

encasing the nest is thin and the oocysts are often non-sporulated. The sporonts occupy a relatively small space inside the oocysts, being 18 μ in diameter. Larger nests encountered in the spleen accommodate various stages of oocysts including also freshly sporulated ones with undeveloped sporocysts and sporozoites. In this stage, the oocysts show a particularly conspicuous cytoplasmic granulation. There are also macrogametes without a cyst wall and very early oocysts with developing walls. In general, the younger the oocysts, the smaller they are and the less relationship they have with each other. Macrogametes and early oocysts occur in loose groups without connective tissue casing; in a later phase a thin connective tissue capsule develops accomodating various stages of oocysts; finally mature oocysts enclosed in capsules of a 10—15 μ thick wall, all in an advanced stage of sporulation, are apparent. In the surroundings of the connective tissue capsule, an inflammatory cellular reaction may or may not develop.

So far evidence has been lacking as to the mode of infestation of fish hosts by coccidia. With species colonizing the spleen, for example, oocysts cannot be extruded into the external world as the spleen has no outlet, hence the sole way of the spread of coccidia from one fish to another seems to be the ingestion of the infected dead fishes or their tissues and organs.

Studies on the seasonal fluctuation of infection by *Eimeria metschnikovi* revealed the occurrence of macrogametes and early oocysts in the spleen mainly during spring. In the summer, and namely in the autumn, groups of mature oocysts, encapsulated by connective tissue, were found in the spleen. This implies that in the majority of fishes, coccidian infestation occurs during spring, taking a more severe course during summer and autumn in accordance with the massivity of infection, but reducing markedly, or even expiring, by the winter season.

Anyhow, it seems to be likely that mass infestation of fishes during spring takes place through the uptake of infective material accumulated in their environment during the winter. The further course of endemic fish coccidiosis depends on the degree of infestation. Massively infected fishes may die of coccidiosis or intercurrent diseases, whereas less massively infected ones tend to loose strength until they become an easy prey of predators. In either case, the coccidia released from their spleen disseminate in the environment. In mild infection, only a few oocysts develop in the spleen which are sooner or later eliminated by the organism. These circumstances explain why signs of coccidian infestation are accumulating during spring and why macroscopic nodules of coccidia develop chiefly in late summer and autumn.

Besides *Eimeria metschnikovi*, redescribed above, *Gobio gobio* fishes harbour also the species *Eimeria cheissini* Schulman and Zaika, 1962. This coccidium is distinguishable from *Eimeria metschnikovi* by the biological feature that it resides not only in the spleen, but also in the peritoneum, intestine, air bladder, etc. We have not found this coccidium in our material.

In 1962, Schulman and Zaika described from the spleen of *Gobio albipinnatus*

tenuicorpus a coccidian parasite named by them *Eimeria macroresidualis*. The oocysts of this species are round bodies, 18 μ in diameter. Sporocysts measure 10.5—12 by 5—6.5 μ and comprise an enormous, coarsely granular inner residual body, 5.2—6.5 μ in diameter. Since the oocysts of the species *Eimeria metschnikovi* and *E. macroresidualis* differ hardly in their morphology and both of them reside in the spleen, their possible identity should be taken into consideration.

SCHULMAN and ZAIKA (1962) described also another fish coccidium, *Eimeria siliculiformis*, from *Gobio albipinnatus*. The round oocysts of this species measure 15.4—17 μ in diameter. Since they do not occur in the spleen, they are evidently not identical with *Eimeria metschnikovi*. *Eimeria metschnikovi* sporocysts differ conspicuously from *E. siliculiformis* ones not only in shape but also in the presence of a large, coarsely granular, residual body, missing in the latter. Based on this morphological dissimilarity and on the fact that *E. siliculiformis* does not occur in the spleen, the validity of the two species is undisputable.

Eimeria scardinii sp.n.

Out of 32 *Scardinius erythrophthalmus*, collected from the Lake Velence, coccidian infestation was stated in four cases. Oocysts occurred in the renal parenchyma, individually or in groups of varying sizes, occasionally surrounded by a connective tissue capsule.

The oocysts are round or short elliptic in shape, with a smooth wall, and a diameter from 21 to 24 μ (Plate IV. Fig. 1.) In younger oocysts a round sporont, constituted by refractive granules, is apparent (Fig. 1.). Its diameter measures 12—14 μ, leaving a relatively large unoccupied space inside the cyst wall.

The sporont gives rise to 4 round sporoblasts (Fig. 2.) with a granular cytoplasm. Later 2 sporozoites are making appearance in each of them. The roughly elliptoid sporocysts have a very thin wall, seen only at a certain adjustment of the micro-

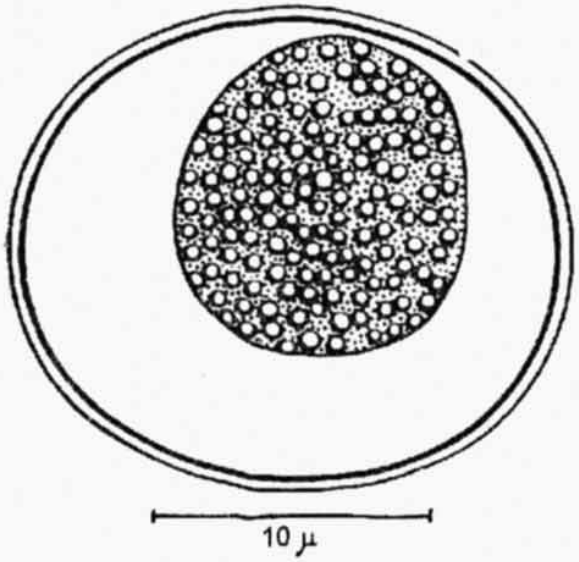

Fig. 1. Unsporulated oocyst of *Eimeria scardinii* sp.n.

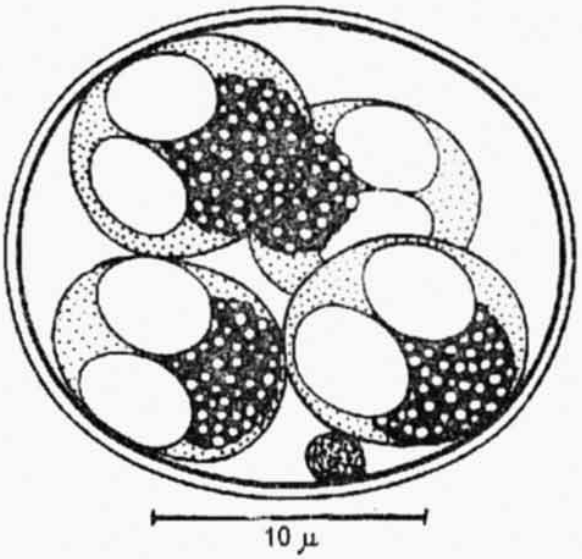

Fig. 2. Roundish sporocysts, abundant secondary residuum in *E. scardinii* sp.n. oocyst

scope, which never attaches closely to the sporozoites. The sporocysts measure 14—17 by 6—7 μ. There is no outer residual body, only 1—3 refractive polar bodies being present. Freshly sporulated oocysts show a rich granular inner residual body, which later on looses bulk and its granules become scattered inside the sporocyst. By the end of sporulation, the inner residual body consists of 10—15 granules (Figs. 3—4).

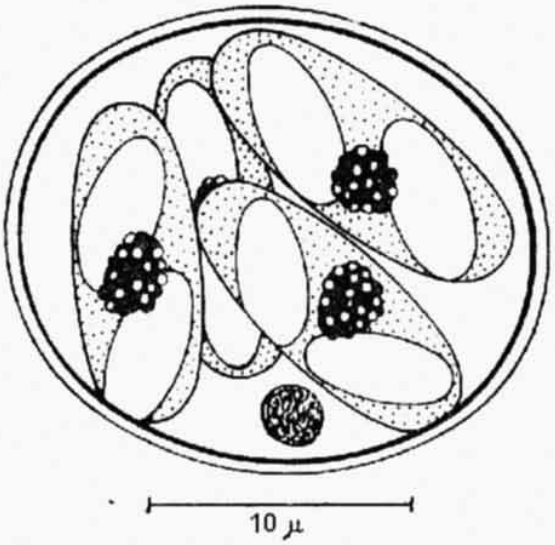

Fig. 3. Elongated sporocysts with small residua in *E. scardinii* sp.n. oocyst

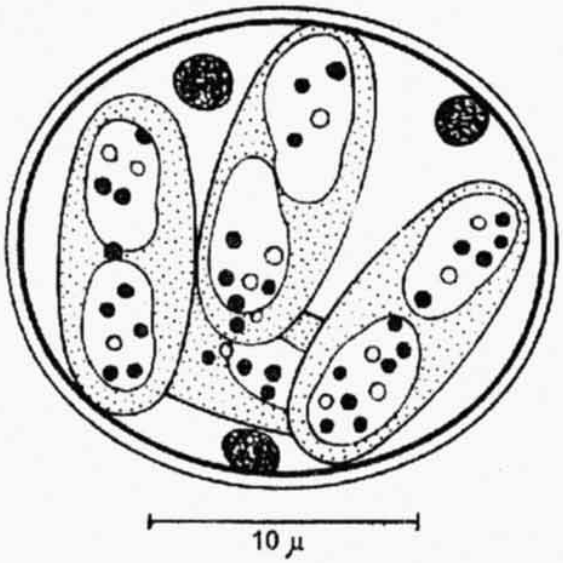

Fig. 4. Mature oocyst of *E. scardinii* sp.n. Granules of secondary residuum scattered in sporocysts. Shape and position of sporozoites are characteristic

The sporozoites localize along the longitudinal axis of sporocysts, being shifted slightly towards both ends of the sporocysts. They measure 8 — 13 × 3.5 — 5 μ and are of a slightly invaginated elliptic shape.

Groups of oocysts usually reside among the renal tubuli in connective tissue capsules. Larger nests (nodules) sometimes exceeding 200 μ in diameter, have a 20—30 μ thick connective tissue wall and their cross sections comprise 16—30 oocysts in histological sections. Also smaller oocysts have been encountered with a thinner wall, consisting of only few connective tissue fibers. They comprise not more than 3—5 early oocysts. Around the nodules usually an inflammatory cellular reaction develops.

In serial histological sections oocysts in different stages of maturity (sporulation) have been encountered. From these findings the parasite's hypothetical life cycle has been derived. The youngest oocysts (macrogametes) have no wall and are found in the smallest nests. The earliest oocysts surrounded by a visible wall measure 24 × 20 μ, their sporonts being 12 × 10 μ in size. Later the sporonts occupied gradually all the space inside the oocyst, giving finally rise to sporoblasts which developed to sporocysts. The latter were initially stout bodies enclosing stout oval sporozoites. In a later stage of sporulation, the sporocysts became elongated, the sporozoites drew appart towards its two ends and the inner residual body arranged as outlined in the foregoing text. The slightly

invaginated elliptoid shape of the sporozoites in fully sporulated oocysts is a typical feature. Fully sporulated oocysts occurred inside the largest nests (Plate IV. Fig. 2).

The particular arrangement of sporocysts and sporozoites in mature *E. scardinii* oocysts is a feature distinguishing them from all other coccidia hitherto described from *Scardinius* fishes. So far 4 further coccidian species have been found in *Scardinius erythrophthalmus*, *Eimeria alburnis* (Stankovitch, 1920), Yakimoff, 1929, has round oocysts 19—20 μ in diameter and its sporocysts have a thick (Goussia-type) wall. This parasite resides in the periintestinal fat tissue and is evidently not identical with *Eimeria scardinii*, parasitizing the renal parenchyma.

An intestinal coccidium, distinguishable by its location from the above described new species is *Eimeria cyprinorum* Stankovitch, 1929. A further intestinal coccidium is *Eimeria stankovitchi* (Stankovitch, 1920), Pinto, 1928. The oocysts of this species differ also morphologically from *Eimeria scardinii* oocysts, being only 10 μ in diameter and showing a Goussia-type bilobular sporocyst sheath.

The fourth known coccidian parasite of *Scardinius erythrophthalmus*, *Eimeria pigra* Léger et Bory, 1932, is also an intestinal coccidium which is distinguishable from *Eimeria scardinii* also by morphological features such as tapering spindle-shaped sporocysts, absence of inner residual body, etc.

Besides the above fish hosts, full parasitological sectioning has been carried out also on *Rutilus rutilus* (60), *Blicca björkna* (28) and *Chondrostoma nasus* (24) fishes. Out of them 8, 4 and 2 specimens, respectively, harboured coccidia in their kidneys. These parasites bore a striking morphological resemblance to *Eimeria scardinii* and also the reaction of the host's organism was similar. These features being not conclusive enough, we are referring only to the kidney-dwelling coccidia encountered in the course of this study in 3 different fish hosts. Their identification and systematic classification warrants further investigation, based chiefly on biological characters.

The most certain biological feature is host specificity, very pronounced with the majority of coccidian parasites, and particularly strict with *Eimeriae* so that hitherto all experimental attempts to state the opposite have failed. If host specificity were a regular biological feature also of the Eimerian parasites of fishes, it can be identified in cross infection experiments, however difficult similar studies on fish may be.

Note. All histological sections shown in the photomicrographs have been stained with hematoxylin and eosin.

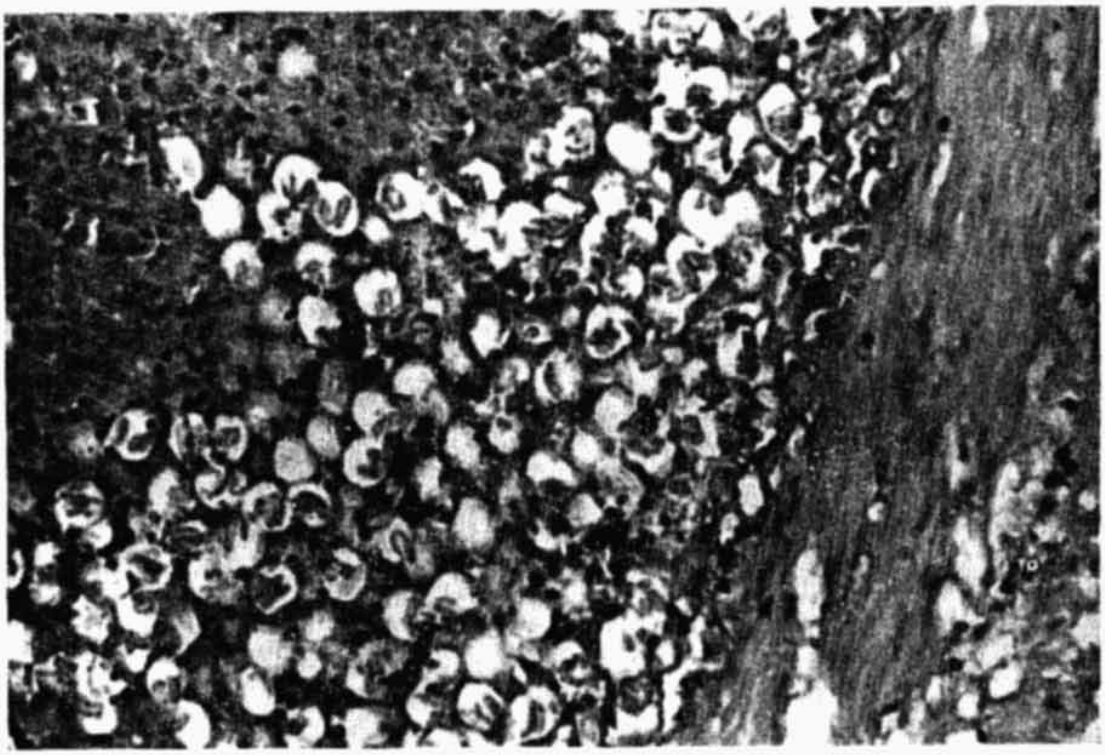

Fig. 1. Groups of oocysts of *E. subepithelialis* deep in the intestinal wall of a carp (× 300).

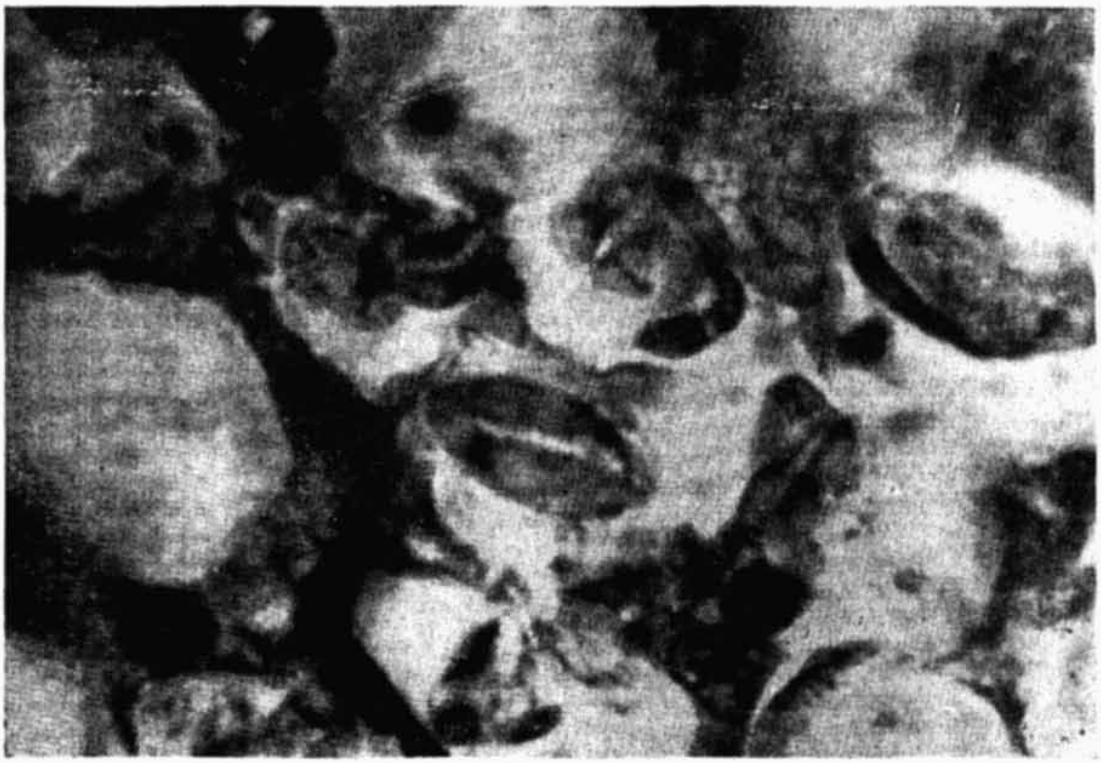

Fig. 2. Sporocysts and sporozoites of *E. subepithelialis* (× 2,000).

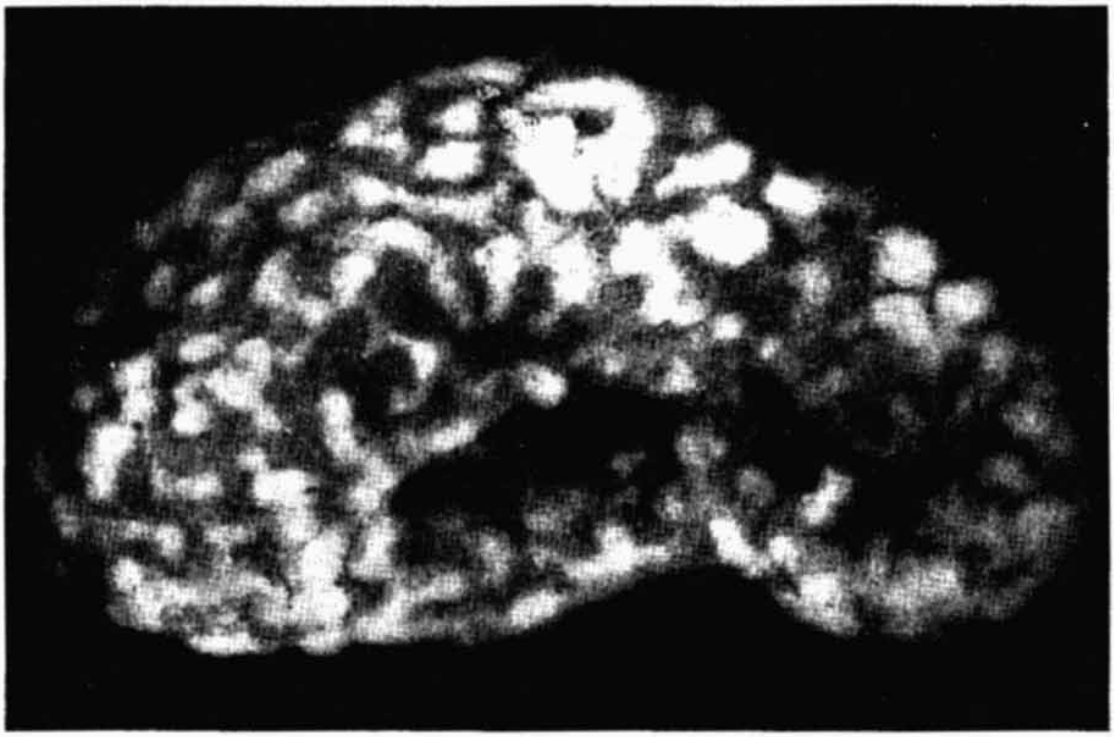

Fig. 1. Spleen of *Gobio gobio* showing multiple nodules of *E. metschnikovi* (×15).

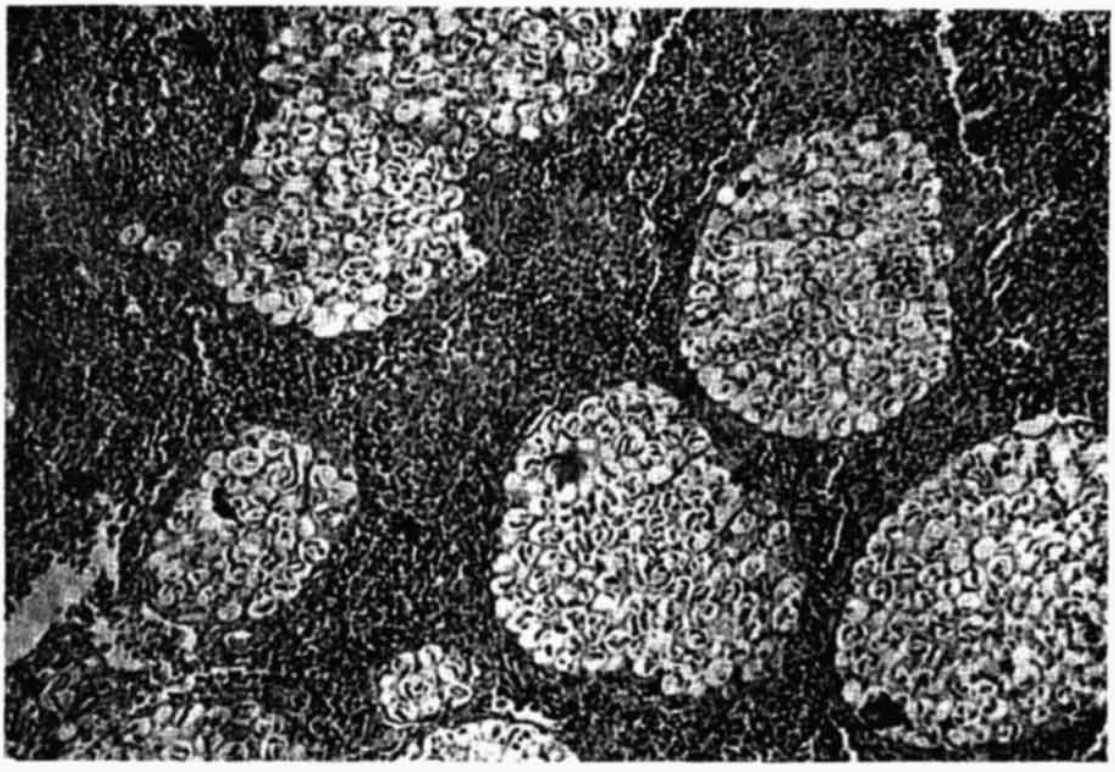

Fig. 2. Nodules of oocysts of *E. metschnikovi* in the spleen of *Gobio gobio* (×150).

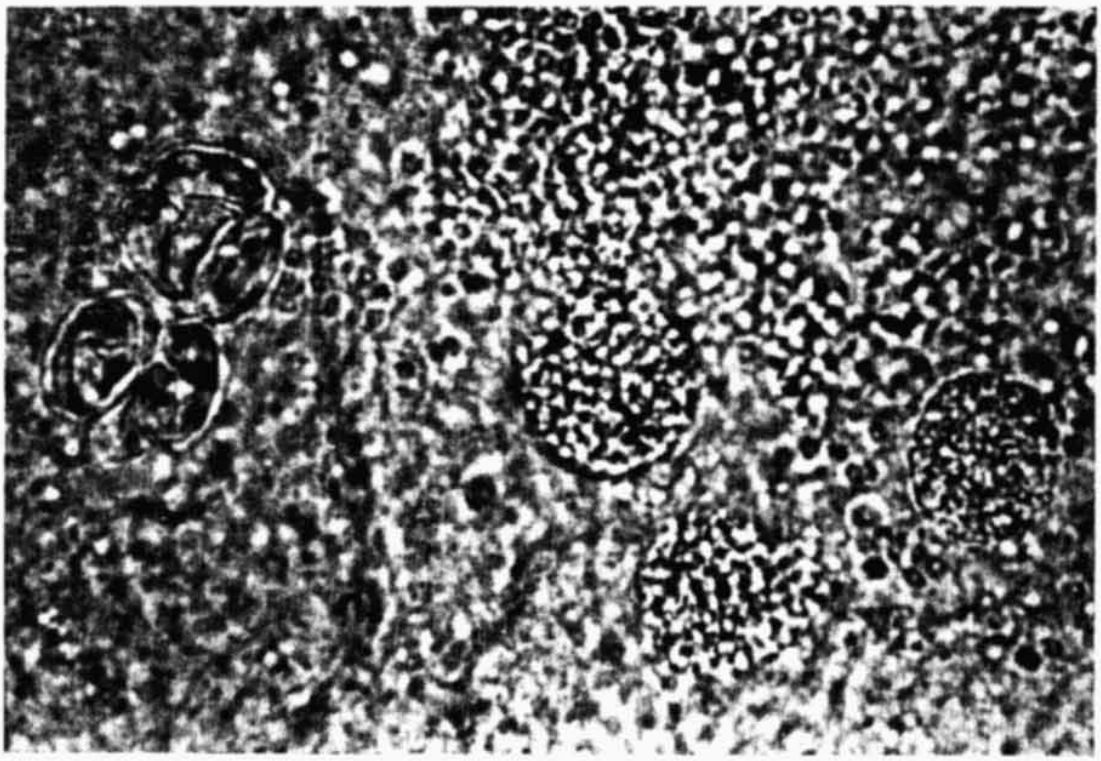

Fig. 1. Macrogametes and oocysts of *E. metschnikovi*. Unstained (×1,000).

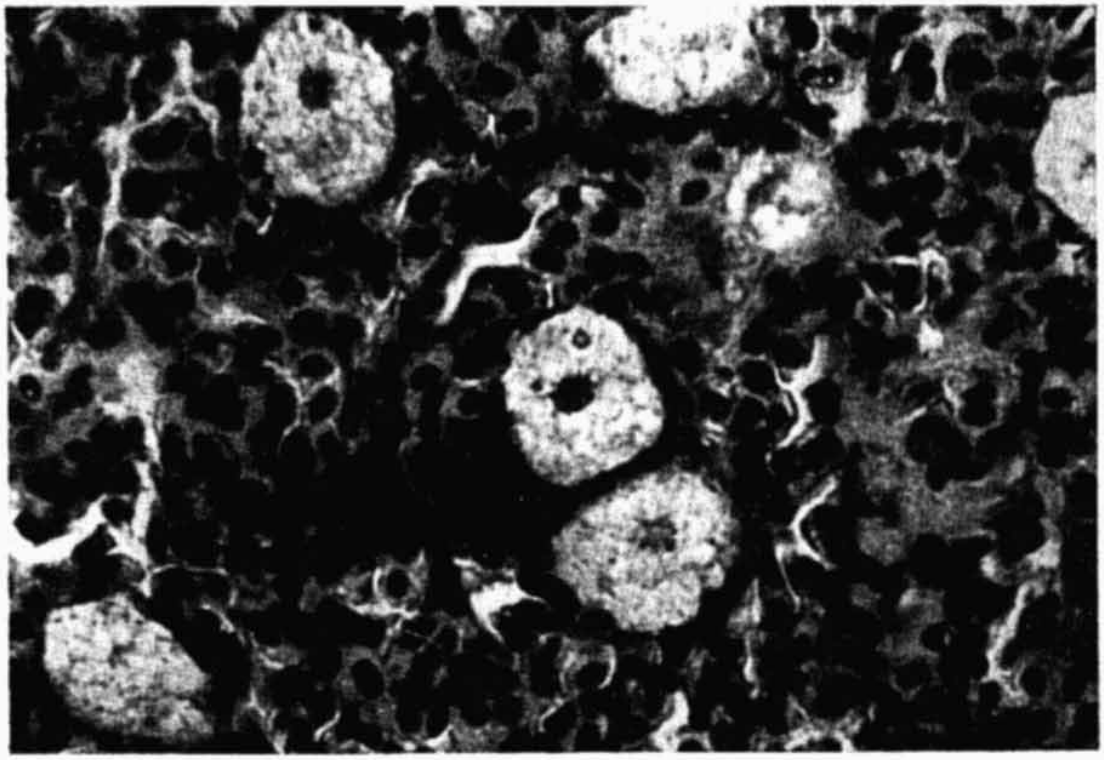

Fig. 2. Macrogametes in the spleen of *Gobio gobio* (×1,000).

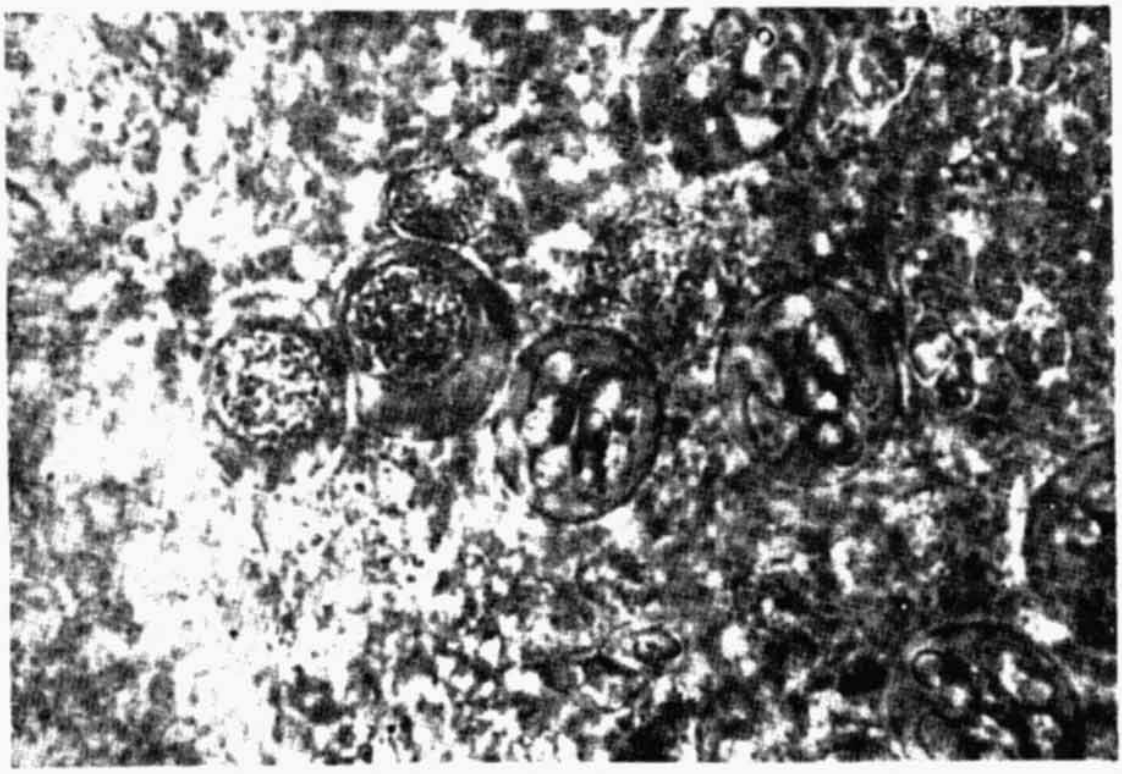

Fig. 1. Mature and immature oocysts of *E. scardinii* sp.n. (× 1,000).

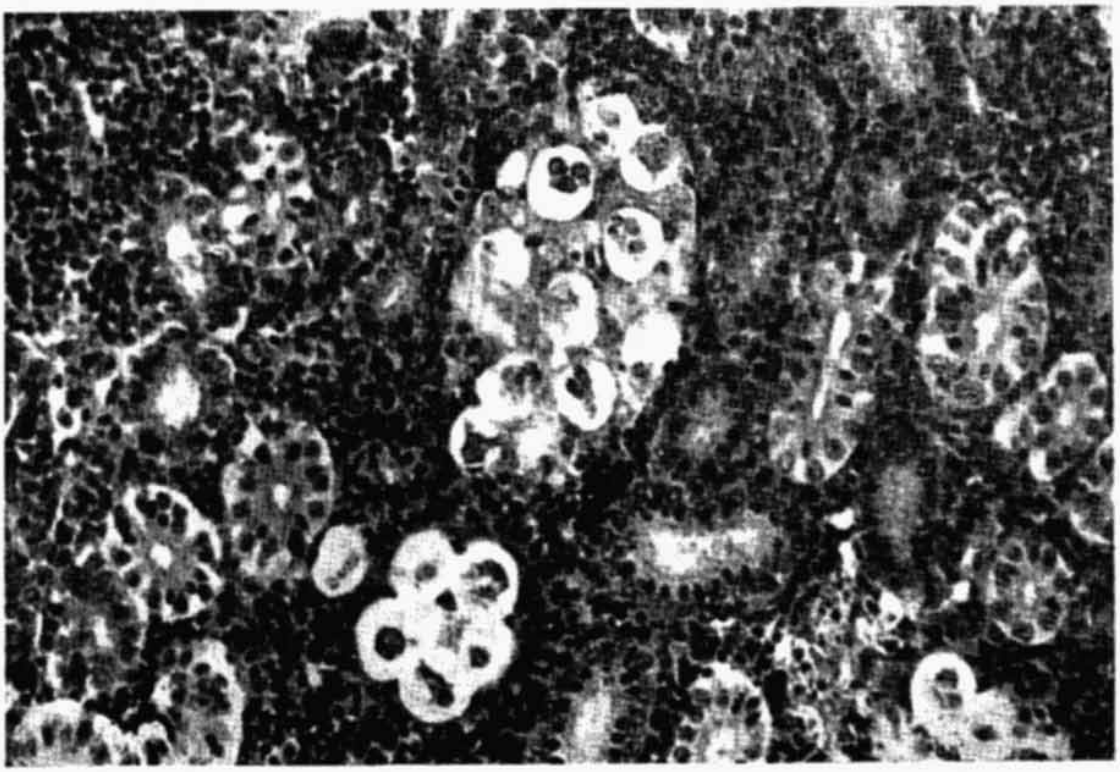

Fig. 2. Groups of oocysts of *E. scardinii* sp.n. in the kidney (× 400).

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L. P., Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Hungária Körút 21, Budapest XIV, Hungary.

OECEOPTOMA THORACICA L., A NEW INTERMEDIATE HOST OF THE CESTODE *NEOSKRJABINOLEPIS SINGULARIS* (CHOLODKOWSKY, 1912).

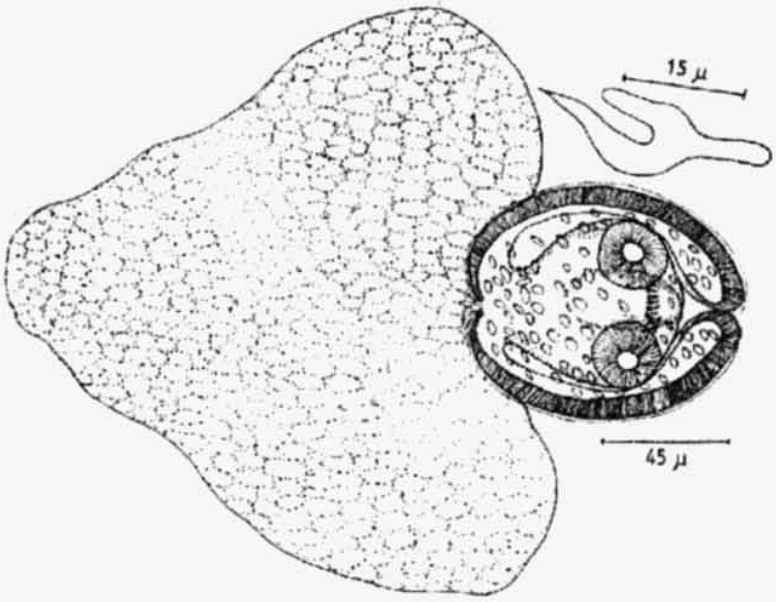

Fig. 1

While searching for larval forms of tapeworms parasitizing small mammals, we dissected insects collected near our field station Klec, district J. Hradec, southern Bohemia. In *Oeceoptoma throacica* we found several cysticercoids, which we identified as larvae of the cestode *Neoskrjabinolepis singularis* (Cholodkowsky, 1912). This species is widely distributed in Czechoslovakia, its definitive hosts are some insectivores of the family Soricidae. In various years we examined more than 2,000 beetles; only in May and June 1967 we found cysticercoids of *N. singularis* in 4 out of the 114 beetle specimens examined. *Oeceoptoma thoracica* is a new intermediate host of this cestode.

Until the present, the occurrence of cysticercoids in beetles has been recorded only by KISIELEWSKA (Bull. Acad. Pol. Sci. Cl. II/VI/5: 205–208, 1958), who found them in *Catops sp.* at the National Park of Bialoweza in Poland. **Description**: shape of the cysticercoid almost spherical or somewhat obovoid (Fig. 1), size 88–92 × 70–74 μ. Tail relatively short, greatly extended at the base, shovel-shaped, size 120–140 × 130–150 μ. The cyst is covered with 5 layers. The cuticular cover bears fimbrialike appendages resembling fimbria of an indistinct structure. Calcareous corpuscles are irregularly dispersed in the last intermediary, parenchymatous layer. The scolex inside the cyst bears four very motile suckers, changing their shape from spherical to elongated. Width of scolex 80–104 μ, length 120–130 μ. Size of suckers 40–50 × × 32–38 μ. The rostellum, resting on a large barrel-shaped pouch (width 53–60 μ) is armed with 10 hooks (Fig. 1) (length 32 to 34 μ).

J. PROKOPIČ, Institute of Parasitology, Czechoslovak Academy of Sciences, Prague