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FURTHER STUDIES ON COCCIDIA OF FRESHWATER FISHES IN HUNGARY

By

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(Received March 5, 1969)

In a previous paper (PELLÉRDY and MOLNÁR, 1968) we have described some coccidia recovered from freshwater fishes in Hungary. Having undertaken further studies in 1968, on the one hand coccidian oocysts were recovered from some additional fish hosts and, on the other, additional coccidia were found in hosts examined earlier.

Though the relevant examinations were carried out on several hundreds of fishes belonging to more than 30 species, in the present paper exclusively the coccidia from those fish species are described in which identifiable coccidia occurred frequently. As to the hosts, statistical evaluation was made solely of the data of species which were examined specially for coccidia, *viz.*, *Abramis brama* (40), *Alburnus alburnus* (70), *Blicca bjoerkna* (60), *Carassius carassius* (14), *Cyprinus carpio* (70), *Chondrostoma nasus* (14), *Rutilus rutilus* (16) and *Scardinius erythrophthalmus* (25).

In every case, freshly killed fishes were examined. Of the organs crush preparations were made and examined microscopically. The dimensions and morphological properties of the oocysts were determined in unstained preparations. In addition to depicting the oocysts, in the majority of cases microphotographs were taken of the smears and histological slides.

1. Coccidia from the kidneys of fishes

Of the hosts examined in 1968, renal coccidia were encountered in 22 *Blicca bjoerkna*, 3 *Rutilus rutilus*, 7 *Alburnus alburnus*, 4 *Scardinius erythrophthalmus* and 8 *Abramis brama* fishes. Part of them corresponded morphologically to *Eimeria scardinii* PELLÉRDY and MOLNÁR (1968), part were similar to *Eimeria leucisci* SCHULMAN and ZAIKA (1964), though not in every respect. From the host *Chondrostoma nasus* exclusively *Eimeria scardinii* was recovered.

The original description of *Eimeria leucisci* was published in 1962, but two years later SCHULMAN and ZAIKA presented a revision to eliminate some errors. Yet that second description still has shortcomings, as, *e.g.*, no secondary residual body is mentioned, though it is clearly depicted on the attached drawing and ZAIKA himself (1965) reported residual bodies of 4—4.5 μ diameter in the sporocysts.

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A revised description of *Eimeria leucisci* is given below:

Host: *Leuciscus leuciscus baicalensis*

Localization: kidney, wall of gallbladder

The oocysts are round and 35—36 μ in diameter. They enclose each 4 elongated-ovoid sporocysts of 16.5—18.5 μ by 7—8.5 μ dimensions, which leave relatively much free space inside the oocyst. The sporozoites are worm-

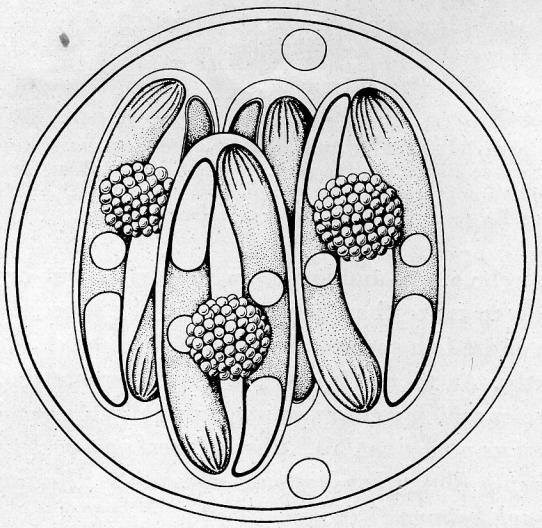

Fig. 1. Eimeria leucisci oocyst from the renal tubuli of *Blicca bjoerkn a.* ×4000. Original

shaped, measure 17—18 μ by 1.5—2 μ *(sic !)*, have a tapering process on one end, while the other is bluntly rounded and near to it an elongate, 5.5 μ long, homogeneous refractive area is seen.

According to our own experience, the oocysts recovered from the above 5 hosts have slightly differed from the cited description of *E. leucisci* (Fig. 1). We measured the dimensions of the roundish-ovoid oocysts, having an 0.5—1 μ thick wall, as 24—29 μ by 21—26 μ; that is, they were somewhat larger than those described by SCHULMAN and ZAIKA. The dimensions of sporocysts (17—19 μ by 6—7 μ) differed hardly, though we found sporozoites much wider (3—3.5 μ in width) than the cited authors. It should be mentioned in this context that the disproportionality of the 1.5—2 μ width given by SCHULMAN and ZAIKA is obvious also from the drawing attached to the original description. Our values for the inner residual bodies (3.5—5.5 μ) agree well with those given by ZAIKA (1965) for *Eimeria leucisci.*

We recovered the oocysts from the renal tubuli in a mature state. They contained elongate-ovoid sporocysts with thin walls, in a roughly parallel

arrangement. In the relatively large, empty space within the oocyst, 1 or 3 refractive bodies (polar bodies) were apparent.

The sporozoites arranged within the sporocysts collaterally in head-to-tail position, with the concave borders turned towards each other. In the centre of the sporocysts there was a markedly refractive granular residual body. Some granules from it were occasionally seen to scatter among the sporozoites. We suppose that such spreading occurs in aged oocysts.

Inconsistently with the afore-mentioned description, we failed to observe the tapering pole at one end of the sporocysts, neither observed the reflected ends of the sporozoites. But with appropriate adjustment of the microscope, we saw a conspicuous longitudinal striation of the sporozoite wall toward the tapering pole, not mentioned in the description.

The morphological features outlined above were unsatisfactory to identify the coccidium in question as *Eimeria leucisci*, while the morphological deviations from the latter were insufficient to classify it as a new species.

In kidney specimens oocysts resembling *Eimeria leucisci* were well distinguishable at low magnification from *Eimeria scardinii*, whose occurrence in one and the same host in often mixed infections was reported earlier (PELLÉRDY and MOLNÁR, 1968). The sporozoites of *Eimeria scardinii* are short and stout (potato-like) rather than elongate structures. Another important feature, seen primarily in histological preparations, is that while the *Eimeria scardinii* oocysts are consistently seen in extratubular location in the renal haemopoietic tissue and/or in its surroundings and are separated from the adjacent tubuli by a connective tissue capsule, the leucisci-oocysts are invariably found inside renal tubuli, occasionally filling the entire lumen and flattening the epithelial lining of the dilated tubular wall (Figs 1 and 2).

2. Oocysts from the serosa of fishes

In the specimen each from 4 fish species originating from different habitats, we found oocysts which morphologically differed only little from *Eimeria siliculiformis* SCHULMAN and ZAIKA, 1962. Their hosts were *Scardinius erythrophthalmus* (from the River Körös), *Alburnus alburnus* (Lake Balaton), *Rutilus rutilus* (River Tisza) and *Abramis brama* (River Danube).

The original description of *Eimeria siliculiformis* is related below:

Host: *Gobio albipinnatus tenuicorpus*

Localization: Air bladder, intestine, kidney

The oocysts are round, 14.4—17 μ in diameter and contain no outer residual body. The sporocysts have fairly thick walls and measure 11.7—13 μ by 5.2—6.5 μ. The sausage-shaped sporozoites lie along one side of the sporocysts and the remaining large space is filled up by an abundant secondary residual body.

In the hosts examined by us, the supposed *Eimeria siliculiformis* oocysts were round in shape, had a 1 μ thick smooth wall and a diameter of 20—21 μ; thus they were somewhat larger than the proper *E. siliculiformis* oocysts (Fig. 2). Their inside was nearly filled up by the 4 sporocysts leaving only little free space. The cylindrical sporocysts, tapering conically at both ends not mentioned in the original description but being well seen in the attached

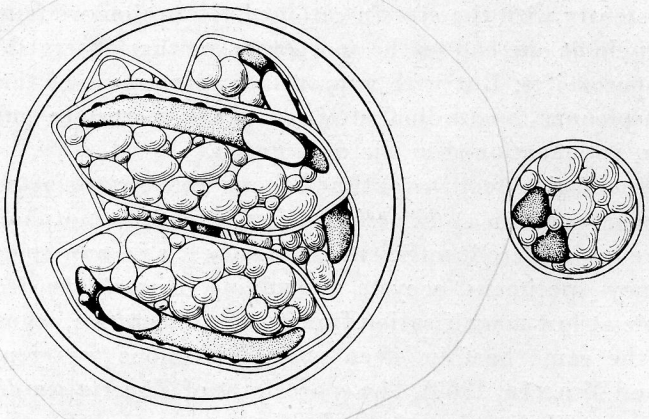

Fig. 2. Sporulated oocyst and transsection of sporocyst of *Eimeria siliculiformis* from the renal serosa of *Scardinius erythrophthalmus.* ×4000. Original

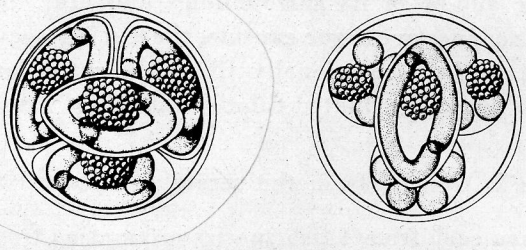

Fig. 3. Eimeria carpelli oocyst from *Cyprinus carpio*, showing the commonest arrangement of the sporocysts. ×4000. Original

drawing are highly characteristic. The dimensions of the thick-walled sporocysts were 14—15 μ by 7—8 μ, *i.e.*, somewhat larger than those described by SCHULMAN and ZAIKA.

The sporocysts contained two sausage-shaped sporozoites and a large granular residual body. The coarsely granular copious residuum appeared particularly typical of siliculiformis-sporocysts (Fig. 3). The sporozoites located along one side of the sporocyst adjacently in head-to-tail position and measured 10—12 μ by 2 μ. The large inner residual body consisted of 7—12 larger granules and many small granules. A residuum consisting of a row of small granules was seen also between the two sporozoites.

In the above hosts the oocysts in question occurred consistently on the serosa, chiefly in the parts covering the kidneys, intestines, testicles and air bladder. The small microscopic specimens permit generally no definite conclusion as to whether the oocysts locate exactly on the serosa; evidence is obtained exclusively by aimed investigations. It is, therefore, supposed that *Eimeria siliculiformis* oocysts do not locate in the organs listed in the original description but rather in the covering serous membranes.

Examining an *Abramis brama* specimen from the River Danube, oocysts corresponding both morphologically and in dimensions to those described above were encountered. In specimens from the intestinal serosa, the oocysts were seen encased by well demarcated connective tissue. In unstained preparations the connective tissue capsule appeared to be embedded in a homogeneous, yellowish-red substance. Inside the connective tissue capsule oocysts and many free sporocysts were seen. On mild compression, the sporocysts readily split into two parts along their longitudinal axis (*Goussia* type). In some oocysts also spontaneously split sporocysts were seen. In some sporocysts the two sausage-shaped sporozoites were still clearly apparent, but only a remnant of residual body, vacuolized or composed of 2 or 3 granules only, making not more than 1/6th of the original residuum, was left. Such oocysts seemed to be aged, deteriorating stages.

The original description of *Eimeria siliculiformis* and our own findings do not seem to be as inconsistent as to justify the segregation of a new species. Until closer studies will have been finished, the present data are just contributions to the knowledge of the genuine, or the present supposed, *Eimeria siliculiformis*.

3. Oocysts from the intestine of fishes

Coccidian oocysts were found in intestinal contents and scrapings from a great many fish hosts, but precise observations were made on Eimerian parasites of only 5 species as follows: *Cyprinus carpio*, *Carassius carassius*, *Abramis brama*, *Scardinius erythrophthalmus* and *Alburnus alburnus*.

In every case, the diameters of the round oocysts varied between 8 and 12 μ, and the conspicuous sporocysts contained two worm-shaped sporozoites with reflected ends. As to other dimensional and morphological features, three types of oocysts could be distinguished.

(a) No distinction could be made between the oocysts parasitic in the intestines of *Cyprinus carpio* and *Carassius carassius*. Oocysts from both hosts were characterized by tapering-ovoid sporocysts filling the entire inside of the oocyst and in 90% of the cases examined, the fourth sporocyst was located above, or below, the other three clustered in one row (Fig. 3). In the majority of the cases one to five oocysts were found to locate in the so-called "yellow-bodies", corresponding to necrotic epithelial remnants.

(b) Oocysts from *Abramis brama* and *Scardinius erythrophthalmus* hosts are characterized by bluntly elliptic shape, somewhat loose arrangement of sporocysts and by an elongate residual body, as compared to the round oocysts, densely-packed sporocysts and chiefly roundish residual body of the former type. In the majority of the cases, two sporocysts each were seen to arrange at one level (Fig. 4). The oocysts occurred free in the intestinal contents.

(c) The oocysts recovered from *Alburnus alburnus* have a biconical cylindrical shape as contrasted to the ellipsoid shape of the oocysts described under (b). Otherwise the arrangement of sporocysts and the shapes and dimensions of sporozoites as well as the inner residual body were identical to type described under (b) (Fig. 5).

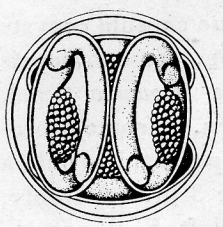

Fig. 4. Eimeria cyprinorum oocyst from the intestines of *Abramis brama*. ×4000. Original

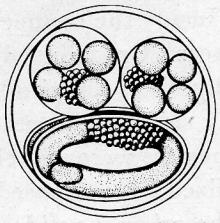

Fig. 5. Eimeria cylindrospora. Oocyst and sporocyst viewed from the longitudinal axis. ×4000. Original

Of the intestinal coccidia encountered, those recovered from *Cyprinus carpio* and *Carassius carassius* have been identified as *Eimeria carpelli*, those from *Abramis brama* and *Scardinius erythrophthalmus* as *Eimeria cyprinorum*, those from *Alburnus alburnus* as *Eimeria cylindrospora*, according to the forthcoming description:

Eimeria carpelli Léger & Stankovitch, 1921

The oocysts are round, with smooth double-layered wall not exceeding 0.5 μ in thickness. Oocyst varies from 8 to 12 μ in diameter.

The oocysts contain four sporocysts, tapering at both ends and widening at the middles. The sporocysts are closely packed inside the oocyst. Sporocyst dimensions: 7—8.5 μ by 5 μ.

Each sporocyst contains two vermiform sporozoites in head-to-tail position, reflected at both ends. Between the sporocysts a round, less often ovoid, homogeneous granular residual body is seen, measuring 2—2.5 μ by 2 μ.

Eimeria cyprinorum Stankovitch, 1921

The double-layered oocyst wall is smooth and thin, not exceeding 0.5 μ in thickness. Oocyst diameter: 8.5—11 μ.

The oocyst contains 4 elongate-ovoid sporocysts, which do not fill up the entire cavity within the cyst wall. The sporocysts measure 6—8 μ by 3.5—4.5 μ.

The vermiform sporozoites with one reflected end lie in head-to-tail position. Between them there is an ovoid or elliptic granular residual body, 3—1.5 μ by 2 μ in size.

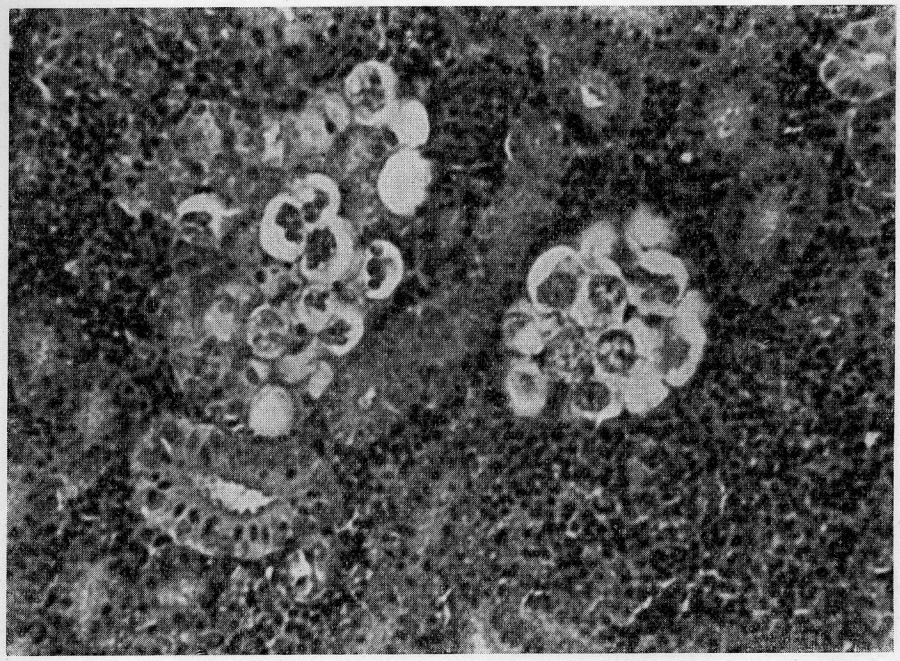

Fig. 6. Eimeria scardinii oocysts surrounded by haemopoietic tissue in the renal parenchyma of *Scardinius erythrophthalmus*. About ×200. Original

Eimeria cylindrospora Stankovitch, 1921

The smooth, double-layered, thin cyst wall does not exceed 0.5 μ in thickness. Oocyst diameter is 10—11 μ.

The oocysts contain four cylindrical sporocysts with tapering ends which do not fill the entire cavity within the cyst walls. Sporocyst measurements: 7—8 μ by 3.5—4.5 μ.

The sporocysts contain each two vermiform sporozoites, with one end reflected, in head-to-tail position. Between them there is an ovoid or elliptic homogeneous granular residual body, measuring 3—4 by 2—2.5 μ.

Faunistic descriptions have often referred to the occurrence of *Eimeria carpelli*-type coccidia. The oocysts recovered from various fish hosts have

been regarded *Eimeria carpelli* by the majority of authors, presumably because SCHULMAN (1962) has considered three species, *Eimeria cyprini* Plehn, 1924, *Eimeria wierzejskii* Hofer, 1904 and *Eimeria cyprinorum* Stankovitch, 1921, to be synonyms of *Eimeria carpelli* Léger and Stankovitch, 1921.

Based on our own experience we hold the view that consistently with SCHÄPERCLAUS (1954), the *Eimeria carpelli* and *Eimeria cyprini*, parasitic in

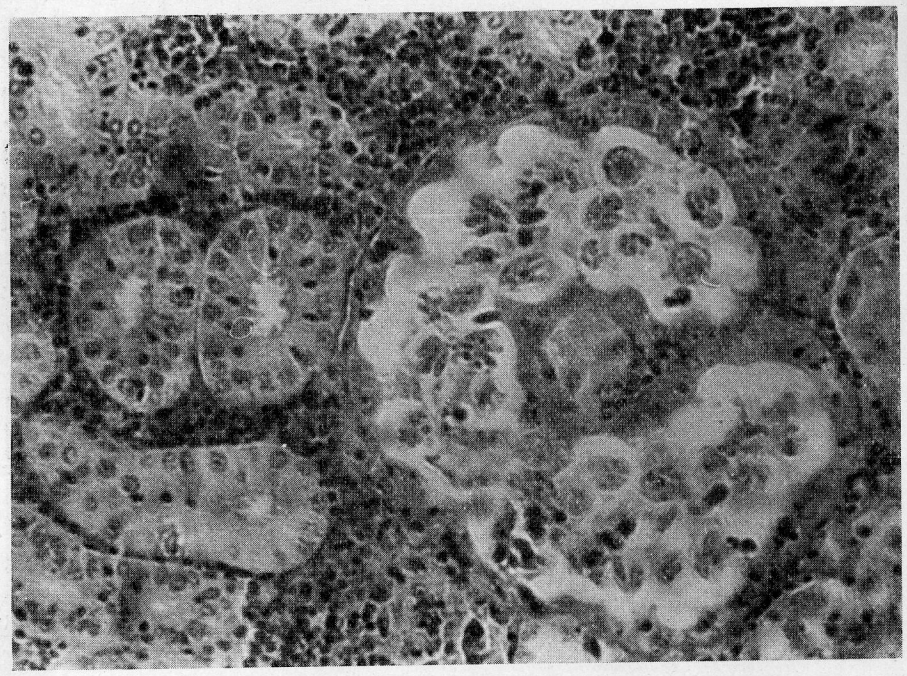

Fig. 7. Eimeria leucisci oocysts from a dilated renal tubule of *Blicca bjoerkna*. Approx. ×200. Original

one and the same host and showing only moderate dimensional differences should be regarded synonyms.

We are convinced of the validity of the species *Eimeria cyprinorum*, as it differs from *Eimeria carpelli* not only in respect of its host, but also in the morphological features outlined above. The validity of *Eimeria cyprinorum* may be doubted, if at all, only in relation to *Eimeria stankovitchi:* but it differs from the latter not only by the more or less conspicuous sporocyst sutures, but also by the circumstance that, according to the original description, the *Eimeria stankovitchi* oocysts contain neither outer nor inner residual bodies.

Particular attention should be paid to *Eimeria wierzejskii*, which by WIERZEJSKI's description (1898) has been identified by many investigators as

Eimeria carpelli. On WIERZEJSKI's drawing two coccidian oocysts locating in a yellow body and one Myxobolus spore are depicted rather than coccidia located in a Myxobolus pansporoblast as interpreted by HOFER (1904). Owing to the incomplete description and the unsatisfactory drawing, *Eimeria wierzejskii* should be preferably regarded a *nomen nudum*.

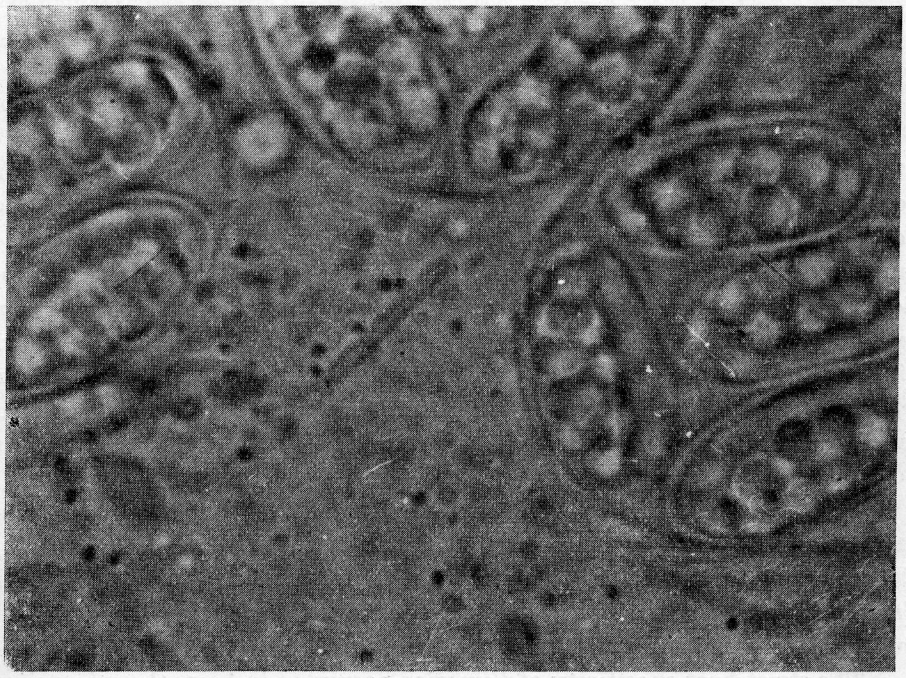

Fig. 8. Eimeria siliculiformis oocyst from the renal serosa of *Scardinius erythrophthalmus*. ×3000. Original

We classify with the carpelli-type also the species *Eimeria cylindrospora*, which is indistinguishable by common methods from the above-described carpelli-type species.

In the course of our examinations, the oocysts recovered from the intestines of *Carassius carassius* have been identified by morphological features as *Eimeria carpelli*, while those recovered from *Abramis brama* as *Eimeria cyprinorum*, which both represent new hosts of the coccidia in question. Nevertheless, we do not exclude the possibility that the newly detected coccidian parasites of the above hosts are independent species.

A list of the carpelli-type Eimeriae discussed in this paper is given below:

Eimeria carpelli Léger and Stankovitch, 1921. Syn.: *E. cyprini* Plehn, 1924. Host: *Cyprinus carpio*, *Carassius carassius*.

Eimeria cylindrospora Stankovitch, 1921. Host: *Alburnus alburnus.*

Eimeria cyprinorum Stankovitch, 1921. Host: *Scardinius erythrophthalmus*, *Rutilus rutilus*, *Barbus barbus*, *Phoxinus phoxinus* and *Abramis brama.*

Eimeria stankovitchi Pinto, 1928. Syn.: *Goussia légeri* Stankovitch, 1920. Host: *Alburnus alburnus*, *Scardinius erythrophthalmus.*

Discussion

Studies on protozoan parasites of fishes have disclosed a greater number of coccidia than expected. The reason of this may be that the majority of authors have been concerned primarily with intestinal coccidia, disregarding the species frequently parasitizing the parenchymatous organs of fishes. Indeed, the latters are difficultly demonstrable with the common methods, as scanty specimens can be detected and even those mostly only by tenacious microscopic examination. A further difficulty has been imposed by the distinct seasonality of the majority of coccidia parasitic in the parenchymal organs; this applies also to *Eimeria metschnikovi*, *E. scardinii*, *E. leucisci* and *E. siliculiformis* studied by us. During spring mostly developing sporogonic stages, during summer mature oocysts and during autumn aged deteriorating oocysts were recovered.

The differentiation of coccidia by oocyst morphology is often impossible. Although we found oocysts supposedly specific for a given fish host, *e.g.*, *Eimeria metschnikovi* in *Gobio gobio*, *Eimeria subepithelialis* in *Cyprinus carpio*, *Eimeria cylindrospora* in *Alburnus alburnus*, in the majority of the cases the morphologically indistinguishable oocysts occurred in various fish species.

The strict host specificity of Eimeriae occurring in homothermic animals has been extensively proven. This property is one of their most convincing species features. But as in a biological respect the Eimerian parasites of fishes differ considerably from the coccidia of warm-blooded animals, to mention only that their sporogony takes place in the host organism instead of the external world, the strict host specificity generally accepted for the latters cannot yet be extended to the formers* in want of cross infection experiments. Accordingly, the Eimerian parasites recovered during the present examinations from identical organs of different hosts have been tentatively regarded by us as belonging to one coccidian species.

* Based on the distinct difference between the biological properties of the Eimerian parasites of fishes and homothermic animals, at the II. International Congress of Protozoology (London, 1965), one of us (PELLÉRDY) has suggested that within the genus Eimeria subgenera be established, one of which should, under the name *Gousseimeria*, comprehend the Eimerian parasites of fishes. So far no final decision has been reached to this end.

SUMMARY

Prior to experiments aimed at proving or disproving the host specificity of fish coccidia, preliminary examinations were carried out on several hundreds of freshwater fishes belonging to 8 genera recovered from different habitats in Hungary. In the course of these studies, some new hosts of known fish coccidia, identified exclusively by morphological characters, were detected. *Blicca bjoerkna*, *Abramis brama*, *Alburnus alburnus*, *Rutilus* and *Chondrostoma nasus* were found to harbour the kidney parenchyma coccidium *Eimeria scardinii*. *Eimeria leucisci* was found to parasitize the renal tubuli of *Blicca bjoerkna*, *Abramis brama*, *Alburnus alburnus* and *Rutilus rutilus*, *Eimeria siliculiformis* the surface of the serosa of *Abramis brama*, *Alburnus alburnus* and *Scardinius erythrophthalmus*, and the intestinal coccidia *Eimeria carpelli* and *Eimeria cyprinorum* the intestines of *Carassius carassius* and *Abramis brama*, respectively.

Based on our findings, we regard *Eimeria cyprini* a synonym of *Eimeria carpelli*, consistently with SCHÄPERCLAUS, while inconsistently with SCHULMAN (1962), we advocate the validity of *Eimeria cyprinorum*. Owing to the incomplete description, we suggest that *Eimeria wierzejskii* Hofer, 1904, be considered a *nomen nudum*. The present studies resulted also in demonstrating the occurrence of *Eimeria cylindrospora* in Hungary.

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